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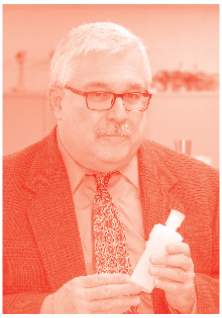



Capsicum
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Meet the editor

Aman Dekebo is currently a professor in the Department of Applied Chemistry, Adama Science and Technology University, Ethiopia. He obtained his first and second PhDs in Natural Products Chemistry from Addis Ababa University, Ethiopia in 2002 and Bio-organic Chemistry from Ehime University, Japan in 2007, respectively. He worked as a researcher during his sabbatical leave at Andong National University, South Korea (2017–2019). His research interests include isolation and synthesis of bioactive compounds, chemical ecology, and apiculture. He has authored more than fifty peer-reviewed scientific papers and served as editor of the book *Plant Extracts* (2019). He has also participated and presented his works in several conferences in many countries. He is co-author of *Phytochemistry of Turmeric Rhizomes* (*Curcuma longa*): *Isolation and Characterization of Compounds from Foots of Turmeric Rhizomes* (*Curcuma longa*) (2012). He serves as a reviewer for many reputable journals. He is recipient of best researcher awards and certificates from School of Applied Natural Sciences, Adama Science and Technology University, Korean Society of Applied Entomology, and Korean Apiculture Association.

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Preface

Capsicum (bell pepper or chili pepper) is a genus of flowering plants in the nightshade family Solanaceae. *Capsicum* is one of the most economically important vegetable crops throughout the world. It has great importance for its nutritional characteristics and its antioxidant content to reduce food spoilage. The fruits of this plant are used as seasoning for food because of their characteristic flavor, aroma, and pungency. The unique flavonoids of this genus are capsaicinoids and capsainoids, which have been linked to many beneficial biochemical and pharmacological effects including antioxidant, anti-inflammatory, and anti-cancer activities. This book provides relevant and practical information for readers interested in this crop, including biochemists, natural product chemists, pharmacologists, pharmacists, molecular biologists, researchers, students, ethnobotanists, ecologists, nutritionists, organic gardeners and farmers, and those interested in herbs and herbal medicine.

Chapter 1 begins with a discussion of the diversity and potency of *Capsicum* species grown in Indonesia. It describes their botanical information, distribution area, and nutritional and flavor aspects, including their bioactive compounds. It also examines the utilization of this crop in the food and pharmaceutical industries.

Chapter 2 focuses on different biotic constraints, particularly anthracnose disease, which is major limiting factor affecting yield and production of *Capsicum*. Different symptoms associated with the disease include fruit rot, leaf spots, dieback on stem, seedling blight or damping off. The chapter illustrates proper management strategies for controlling this disease. Chapter 3 reviews the updated information available regarding the effect of hot water treatment on growth, disease incidence, and increase in yields of vegetables and crops including *Capsicum*. Chapter 4 deals with biological control, based on the use of microorganisms, as an efficient and sustainable alternative for *Capsicum* cultivation compared to the traditional uses of chemical pesticides that have negative consequences on the environment and human health. Chapter 5 reviews current options for managing different viruses attacking *Capsicum* and other vegetables and crops. These viruses include the *Pepper veinal mottle virus* (PVMV), *Potato virus Y* (PVY), and *Cucumber mosaic virus* (CMV), which are endemic in many African countries including Uganda, Mali, Cameroon, Morocco, and Nigeria.

Chapter 6 describes how the micronutrients provided by incorporating nutrient-rich pepper into an individual's diet help in treating nutrient deficiencies. Chapters 7–10 deal with bioactive compounds of *Capsicum* known as capsaicinoids. Capsaicinoids, a group of chemical principles present in matured *Capsicum* pods, are responsible for the pungency as well as pharmacological properties of capsicum. Some examples of important capsaicinoids include capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. Chapter 8 discusses the combinational use of capsaicin with other natural dietary compounds that have synergistic anticancer activities. Chapter 9 reviews bioactive compounds from *Capsicum* seeds, their biological properties, extraction systems, and their industrial application. Chapter 10 addresses the aspects of pepper spraying technology and use of capsaicin for non-lethal defense technology. Chapter 11 details a study on the importance of capsaicin, which is neurogenic and may affect neuronal functions. The study investigated the effects of consumption of chili pepper and capsaicin diets on the neurobehavior of CD-1 mice. The neurobehavioral parameters assessed were anxiety, motor coordination, pain, social behavior, learning, and memory.

The contributors to this book have critically reviewed the literature and presented their original research. The editor worked closely with all authors to ensure that the individual chapters form a cohesive book. I would like to thank everyone who contributed to the publication of this volume.

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Section 1

Diversity and Potency of *Capsicum*

Chapter 1

Diversity and Potency of *Capsicum* spp. Grown in Indonesia

Cristofora Hanny Wijaya, Manik Harda and Bunga Rana

Abstract

Capsicum spp., popularly known as chili pepper, is abundantly cultivated in Indonesia. Chili pepper has deeply integrated into Indonesian culture, even turned into inseparable ingredients in the local diets. Adapted to the Indonesian environment and timely selected according to the local palate, vast variation of appearance, color, taste, aroma, and pungency of chili pepper transformed into a variety of cuisines and medicinal purposes. This chapter covers a selection of chili peppers in Indonesia, introduced as "commonly available in the market," and some other variants, which are less popular; however, due to their unique flavors, they will be interesting to be introduced and addressed as "exotic *Capsicums*." This chapter will describe their botanical information, distribution area, and nutritional and flavor aspects, including their bioactive compounds. Furthermore, the utilization of genus *Capsicum* in Indonesia, from the food industry, pharmaceutical, and ornamental plants, will be described.

Keywords: bioactivity, *Capsicum* spp., chili pepper, flavor, Indonesia

1. Introduction

Portuguese brought *Capsicums* to the eastern part of Indonesia, Maluku, in the seventeenth century and then widely distributed to other parts of the archipelago. It is assumed that the four species (*C. annuum*, *C. frutescens*, *C. chinense*, and *C. pubescens*) were introduced to Indonesia before World War II [1]. During that time, the Javanese called the plant "godong sabrang," which means a plant from a foreign country [2]. Later, in each region of Indonesia, *Capsicums*, especially chili or hot peppers might have different names, like "cengek," "lado," "lada," "cabe," "cabi," "lombok," "ricagufu," "serbeh ulom," "serbeh," and many others. However, officially, pungent members of *Capsicums* or hot chili peppers in Indonesia are called "cabai," while the sweet and nonpungent ones are called "paprika." From then on, in this text, we use the term "cabai" for hot peppers or chili peppers and "paprika" for sweet peppers.

The *Capsicums* currently cultivated in Indonesia are quite different from those first carried by the Europeans since they have been crossbred and undergone genetic changes due to adaptation to local environments and natural and human selection. The seeds of *Capsicums* cultivated in Indonesia are also distributed to neighboring countries and even to Macau, China [3, 4]. During that time, local inhabitants of Java Island used *Capsicum* fruits in their cooking, while the leaves were used for topical ointment.

Indonesia also included *Capsicums* as part of their traditional ceremony and ritual. In some ceremonies in Java, sometimes, they put *Capsicums* on top of *tumpeng*, a traditional dish consisting of cone-shaped yellow-colored rice complemented with other side dishes and vegetables. Examples of traditional Indonesian dishes using *Capsicums* are *sambal terasi* (Java), *balado Padang* (West Sumatra), *rica-rica Manado* (North Sumatra), and *lawar* (Bali). *Lawar* from Bali was involved in wedding, death, and other religious ceremonies [2].

Capsicum has become an essential commodity in Indonesia, where its price might influence political and socioeconomic conditions. Similar to what happened in South Korea in 1978–1979, where the people's unrest was subjected to the government due to a shortage of *Capsicums* [5], in Indonesia, the price of *cabai* could be one of the parameters to measure the government performance in establishing food security. For the majority of Indonesians, regardless of social class or economic status, *Capsicum* has become an inseparable part of their daily consumption and may serve as a source of vitamin C, E, and folate, as well as carotenoids, phenolic compounds, flavonoids, and capsaicinoids [6, 7]. Even with the decrease in household incomes or an increase in household expenses, consumers still tend to buy *cabai* [8, 9]. Like India, Thailand, and South Korea [5, 10], the consumption of *Capsicums* in Indonesia was also high. Among other vegetable plantations, *cabai* occupied 416,3981 ha of cultivation area in 2019 [11], the second largest in the country after cabbage.

Cabai prices could influence the price of other food products, affecting the economy, consumption, and buying habits of the consumers. Hence, the fluctuation of *cabai* prices could trigger inflation. For example, in the rainy season, there is a possibility of decrease in *cabai* production due to the crop failure. The unmet high demand, causing a double increase in price for *cabai*, resulted in inflation. This phenomenon has already occurred for several years. *Cabai* contributed 0.09% of 0.28% Indonesian inflation level in the early rainy season in October 2018, and *cabai* was also reported as the leading cause of Indonesian economic inflation in 2006 based on data from Statistics Indonesia [13].

2. Types of *Capsicum* species in Indonesia

From the genus *Capsicum*, there are 27 species members, including five domesticated species (*C. annuum, C. frutescens*, *C. chinense*, *C. pubescens*, and *C. baccatum*) around the globe [14]. *Capsicum* species commercially cultivated in Indonesia are *C. annuum* and *C. frutescens* as seen in **Figure 1**. The other species like *C. chinense* and *C. pubescens* can only be found in several districts and usually cultivated by

Figure 1.
Capsicums *commonly available in Indonesian market.*

hobbyists [4]. From the distribution of *C. chinense* in Indonesia, though it is very limited in Southeast Asia, it is assumed that "Indonesia had more potential and genetic resources to breed species of *Capsicum* than other countries in Southeast Asia" [1].

In Indonesia, there are *Capsicum* hybrid lines produced by seed agents, like "cabai sultan," "hot beauty," "megahot," "Carvi Agrihorti," and "cabai trisula." *Capsicum* hybrid lines were most farmer's favorites, due to their high productivity beside their relatively high prices. However, as progenitors, they might not be good candidates due to their variability in the progenies. Meanwhile, the local and open-pollinated cultivars might not produce as much yield as hybrid lines, but they are generally more resistant to pests and disease, cheaper, and fit to use as progenitors [15]. The cultivated variety is selected based on market demand (color, aroma, taste, pungency, appearance, and size), high productivity, resistance to pests and disease, and adaptability to the local environment. Meanwhile, the consideration in choosing seeds is the availability of the seed's certificate, moisture content, seed purity, germination, absence of contaminants, and seed's health.

2.1 Commonly available in the market

*2.1.1 Cabai keriting (*Capsicum annuum *L. var. Longum)*

Cabai keriting is a highly marketable commodity. The fruits are thin-skinned, long (around 12 cm), slender, curly, or wavy in appearance with pointed tips as shown in **Figure 2** [16]. There are many seeds inside the fruit. The plants usually have a productive age up to 6 months in relatively high humidity with a temperature around 16–32°C, soil pH around 5–7 [17], and relatively resistant to plant diseases and can be harvested all year round [4]. On average, each plant can produce up to 0.75–1 kg fruit/harvesting period, i.e., 6 months. The fruits are harvested both in the immature stage (green) and the mature stage (red) [18].

This cultivar of *Capsicum* is one of the superior and popular cultivars in Indonesia due to the plants' productivity and relatively high price, and their thin skin and lower moisture than *cabai merah besar* make them not so prone to rot and have longer shelf-life compared to *cabai merah besar* [4, 17]. Some known varieties are *cabai keriting Solok*, *cabai keriting Cirebon*, *cabai keriting Irian*, *cabai keriting ungu*, *cabai keriting Lampung*, *cabai keriting Padang*, *cabai keriting Bengkulu*, *cabai*

Figure 2.
Cabai keriting *(*C. annuum *L. var. Longum).*

keriting Lembang, *cabai keriting Pangalengan*, and *cabai keriting Medan* [19, 20]. In 2011, there were 86 cultivars of *cabai keriting* in total [21]. From the plant morphology, there were 13 parameters to consider in choosing cultivars, i.e., leaf shape, leaf tip, the color of the anther, the color of pistil, fruit surface, the color of young fruit, plant height, dichotomous height, canopy width, fruit length, fruit diameter, and fruit weight [15]. Examples of popular *cabai keriting* seeds cultivated in Indonesia are Taro, Lado, Bagayo, Tanamo, Jago, New Taro, and Laris. A picture of *Cabai keriting* grown in Indonesia is shown in **Figure 2**.

Distribution areas. *Cabai keriting* is usually cultivated in West Java and Sumatra Island (Bengkulu, Lampung, West Sumatra, and North Sumatra) [16, 19].

Flavor characteristics. The taste of *cabai keriting* is relatively hotter than *cabai merah besar* (*C. annuum*) but not as hot as *cabai rawit* (*C. frutescens*). There were several varieties of *cabai keriting* in Indonesia, e.g., *cabai keriting ungu*, *cabai keriting Lampung*, *cabai keriting Padang*, *cabai keriting Bengkulu*, *cabai keriting Lembang*, *cabai keriting Pangalengan*, and *cabai keriting Medan*, according to the order of their pungency from low to high with Scoville heat unit (SHU) varied from 30,000 to 170,000 [19]. Since the flavor components change during maturation [22], it would be important to notice that the green and red maturing stage of cabai keriting might alter the character of the dishes. For example, *ayam cabe hijau* was a traditional cuisine that is uniquely characterized by the use of immature stage (green) of *cabai keriting* as the main seasoning for cooked chicken. Meanwhile, red *cabai keriting* is usually used in cooking for its attractive red color and relatively hot pungency.

Bioactivity and health benefits. *Cabai keriting* was believed to have antimicrobial effects; thus, the inhibitory effect of the oleoresin of *cabai keriting* against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* had been reported [23]. The oleoresin contained tetramethyl hexadecenol, tetramethyl hexadecene, tetramethyl hexadecapentaene, ethyl decahexanoate, ethyl linoleate, methyl linoleate, palmitoyl chloride, hexanedioic acid, octadecadienoic acid, ethyl linoleolate, butyl isopropylphenyl oxalate, and capsaicin.

*2.1.2 Cabai merah besar (*Capsicum annuum *L.) and (*Capsicum annuum *L. var. Tanjung)*

The fruits of *cabai merah besar* are quite long, big, and bulky with a smooth surface and thick skin [12]. Some fruits have sharp points, and the others have round points [24]. On average, each plant can produce up to 1–1.2 kg fruit/harvesting period [18], but this number depends on the varieties, agricultural practices, and plants' environments. The farmers harvest both immature fruits (green) and mature fruits (red) of *cabai merah besar*.

Some known varieties of *cabai merah besar* are Ciko, Lingga, Tanjung, Kencana, Tit Super, Paris, and Jati Laba. In 2011, there were 87 cultivars of cabai merah besar in total [21]. Examples of popular *cabai merah besar* seeds cultivated in Indonesia are Profit, Arimbi, Horison, Imperial 10, Jet Set, Imola, Gadewa, Gada, Elegance, and Gada MK. *Cabai merah besar* is shown in **Figure 3**.

Distribution areas. *Cabai merah besar* is cultivated in almost every province in Indonesia especially in West Java, Central Java, North Sumatra, East Java, West Sumatra, Aceh, Lampung, South Sumatra, South Sulawesi, and Bengkulu [25].

Flavor characteristics. There were several varieties of *cabai merah besar* in Indonesia, e.g., Tit Super, Paris, and Jati Laba, with pungency around 12,500 SHU [19]. *Cabai merah besar* is the least hot compared to *cabai keriting* and *cabai rawit* (*C. frutescens*). The popular cultivar of *C. annuum* was less hot compared to *C. frutescens* [26]. Since their pungency is considered low, *cabai merah besar* is usually used due to its attractive color, texture, and aroma, not because of their pungency.

Figure 3.
Cabe merah besar *(*C. annuum *L.) in the market.*

The fruits have a crunchy texture and higher moisture content compared to *cabai keriting*. However, the same with *cabai keriting*, both green and red maturing stages of *cabai merah besar* are also used, but the application is different depending on the intended dishes. The green stage of *cabai merah besar* is usually boiled, stir-fried, or fried before consuming, to remove unwanted aroma.

Nutrition component. *Cabai merah besar* contains moisture (90%), energy (32 kcal), protein (0.5 g), fat (0.3 g), carbohydrate (7.8 g), fiber (1.6 g), ash (0.5 g), calcium (29.0 mg), phosphorus (45 mg), iron (0.5 mg), vitamin A (470 IU), vitamin C (181 mg), thiamin (0.05 mg), riboflavin (0.06 mg), and niacin (0.9 mg) per 100 g red fruits [27–29], while the green fruits contain energy (23 kcal), protein (0.7 g), fat (0.3 g), carbohydrate (5.2 g), calcium (14 mg), phosphorus (23 mg), iron (0.5 mg), vitamin A (260 IU), and vitamin C (84 mg) per 100 g fruits [24].

Bioactivity and health benefits. It is reported that the ethanolic extract of *cabai merah* with a dosage of 200, 400, and 600 mg/kg was effective in lowering blood sugar levels in male white mice hyperglycemia [30]. The most effective duration in lowering blood glucose levels was 21 days. The possibility of luteolin extracted from *C. annuum* L. as an alternative to treat autism spectrum disorder (ASD) using the reverse docking technique has been explored; the result showed that luteolin had a potency to work as an MMP-3 antagonist. MMP-3 (matrix metalloproteinase) was suspected of having a role in the neuropathology of ASD [31].

A study about *cabai merah* effects towards hypercholesterolemia showed that 200 mg/kg ethanolic extract of *cabai merah* had a cholesterol-lowering effect on hypercholesterolemia mice with the best administration duration in lowering blood cholesterol of male albino mice of 14 days [32]. The conversion of this dose to humans with the average body weight of 70 kg results in as much as 173.15 g of *cabai merah* needed to get the expected effect. This bioactivity might be attributed to several compounds such as capsaicin, vitamin C, saponin, and flavonoid compounds. It is reported that dietary capsaicin improved endothelium-dependent vasorelaxation and prevented hypertension [33].

The inhibitory effect of oleoresin of *cabai merah besar* against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *and Pseudomonas aeruginosa* was also reported. Oleoresin contained tetramethyl hexadecenol, tetramethyl hexadecene, tetramethyl hexadecapentaene, ethyl hexadecanoate, linoleic acid, methyl linoleate, azacylotridecanone, hexanedioic acid, octadecadienoic acid, ethyl linoleolate, octadecenyl acetate, and capsaicin [23].

*2.1.3 Paprika (*Capsicum annuum *var. grossum)*

The fruit color and shape vary as shown in **Figure 4**, but mostly they have bell-shaped, long with pointed tips or almost square with hollow space inside, and thick skin (around 0.5 cm). The fruit is relatively big compared to *cabai merah besar* [12, 34]. The seeds are clustered together and attached to the placenta [35]. On average, each plant can produce around 3.5–3.7 kg fruit/harvesting period, i.e., 12 months [18]. There are many paprika seed varieties cultivated in Indonesia, for example, Jumbo seed, Takii ace, Green Horn, New Ace, Wonder Bell, Top Star, Blue Star, Uranus, Big Star, Queen Star, Beauty Bell, Sunny Star, Lucky Star, Colombo, Mayata, Golden California Wonder, Skipper, Melody, Fortuna, Yellow Star, and Virgo [35].

Distribution areas. Paprika is usually cultivated in North Sumatra, Riau, West Java, East Java, Bali, West Nusa Tenggara, East Nusa Tenggara, Central Sulawesi, Southeast Sulawesi, Maluku, and West Papua [25].

Flavor characteristics. The fruit tastes mildly sweet with a crunchy texture. The fruit aroma has a pungent impression but does not taste hot at all [34]. The fruit slices are usually cooked with meat or as pizza toppings.

Nutrition components. The fruit contains protein 0.9%, fat 0.3%, carbohydrate 4.4%, calcium 7 mg/100 g, phosphorus 22 mg/100 g, iron 0.4 mg/100 g, vitamin A 22 IU/100 mg, vitamin B1 540 mg/100 g, vitamin B2 0.02 mg/100 g, vitamin C, 160 mg, and niacin 0.4 mg/100 g [35].

Bioactivity and health benefits. As an herbal medicine, consuming paprika is suggested to control cholesterol and triglycerides and prevent atherosclerosis, hypertension, flu, sinusitis, and digestion problem [36]. One study found that the ethanolic extract of paprika exhibited antimicrobial activity towards pathogen *Enterococcus faecalis* [37]. Another study reported that *Capsicum annuum* var. grossum could be

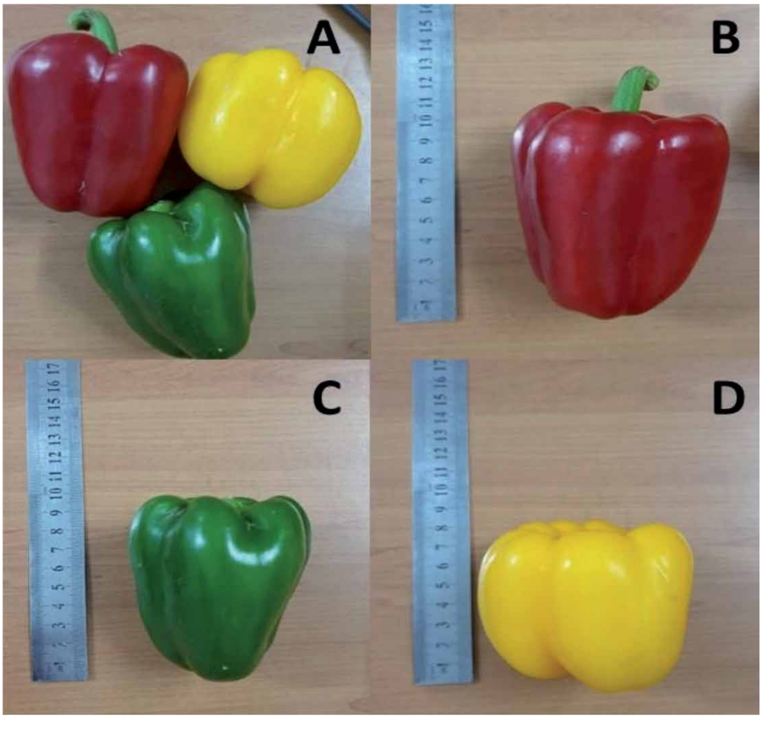

Figure 4.
(A) Some paprikas commonly available in Indonesian market, (B) red paprika (C) green paprika, (D) yellow paprika.

an alternative intervention for Alzheimer's disease (AD) by inhibiting β-secretase activity and β-Amyloid$_{1-40}$ aggregation [38]. It is stated that phenolic extracts from the fruits could counteract the initial aggregation of Aβ_{1-40}, as well as prevent further aggregation of preformed fibrils. The deposition of amyloid protein as senile plaques produced by the sequential cleavage of the amyloid precursor protein by secretases was the key signature of Alzheimer's disease. These inhibitory activities were attributed to the presence of phenolic constituents in the fruits [38].

*2.1.4 Cabai rawit (*Capsicum frutescens*)*

As it is shown in **Figure 5**, *cabe rawit* fruits are small, around 3.7–5.3 cm long, and the seeds are usually light brownish [12]. On average, each plant can produce a 0.5–0.6 kg fruit/harvesting period [18]. There are several cultivars of *cabai rawit* in Indonesia. A bit bulky with round tips is *cabai ceplik*. Immature *cabai ceplik* in Indonesia turned color from bright green to dark red during maturation. *Cabai rawit* with relatively higher pungency than *cabai ceplik* is *cabai jemprit*. The fruit is small and short and has a sharp point. The fruit turns color from dark green to red during maturation. *Cabai putih/cabai rawit domba* looks similar to *cabai jemprit*, but the color is yellowish and turns bright reddish orange during maturation [24]. There are also other cultivars called *cabai kathur* because the fruit's tip points to the sky and *cabai burung* or bird eye pepper with small fruits. There are several seed varieties cultivated in Indonesia, e.g., Pelita 8, Sonar, Bara, Taruna, Dewata, Nirmala, Lentera, Raga, Bhaskara, Maruti, Cakra Putih, and Cakra Hijau.

Distribution areas. *Cabai rawit* is cultivated in almost every province in Indonesia, especially in West Java, Central Java, East Java, Aceh, West Nusa Tenggara, and Bali [25].

Flavor characteristics. The taste of *cabai keriting* is relatively hotter than *cabai merah besar* (*C. annuum*) but not as hot as *cabai rawit* (*C. frutescens*). There were several varieties of *cabai rawit* in Indonesia, e.g., *rawit putih*, *cabai rawit hijau*, *cabai rawit Lampung*, and *cabai rawit Kalimantan*, according to the order of their pungency from low to high with 82,500–480,000 SHU [19], the major organic acids of *C. frutescens* were malic, citric, and ascorbic acid. In contrast to *C. annuum* in which citric acid was the primary organic acid, in *C. frutescens*, the dominant organic acid

Figure 5.
*Cabai rawit (*C. frutescens*).*

was malic acid [26]. The volatile compounds of *C. frutescens* consisted of aliphatic esters and methyl salicylate imparting fruity and fresh impressions. However, as the fruit turned to red, the flavor changed towards more pungent and citrus-like aroma. The pungency also increased as the fruit matured [39], but the pungent compound might decrease at later stages of maturation [40]. The immature fruit, having less pungency than the mature ones, is used to be consumed raw with vegetable fritter snack, fried tofu, and tempeh or put into rissoles.

Nutrition components. *Cabai rawit* contains energy (103 kcal), protein (4.7 g), fat (2.4 g), carbohydrate (19.9 g), fiber (1.6 g), ash (0.5 g), calcium (45.0 mg), phosphorus (85 mg), vitamin A (11,050 IU), and vitamin C (70 mg) per 100 g fruits [24].

Bioactivity and health benefits. *Cabai rawit* contains phytochemicals such as flavonoids, capsaicinoids, and other phenolic compounds [41]. Capsaicinoids have been reported to have various health benefits such as antioxidant, anticarcinogenic, anti-inflammatory, and relieving neuropathic pain [42–45]. The locals believe that *cabai rawit* is good for the eyes, can cure cancer, alleviate joint pain, improve blood circulation, and stimulate appetite [46]. The potency of *C. frutescens* extract for anti-obesity in 3 T3-L1 cells, due to its higher content of phenolic compounds and antioxidant activity compared to *C. annuum*, was explored [39].

2.2 Exotic *Capsicum*

*2.2.1 Cabai gendot (*Capsicum pubescens*)*

Before 1916, this *Capsicum* was introduced to Indonesia, and the locals called this *cabai* as "cabai gendot," "cabai bendot," "cabai gondol," "cabai gombol," "cabai Bandung," or "cabai Dieng" [47]. The fruit shape varies from round to globular, 3.5–4 cm long, and 2.5–4 cm in diameter with thick flesh [40]. The fruit color changes from green (immature) to red (mature). The seeds are relatively small and few. Some people might confuse this *cabai* as *C. chinense*. The picture of *cabai gendot* can be seen in **Figure 6**.

Distribution areas. *Cabai bendot* is usually cultivated in Bandung (West Java), Dieng (Central Java), Kota Batu, and Pasuruan (East Java). This *cabai* is rarely found in the market [16, 47].

Flavor characteristics. The flavor of *cabai gendot* differs from *C. annuum*. This *Capsicum* cultivar is relatively juicier and hotter than *cabai merah besar*, around 100,000–350,000 SHU. The fruit has a fruity and floral aroma [34]. Due to their hotness, some people rarely eat fruit raw. People used to put both mature and immature stages of *cabai gendot* in *sambal* and mix them in stir-fry vegetables (*oseng-oseng*), or other plant-based dishes (e.g., *sayur tahu*, *sayur asem*). The fruits also have a strong and unique pungent aroma [47].

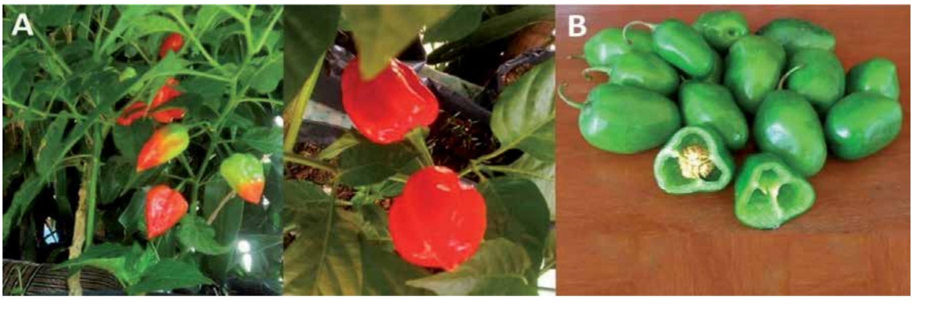

Figure 6.
*Cabai gendot (*C. pubescens*) (A) red cabai gendot (B) green cabai gendot.*

*2.2.2 Cabai katokkon or cabai kotokkon (*C. chinense*) or (*Capsicum annuum *L. var. sinensis)*

The name *cabai katokkon* was derived from the word "katokkon," which means "big fruit." This *cabai* is classified as *C. chinense* [1] or *C. annuum* L. [48]. The fruits are shaped like paprika but smaller (around 3–4 cm in length and 2–3.5 cm in diameter), round, and bulky as shown in **Figure 7**. The immature fruit is purplish green and turned bright red when mature. This plant can grow well on 200 m above sea level with adequate sunlight and watering. The plant can be harvested 3 months after seed planting, and on average, the plant can produce 0.8–1.2 kg fruits/harvesting period, i.e., 8–10 months [49].

Distribution areas. *Cabai katokko*n is cultivated in Rantepao, Tana Toraja (South Sulawesi) [1].

Flavor characteristics. The fruits have a unique floral, fruity and strong pungent aroma, and higher pungency than commonly consumed *Capsicums* [1]. By calculating the concentration of capsaicin (without taking account of dihydrocapsaicin), the pungency level of cabai kotokkon dried powders was reported to be around 42.914 SHU (immature stage) and 48.778 SHU (mature stage) [50]. Other sources reported the pungency level of 400,000–691,000 SHU [48].

Bioactivity and health benefits. Besides containing vitamin C and provitamin A that have antioxidant capacity, in Tana Toraja, South Sulawesi, people believe that the fruits could increase appetite, prevent aging, reduce stress, help to alleviate joint pains, lower cholesterol, improve blood circulation, prevent stroke, and alleviate wet cough, nasal congestion, and migraine [49].

*2.2.3 Cabai domba (*Capsicum frutescens*)*

The fruit shape is small but a little bit bigger than common *cabai rawit*. It has a round tip as shown in **Figure 8**. The color of the fruit varies from greenish white (immature stage), pale green, and orange [51]. Some people put them in the same category as *cabai rawit*; *thus*, it is called "cabai rawit domba" or "cabai putih," and some other people confused cabai domba as *Capsicum annuum* L. var. abbreviata Fingerhuth.

Distribution areas. *Cabai domba* is cultivated in Bandung, West Java [51].

Flavor characteristics. The pungency of the fruit is around 50,000–100,000 SHU [49]. Due to their relatively hot taste, the fruit is rarely consumed raw; instead,

Figure 7.
*Cabai katokkon (*C. chinense*) growth in the garden.*

Figure 8.
*Cabai domba (*C. frutescens*).*

Figure 9.
*Cabai hiyung (*C. frutescens*) [54].*

people mixed it in their cooking or put in *sambal*. They have a strong pungent smell, especially when exposed to heat.

*2.2.4 Cabai hiyung (*Capsicum frutescens *L.)*

Cabai hiyung has an appearance similar to *cabai rawit*, around 2–3 cm in length, and relatively thin skin with the weight of each fruit is around 0.7 g [52]. The plant can be harvested at the age of around 3–4 months with harvesting period of 6–7 months. The fruits are harvested when they turn brownish yellow or red [53]. The picture of *cabai hiyung* [54] can be seen in **Figure 9**.

Distribution areas. *Cabai hiyung* is cultivated in Hiyung village, South Kalimantan [53].

Flavor characteristics. The fruit was reported as having many times higher pungency than the common cultivar of *C. frutescens* in Indonesia; therefore it was named as the hottest *C. frutescens* in Indonesia [55]. Though we could not find the level of pungency in SHU, the fruits reported having 802 ppm of capsaicin [56].

Nutrition components. The fruit was reported containing vitamin A (11.89 IU/100 g), 82–92 mg/100 g vitamin C, and 1.2–1.5% protein [53, 56].

*2.2.5 Cabai ceremai (*Capsicum chinense*)*

This *cabai* is a habanero-type pepper (*C. chinense*), in which the colors vary from green, yellow, bright orange, and orange. Different names have been attributed to this

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fruit, such as "cabai ceremai," "cabai cermai," "cabai kancing," "cabai tomat," "cabai belimbing," "baby chili Haba," and "cabai tawau." The fruits are generally clustered into three to five pieces in each branch. The fruit shape varies from round shape with a papillae tip to bell and cone and long shape with fruit length up to 12 cm. The fruit skin wrinkles, but sometimes it also has smooth skin. The colors of the fruits vary from green, red, orange, pink, yellow, or brown. The seeds have a pale color [1, 4].

Distribution areas. The distribution of *cabai ceremai* in Southeast and East Asia was still limited. *Cabai ceremai* can be found in Jakarta, Bogor, Sukabumi (West Java), Jepara, Tawangmangu (Central Java), Malang (East Java), Bangka Island, Bali, Putu Sibau (West Kalimantan), and Tarakan (East Kalimantan) [1, 4].

Flavor characteristics. The fruit was reported to have high pungency [1, 4], but we could not find the data on the level of pungency in SHU. *Cabai ceremai* was not popular in the market since consumers preferred to buy and consume *cabai* that have elongated shape [57].

3. *Cabai* utilization and applied products in Indonesia

Since its first coming to Indonesia, *cabai* has been widely utilized in the food and culinary industry. Besides that, *cabai* in Indonesia was also utilized in the pharmacy as transdermal medicine (plaster, oil, balm, and cream) and as ornamental plants due to its attractive and unique fruit colors.

3.1 *Cabai* in Indonesia food and culinary culture industry

Cabai is considered as an important ingredient since it is inseparable from Indonesian food culture as is shown in some of Indonesia dishes in **Figure 10**. During a meal, if *cabai* is not included in the dish seasoning, it will be served separately as "*sambal*," crushed *cabai* mixed with various other ingredients and commonly eaten alongside the main course, or the *cabai* will be served to be eaten raw. Some people even consider something missing in their meal, and it would not be "complete" if their food is not spicy or there is no *sambal* to be eaten with the main course. Therefore, their satisfaction with the food will decrease. Over time, the way of people consuming *cabai* was developed, and *cabai* was transformed into processed food products, such as sauce and powder, as the complementary dish of the main course (**Figure 11**).

The utilization of *cabai* in local cuisines may vary from vegetable-based food such as stir-fry vegetables or as a sauce (or like salad dressings) together with peanut in mixed vegetables, such as *gado-gado*, *pecel*, and *karedok*. *Cabai* is also mixed in meat-, chicken-, and seafood-based cuisines, such as *rendang* (Indonesian famous pungent cuisine), *gulai* (spicy curry), *asam padeh* (spice and acid fish curry), and other dishes. "Balado" is an Indonesian dish that combines any type of food ingredients with *cabai merah*, for example, egg *balado*, fish *balado*, chicken *balado*, *dendeng* (dried meat) *balado*, eggplant *balado*, and shrimp *balado*.

3.1.1 Sambal: An Indonesian traditional crushed cabai

Sambal is an Indonesian famous and essential complementary cuisine made from *cabai* as the main ingredient. It is a crushed or grounded *cabai* with or without any other ingredients. Generally, *sambal* is known to stimulate appetite or to increase food palatability [8]. Before *Capsicum* came to Indonesia, *sambal* was originally made from ginger. Then people started to prepare *sambal* from pepper (*merica*) and *cabai Jawa* (*Piper retrofractum*) [58]. Since *Capsicum* spp. was introduced to Indonesia and cultivated, *sambal* was dominantly made from *Capsicum* spp.

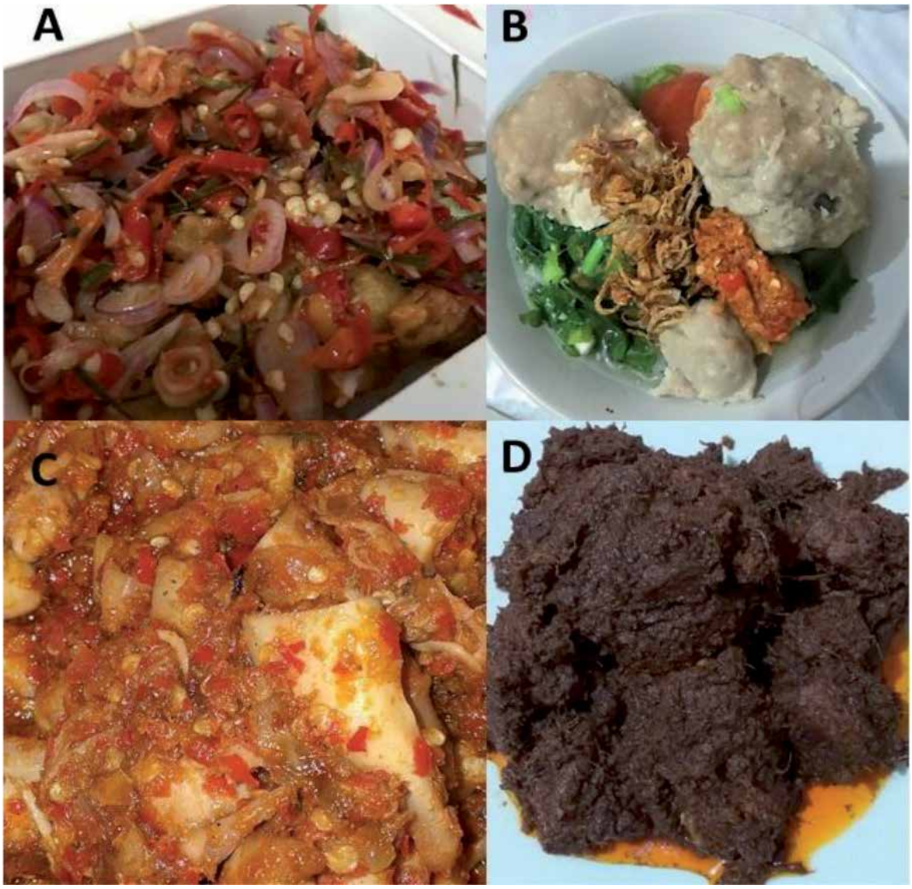

Figure 10.
Cabai *as an inseparable ingredient in Indonesian cuisines. (A)* Cabai *slices are mixed as ingredient in daily cuisine, (B)* Sambal *and* cabai sauce *in meat balls cuisine, (C)* Cumi balado *(calamari* balado*) as an example of* balado *dish, (D)* Rendang, *Indonesian famous spicy cuisine with* cabai *as one of essential ingredient.*

Figure 11.
Common cabai processing product in Indonesia. (A) Sambal terasi, (B) cabai sauce, (C) cabai powder.

Almost every region in Indonesia has their own types of *sambal*. There were about 322 types of sambal in Indonesia, and they varied over the nation with 119 types of raw sambal, 138 types of cooked sambal, and other types of *sambal* [59]. *Sambal terasi,* as seen in **Figure 11(A)**, was made from *cabai merah*, *cabai rawit*, *terasi* (fermented shrimp), onion, garlic, salt, and sometimes tomato. *Sambal roa* from Manado, North Sulawesi, was made from *Capsicum* and smoked *roa* fish, which can be found abundantly in some particular areas. *Sambal tempoyak* from southern Sumatra was mainly made from *Capsicum* and *tempoyak*, a fermented durian. Other examples of *sambal* variants are mango *sambal*, *andaliman sambal*, *matah sambal*, various kinds of fish and seafood *sambal,* and sweet soy sauce *sambal*. The local culture and commodities available in the area influenced the ingredients included in *sambal*.

3.1.2 Cabai sauce

Cabai sauce or "saus cabai," as seen in **Figure 11(B)**, is described as a sauce made from *Capsicum* sp. as the main ingredient and processed with or without the addition of other legitimate food additives [60]. *Saus cabai* is usually made from *cabai*, salt, onion, and garlic, with the addition of cornstarch as a thickening and binding agent [61]. The ingredients are sorted and washed, and then, *cabai* is steamed, mixed, and grounded with other ingredients. Cornstarch solution is added, and the mixture is cooked. Acidity regulators and preservative agents are added and mixed before *saus cabai* are packed and stored [61]. The flavor of *saus cabai* varies from sweet to spicy, but its pungency level is usually lower than *sambal*.

3.1.3 Cabai powder

Cabai powder, as seen in **Figure 11(C)**, is used as an ingredient to bring up spicy taste in savory processed food such as instant soup, instant noodles, and snacks. In 2012, a company developed a mixture of *cabai* powder with other ingredients such as onion, garlic, pepper, and also with particular fish and shrimp. The addition of fish and shrimp has enriched the umami taste of *cabai* powder products in Indonesia. "Tasty chili powder" with umami and spicy taste is developed as an innovation of *cabai* powder, and a new habit of eating *cabai* emerged in Indonesia with this dry powder as a condiment to be sprinkled on top of the food instead of dipping or mixing the food in *sambal*. The spiciness of tasty chili powder varies up to several levels from low to high. In the making of this powder, the ingredients were sorted, blanched, dried, grounded, and packed [61]. Tasty chili powder contained up to 16.8 ppm capsaicin [62].

3.2 Other rare and unique foods from *Capsicum* in Indonesia

3.2.1 Manisan cabai/sweetened capsicum

Manisan cabai is sugar-sweetened *Capsicum* slices, which is commonly eaten as a snack. It is usually made from thick-skinned red *Capsicums* such as *cabai merah besar* (*C. annuum* L.). The processing of sweetened *Capsicum* started from seed removal. The remaining skin is soaked in the salt solution to remove the pungent flavor. Then, *Capsicum* is soaked in $CaCO_3$ solution to harden the texture before being soaked in the warm sugar solution for 6–12 hours to absorb the sugar. The sweetened *Capsicum* may be dried and sprinkled with sugar. Then the resultant products are being packed and stored. Besides altering *Capsicum's* taste by masking the pungency and increasing the sweetness, which makes *Capsicum* likable by children, this process also lengthens the shelf life of *Capsicum* due to high sugar concentration. The example of sweetened *Capsicum* is shown in **Figure 12**.

3.2.2 Capsicum chocolate bar

Capsicum chocolate bars tasted like typical chocolate bars at early bites, but the pungent taste of *Capsicum* will appear after several bites. Although *cabai rawit* (*C. frutescens*) used in the chocolate bar has quite strong pungency level, it can be covered by the high content of chocolate (58–60%) [63]. An example of *Capsicum* chocolate bar can be seen in **Figure 13**.

Figure 12.
*Sugary sweetened Capsicum (*Manisan cabai*) .*

Figure 13.
Capsicum chocolate bars.

*3.2.3 Kopi cabai rawit/*Capsicum frutescens *coffee*

Kopi cabai rawit is a mixture of coffee, milk, ginger, and *cabai rawit* (*C. frutescens*). This beverage can be found in Bandung, West Java, Indonesia. This coffee has a unique taste from the bitterness of caffeine, pungency from ginger, and spiciness from *cabai rawit.* After drinking the coffee, people felt a warmth sensation towards the throat and the body [64].

3.3 Pharmacy

Indonesia is rich in spices and herbs, and the locals have been utilizing them as traditional medicine for years. This tradition has been developed, as the active ingredients from traditional medicine are processed into more convenient products with the same function and characteristics. Plasters/patches, cream, balm, and oil with pungent and mint sensations have been used daily for muscle pain, headache, stomachache, and reducing the pain of toothache. The applications of those products from *Capsicum* are verily nurtured in Indonesian culture.

Capsaicin, responsible for pungent taste in *Capsicum*, also plays a role in the hot sensation in the skin. This hot sensation is utilized in the pharmacy industry as

Figure 14.
Koyo cabai*, the* Capsicum *hot medicated plaster in Indonesia.*

a muscle pain reliever by adding plaster fabric, cream, or gel, together with other supporting ingredients. Besides that, capsaicin cream is useful for chronic soft tissue pain and chronic back pain. It gives beneficial effects as first-line therapy of osteoarthritis pain and decreases post-surgical neuropathic pain [65–67].

3.3.1 Koyo cabai or capsicum transdermal patch/capsicum hot medicated plaster

Koyo cabai or *Capsicum* Hot Medicated Plaster is one of medicated plasters in Indonesia. It is usually used for the treatment to relieve muscle pain or reduce the pain of toothache. *Koyo cabai*, at first imported from a Deutsch company, had been used in Indonesia since 1970. However, in 1983, a local company started to produce *koyo cabai* [68]. The plaster was made from high concentration *cabai* extract mixed with rubber as sticking agent and other ingredients. The sticky mixture then applied and pressed to the plaster fabric layer. The layer was cut and packed. *Koyo cabai* as a famous *Capsicum* Medicated Plaster in Indonesia can be seen in **Figure 14**. One Capsaicin Medicated Plaster may contain 0.37 mg/cm^2 of *Capsicum* extract [69].

3.3.2 Cabai cream and cabai balm

Similar to *koyo cabai*, *cabai* cream or *krim cabai* and *cabai* balm or *balsam cabai* in Indonesia are mainly utilized to relieve muscle pain and headache. It is a common thing that people in Indonesia keep muscle pain-relieving cream or balm as a first aid medicine in their homes or travel kits. *Cabai* cream may contain 1.66% *cabai* oleoresin; meanwhile, *cabai* balm may contain 0.56% *cabai* oleoresin.

3.4 Plant ornaments

Ideally, ornamental *cabai* has bonsai-like quality that is short in height (dwarf) and produces quite a number of fruits with various colors [70]. *Capsicum* species, especially *C. chinense* and *C. pubescens*, are attractive to be used as ornamental plants. *C. chinense* has various fruit shapes that look like a combination of tomato and starfruit and different bright colors during maturation, while *C. pubescens* has purplish fruits and is combined with erect flowers [71].

From the ornamental plants *C. annuum* L., the variety is called "Ayesha IPB" [70]. The plant relatively early blossoms in 13–16 days after planting and can be harvested in 65–70 days after planting. The average plant height is 25 cm with 46–53 cm of canopy width, and the leaves of canopy are nicely clustered. The fruits have round tips, and they would turn from yellow green (immature stage) to orange (intermediate) and orange red (mature stage). The other variety is called

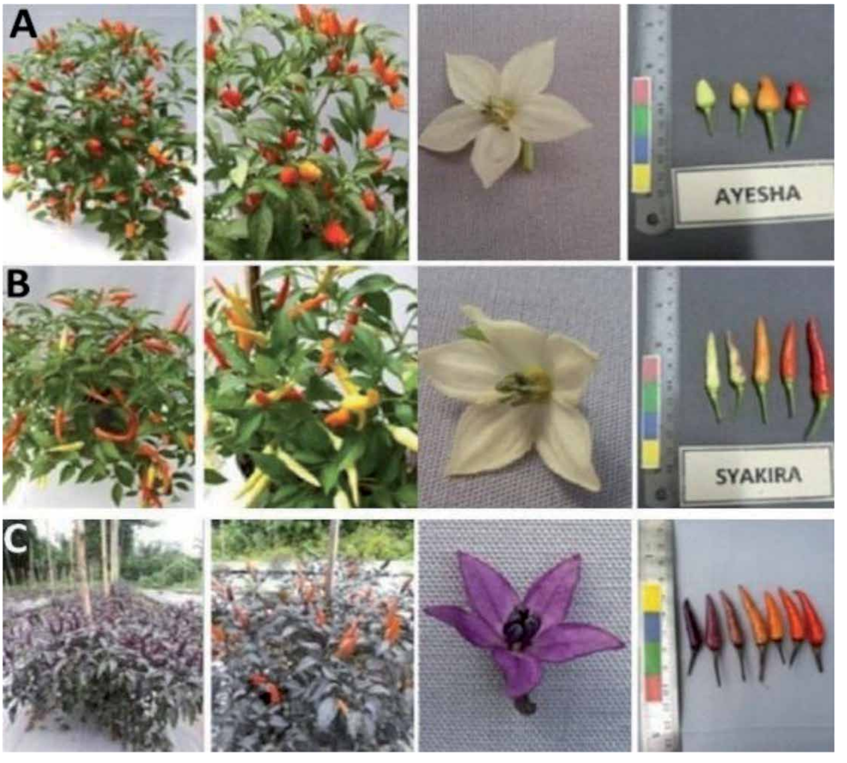

Figure 15.
Some Capsicum ornamental plants in Indonesia (A) Cabai Ayesha *(B)* Cabai Syakira *(C)* Cabai Lembayung *[73].*

"Lembayung IPB" with a short height, purplish stem and stalk, and attractive fruit colors. The immature fruit color is purple and they would turn into dark orange [72]. The fruit tastes relatively hot. Other ornamental cultivars from *C. annuum* are "Namira," "Syakira," "Jelita," and "Ungara." Some types of *cabai* as ornamental plants [73] can be seen in **Figure 15**. Colorful *Capsicum* as ornamental plant is famous to be called as *cabai pelangi* or *rainbow cabai* in Indonesia.

4. Conclusion

The major *Capsicum* species commercially important in Indonesia are *C. annuum* and *C. frutescens*. Cultivars from *C. chinense* and *C. pubescens* were rarely found in the market and can be considered exotic and mostly cultivated for ornaments. *Capsicum* is profoundly intertwined and inseparable from Indonesian culture, from food and pharmaceuticals to ornaments with many variations of the products. The market is still exceptionally receptive to innovations to transform and include *Capsicums* in the products. The variety of *Capsicums* in Indonesia and the potential applications combined with prospective market also make the field of flavor and bioactive compounds of *Capsicums* in Indonesia attractive to be investigated.

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Treatment of *Capsicum* and Its Disease Control

Chapter 2

Anthracnose of Chilli: Status, Diagnosis, and Management

Raj Kiran, Jameel Akhtar, Pardeep Kumar and Meena Shekhar

Abstract

Chilli (*Capsicum annuum* L.) is one of the most economically important vegetable crops in the world. Among different biotic constraints, anthracnose disease is the major limiting factor affecting yield and production of chilli crop. Different symptoms associated with disease are fruit rot, leaf spots, dieback on stem, seedling blight, or damping off. Many species of genus *Colletotrichum* are found associated with the disease worldwide. In India, primarily three important species, namely, *Colletotrichum truncatum*, *C. acutatum*, and *C. gleosporoides*, are responsible for the chilli anthracnose. Accurate identification of pathogen is needed for choosing the proper management strategy for controlling this disease. Both conventional and molecular methods are adapted along with different management strategies, recommended for this disease namely cultural, chemical, and other eco-friendly methods.

Keywords: *Colletotrichum*, biocontrol agents, diagnosis, molecular methods

1. Introduction

The genus *Capsicum* includes many cultivated species, of which *Capsicum annuum* L. is one of the most widely cultivated one; besides this, other domesticated species are *C. baccatum*, *C. chinensis*, *C. frutescens*, and *C. pubescens* [1]. *C. annuum* comprises of both sweet (bell pepper) and pungent (chilli) fruits of numerous shapes and sizes. It is a good source of Vitamin A and C, potassium, and folic acid [2]. Fresh green chilli has more vitamin C than a citrus fruit, whereas red chilli has more vitamin A than in carrots [3, 4]. Besides its wide use as vegetable, spice, and condiments, it is also used in medicines and beverages. Capsaicinoid and caretenoids are the active ingredients of the chilli; the capsaicinoids are nonvolatile alkaloids that make chilli pungent [5], and caretenoids have nutritional value that also provides color to the chilli fruit [6]. In tropical and subtropical countries, chilli is considered the most important constituent of different cuisines. As the native home of chillies are considered to be tropical America, where it is still found growing in the wild state [7]. Its introduction to India is credited to voyage of Columbus who brought seeds from Spain, introducing it to Europe, which subsequently spread to Africa and Asia [8].

India is the world's largest producer of dried chillies and in 2018 India produced 1.8 million tons, out of 4.1 million tons produced worldwide [9]. There are two important commercial qualities that makes Indian chilli world famous are color and pungency levels. Chilli crop is attacked with different pests and pathogens in field and during post-harvest, contamination with mycotoxins are major constraints in chilli production. Worldwide, *Capsicum* is vulnerable to various pests, weeds, fungal, bacterial, and viral pathogens; among the fungal diseases, anthracnose/die-back/fruit-rot of chillies is an important disease causing serious losses in field, transit, transport, and storage [10, 11].

2. Anthracnose disease losses

The word anthracnose derived from Greek language meaning 'coal' it is the common name of plant disease with very dark, sunken lesions and containing fungal spores [12]. Typical symptoms (**Figure 1**) of anthracnose on chilli fruit include dark spots, sunken necrotic tissue with concentric rings of acervuli. Besides fruit rot, it also causes leaf spots, dieback on stem, seedling blight, or damping off. This disease not only affects the quality of fruit by appearance of anthracnose lesion but also reduces dry weight of fruit, and quantity of capsaicin and oleoresin [13, 14].

Losses are caused by this disease worldwide; it is reported that in Vietnam it causes 20–80% yield loss [15], 10% yield loss in Korea [16], 50% yield loss in Malaysia [17] and as high as 80% yield loss (during severe epidemics) in Thailand [18]. In India, a calculated loss of 10–54% has been reported in yield due to this disease [19, 20], and this disease is reported throughout India but it found to be more common and aggressive form in Assam, Bihar, Andhra Pradesh and Uttar Pradesh [10]. The anthracnose pathogen has been intercepted in seed and it has been reported that there is occurrence of pathogen in seed samples, upto 5% infection index indicates its wide spread occurrence in India [21].

Figure 1.
(a) Healthy chilli plant, (b) chilli plant affected with anthracnose disease, and (c) chilli fruits showing anthracnose symptom.

3. Causal organisms

This disease is caused by the species of genus *Colletotrichum*, which belongs to Ascomycetes. Worldwide, different species of *Colletotrichum* are reported to cause chilli anthracnose disease (**Table 1**), In India, among different species known to cause this disease, there are primarily three important species *Colletotrichum capsici* Syd. Butler and Bisby (Synonym *C. truncatum*), *C. acutatum* and *C. gloeosporioides* have been reported to be associated with the disease, however *C. truncatum* causing major damage at the ripe fruit stage of the plant [35, 52–55].

Country	Pathogen	References
Australia	*C. acutatum*, *C. atramentarium*, *C. dematium*, *C. gloeosporioides* var. minor and *C. gloeosporioides* var. *gloeosporioides* and *C. brisbanense*	[22, 23]
Brazil	*C. boninense*	[24]
India	*C. capsici/ C. dematium/ C. truncatum, C. gloeosporioides, C. graminicola, C. acutatum, C. piperatum, C. atramentaum, C. fructicola and C. siamense, C. cliviae, C. coccodes and C. karstii*	[20, 25–35]
Indonesia	*C. gloeosporioides*, *C. truncatum* and *C. acutatum*	[36]
South Korea	*C. acutatum*, C. *gloeosporioides*, *C. coccodes* and *C. dematium*	[37]
Mexico	*C. truncatum*	[23]
Malaysia	*C. truncatum*	[17, 38]
New Zealand	*C. coccodes*, *C. kartsii*, *C. novae-zelandiae* and *C. nigrum*	[39, 40]
Papua New Guinea	*C. truncatum* and *C. gloeosporioides*	[41]
Philippines	*C. gloeosporioides*, *C. truncatum* and *C. scovillei*	[42, 43]
Sri Lankla	*C. truncatum*	[44, 45]
Taiwan	*C. acutatum*, *C. truncatum* and *C. gloeosporioides*	[46]
Thailand	*C. acutatum*, *C. truncatum* and *C. gloeosporioides*	[47, 48]
USA	*C. gloeosporioides*, *C. acutatum*, *C. truncatum* and *C. coccodes*	[49, 50]
UK	*C. acutatum* and *Glomerella cingulata*	[51]
Vietnam	*C. acutatum*, *C. truncatum*, *C. gloeosporioides* and *C. nigrum*	[15]
Zimbabwe	*C. nymphaeae*	[23]

Table 1.
Different Colletotrichum species associated with the disease anthracnose of chilli in different countries.

4. Diagnosis

Identification of *Colletotrichum* species based on morphological characteristics (size and shape of conidia; presence of setae) and colony characteristics is generally used by several workers [56–59]; it is widely used in seed health testing labs for detection of *C. capsici* in germplasm for pest free conservation of chilli seeds [21]. As the pathogen is seed-borne, there is threat of introduction of this pathogen along with import of germplasm (including Chilli) from different countries; therefore, while importing from any other country, there is a need to examine the samples very critically including sensitive molecular diagnostic tools to prevent entry of this pathogen associated with germplasm [60]. Moreover, for the accurate identification of the pathogen at species level molecular methods are widely adapted. Loop-mediated isothermal amplification (LAMP) assay was used for the accurate and sensitive detection of *C. capsici* LAMP primers (β-tubulin gene sequences based) were designed and it was reported that it could detect as little as 10 fg/μl of *C. capsici* pathogen in comparison with only upto 1 ng/μl of *C. capsici* detection using polymerase chain reaction [61]. A sequence characterized amplified region (SCAR) marker was developed for specific and sensitive detection of *C. capsici* in chilli seeds and fruits. This markers did the amplification of an expected 250-bp fragment from genomic DNA and these markers were very much sensitive as it was reported that the marker could detect purified *C. capsici* DNA template up to 1 pg and DNA from *C. apsici* infected chilli fruits up to 25 ng [59]. As these two markers are very sensitive, these may be very useful in detection of the pathogen in imported germplasm in plant quarantine laboratories.

COL1/COL2 primers were used for amplification of the specific internal transcribed spacer region of tested *Colletotrichum* species (*C. acutatum*, *C. truncatum* and *C. gloeosporioides*) with a specific band of 460 base pairs. *C. gloeosporioides* was detected at a low level of 1000 conidia on chilli leaf and fruit by this primer [62]. Another, primer set based on the sequences of the ribosomal internal transcribed spacer (ITS1and ITS2) regions of *C. truncatum* was designed and standardized for the detection of *C. truncatum* in infected plant tissues using PCR assay. The sensitivity was 10 pg of genomic DNA from the pathogen [63]. Machenahalli et al. [64] detected pathogens from different parts of plant like seeds, fruits, infected twig/stem by PCR-based method by using specific primers. *C. truncatum* was amplified by species specific primer (C.cap-f and C.cap-r) as single band at 450 bp. *C. gloeosporioides* was amplified by species specific primers CgInt at 450 and *C. acutatum* by CaInt at 490 bp respectively. The accurate identification is very important for choosing the correct management strategy for this disease.

5. Management strategies

For the management of anthracnose disease of chilli, different strategies are adapted. These are use of cultural practices, chemical control, eco-friendly measures like use of biocontrol agents, plant extracts and use of resistant cultivars. Generally, use of different strategies in combination has been recommended for managing the disease [65]. The summarized information is given from across the world for the management of this disease.

5.1 Cultural practices

Several cultural practices have been reported to manage chilli anthracnose due to the special etiology of the pathogen. These precautionary measures are implemented to reduce the rate of infection and minimize the inoculum pressure even before fruits are mature and harvested. Than et al. [47] and Ali et al. [66] in their review reported that different cultural practices like disease free seeds, weeding, crop rotation, proper drainage, removal of crop residue are being followed for the chilli cultivation. It was suggested that disease free chilli seeds should be planted and elimination of weeds should be done in chilli field and rotation of chilli crop with other crops which are not alternative hosts to *Colletotrichum* spp. after every 2–3 years is very effective for controlling this disease. Good drainage systems on the field to channel out waste water during irrigation regimes, on-farm fruit disinfection such as fruit washing at packing houses and finally removal of plant debris which may serve as source of inoculum are some other clean crop and sanitation practices [47]. If there was history of disease in a particular field, then other crops should be rotated in isolation from other solanaceous plant for at least alternate years [50]. Deep plow is recommended to completely cover diseases plants or removing infected plant debris from the field at the end of growing season [67]. Early planting of chilli or planting cultivars that bear fruit within a short ripening period to allow the fruit to escape fungal infection is also recommended. Other alternative sanitation practices such as weeding, removal of infected or wounded fruits should be carried out regularly to prevent the pathogens from using such wounds as sites of infection.

5.2 Chemical control

Different strategies for managing the disease are recommended and chemical control is found most effective and practical method [68]. As time required for controlling the disease with chemical method is much lesser as compared to the time required for the development of resistant cultivar. Use of protective fungicide like manganese ethylene bisdithiocarbamate (Maneb) is widely recommended for managing this disease. Other dithiocarbamate fungicides like Mancozeb (0.2%), ziram (0.1%), copper oxychloride fungicide (Blitox 50), and Bordeaux mixture (0.5 or 1%) of a copper sulphate fungicide were found effective in managing this disease. Seed dressing with benzimidazole fungicides (Benlate, delsene M) and strobilurin fungicide (azoxystrobin) are recommended [69] and soaking of chilli seeds for 12 h in 0.2% Thiram, a dithiocarbamate fungicide was also found effective for better control of the disease [70].

Among different systemic fungicides recommended Bavistin (carbendazin 50%WP) 0.1%, Plantvax (oxycarboxin) and vitavax (carboxin) were found effective as use of Bavistin resulted in 80.84% disease reduction [71] and Plantvax and Vitavax were reported to reduce the disease by checking the spore germination of *C. truncatum* [72]. Additionally other systemic fungicides from triazole group propiconazole [73], difenoconazole, benzimidazole fungicide (Benomyl) [74] have been used in both pre and post-harvest management of chilli anthracnose, as propiconazole, exhibited the highest level of inhibition of in vitro mycelial growth, biomass production, sporulation and spore germination at concentrations as low as 0.1 mg/ml. Other workers also reported that Tilt (propiconazole) is highly effective in controlling *Colletrotrichum* spp. [75, 76] concentration of Tilt at 150 ppm was found effective in inhibiting the pathogen as it caused 50% inhibition (ED 50) of *C. acutatum* growth in culture media [77]. It is to be noted that Benomyl and its associated fungicides Carbendazim and thiophanate methyl (both of which registered) has raised major health concerns and these are proved unacceptable and dangerous [78]. Different strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint) and pyraclostrobin (Cabrio) have also been recommended for effective management of the disease [47, 79].

Moreover, dependence on only single chemical resulted in the emergence of resistant strains of *C. truncatum* isolates from chilli fruit against different chemicals benomyl, which were cross-resistant to thiophanate methyl and carbendazim [80], resistance of *C. truncatum* to benomyl and strobilurin-fungicides (azoxystrobin and kresoxim-methyl) is also reported [81–83]. Under such circumstances, combined application of Bioagents with chemicals are recommended, *Pseudomonas fluorescens* along with half of the recommended dose of azoxystrobin fungicide has been found effective and viable option to control fruit rot [79]. As use of chemicals are not eco-friendly and it leaves chemical residue in chilli fruits, which hinders the export, and there are numerous reports describing negative effects of using chemicals on farmer's health in developing countries [36]. To overcome the undesirable effects of chemical usage alternate methods such as use of bioagents, plant extracts or use of chemicals in combination with these are recommended to control the infection.

5.3 Biological control

Trichoderma species is the fungal antagonist which is widely applied to control *Colletotrichum* species in chilli [84, 85]. It is also believed that *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success [86–88]. Chloroform extracts of nonvolatile antibiotics (NVAC) of *T. viride* added to the culture media inoculated with *C. truncatum,* showed reduction in biomass and synthesis of RNA, DNA and protein [89]. It has been reported that antifungal metabolites (100 mg/L) secreted from *Trichoderma harzianum* Rifai strain number T-156co5 significantly controlled *C. truncatum* isolated from *C. annuum* [90]. *In vitro* studies indicated that *T. viride* and *P. fluorescens* are very effective in inhibiting mycelial growth of the pathogen [91]. It is suggested that the use of *T. viride* and *P. fluorescens* individually or in combination known to significantly lower the anthracnose disease incidence and should be used as an alternative to chemical control [92].

Other bioagents like *Bacillus subtilis* and *Candida oleophila* (a yeast species) have been tested for efficacy against *C. acutatum* [93]. *Pichia guilliermondii* Wick strain R13 is another yeast species which is reported to reduce the disease incidence on *C. truncatum* infected chilli fruit as low as 6.5%. It has also been proposed that this fungal strain with other yeasts suppressed *Colletotrichum* spp. through multiple modes of action (nutrient competition, competition for space between antagonist and the pathogen, toxin production, induction of plant resistance and hydrolytic enzyme production) [94–96]. Intanoo and Chamswarng [97] reported that DGg13 and BB133 were antagonistic bacterial strains found very effective in controlling *C. truncatum*. *Pseudomonas aeruginosa* FP6 also found effective against *C. acutatum* [98].

Rhizosphere and rhizoplane fungal isolates (*Chaetomium globosum*, *T. harzianum* and *F. oxysporum*) from perennial grasses has been reported to decreased disease incidence and severity in seedlings and mature plants, and promoted plant growth and increased yield in the greenhouse and field [99]. In an experiment crude extracts from *Chaetomium cupreum* CC, *C. globosum* CG, *T. harzianum* PC01, *T. hamatum* PC02, *Penicillium chrysogenum* KMITL44 and antibiotic substances Rotiorinol, Chaetoglobosin-C and Trichotoxin A50 was used against *C. gloeosporioides* isolate WMF01 (the most virulent on all tested varieties of grape). The results revealed that application of all bioproducts significantly reduced the disease incidence on leaves, twigs and fruits of grape in all varieties as compared to the chemical control [100]. *Cordyceps sobolifera* an entomopathogenic fungi have also been reported for use as a biocontrol agent against *C. gloeosporioides* [101, 102].

5.4 Plant extracts

Antimicrobial plant secondary metabolites compounds are one of the best options to controlling plant diseases. In chilli, several workers have shown the efficacy of plant extracts against *Colletotrichum* spp. [103–108]. Among the plant extracts, *Allium sativum* (10%) and *Azadirachta indica* (10%) demonstrated the highest inhibition of mycelial growth of *C. gloeosporioides* [91]. *A. indica, Datura stramonium, Ocimum sanctum, Polyalthia longifolia* and *Vinca rosea* were used against *C. truncatum*. Among the five fermented leaf extracts tested against *C. truncatum, A. indica extract* at 20% concentration highly inhibited the growth of *C. truncatum in vitro* condition. And *in vivo* the application of fermented leaf extract of *A. indica* alone reduced the fruit rot incidence (@3%) and increased plant height, number of fruits and yield significantly [109]. In an experiment the botanicals or plant extracts from *Catharanthus roseus*, *Coleus aromaticus*, *Manilka razapota* and *A. indica* used against fungi, it was concluded that these botanicals confer antifungal effects on the radial mycelial growth of *C. truncatum* [107]. The organic pesticides were prepared from the extract of neem leaves, soursop leaves, lemongrass extract, tuba root extract, and kenikir/*Cosmos caudate* extract [110]. The result indicates that neem leaves are the most effective organic pesticides to control the chilli pepper disease especially in Indonesia.

Nine plants extracts viz., *Lawsonia inermis, A. indica, Bougainvillea spectabilis, Withania sominifera, Ocimum tenuiflorum, Aegle marmelos* L., *Justicia adhatoda* and *Calotropis gigantean were* tested under *in vitro* condition through poisoned food technique against chilli fruit rot pathogen *Colletotrichum* sp., among them *W. sominifera* (10%) was found to highly inhibit the mycelial growth of the anthracnose pathogen up to 84.88% [111]. Further, Singh and Khirbat [112] reported the efficacy of aqueous extract of three wild plants viz., *Albizza lebbeck*, *Acacia arabica* and *Clerodendrum infortunatum* to control chilli fruit rot. Alves et al. [113] reported the efficacy of 1% aqueous or 20% ethanol plant extracts to control bell pepper anthracnose caused by *C. acutatum*. In this study, 6% aqueous garlic, mallow and ginger extracts reduced disease severity by more than 97%. Even though recent research suggests the use of these plant extracts as bio-fungicides, but still more studies on their efficacy in the controlling of chilli anthracnose need to be performed under field conditions.

5.5 Resistant cultivars

As use of resistant or tolerant cultivar is the most cost-effective management strategy. Due to the lack of resistance in the *C. annuum* gene pool, no commercial resistant varieties have been developed in *C. annuum* [114]. The introgression of the resistance gene from *C. baccatum* to *C. annuum* is difficult. There are some studies on introgression of anthracnose resistance into *C. annuum* to develop a new variety [115, 116]. Five lines of *C. annuum* from AVRDC, Taiwan, namely AVPP1102-B, AVPP0513, AVPP0719, AVPP0207 and AVPP1004-B, as the promising lines with good fruit yield and tolerance to anthracnose [117]. Two chilli varieties, Lembang-1 and Tanjung-2, have been reported as moderately resistant from IVEGRI, Indonesia, [118].

In India, some anthracnose-resistant lines listed are LLS, PBC932 (VI047018), Breck-2, PBC80 (VI046804), Breck-1, Jaun, and PBC81 (VI046805) [119]. Other nine resistant varieties (BS-35, BS-20, BS-28, Punjab Lal, Bhut Jolokia, Taiwan-2, IC-383072, Pant C-1 and Lankamura Collection) were identified which could be employed for developing successful resistant cultivars through breeding programs [120]. The information on the resistance varieties against *Colletotrichum* spp. may also be utilized for studying the inheritance of the resistance and also to locate and study the quantitative trait loci (QTLs) maps for resistance [121]. Further studies need to be undertaken to investigate the importance of these distinct genes in the management of chilli anthracnose. Nevertheless, the genetic mechanism associated with chilli resistance to anthracnose is still poorly understood mainly due to lack of information on the defense signaling modules governing the resistance mechanism.

6. Conclusion

Anthracnose of chilli is main constraint for its production in the India as well as worldwide. Detection this pathogen in the seed by the morphological features and with the developed molecular markers are very important especially in quarantine laboratories. The accurate detection of pathogen also helps in choosing the best management strategy for the control of this disease. Involvement of many *Colletotrichum* species in the disease and absence of resistance gene in *C. annum* makes breeding for resistance is more challenging. Moreover, injudicious use of chemicals for the control of this disease leaves residue in the chilli fruit poses threat to the export. Combining the use of resistance cultivars with other disease control measures would enhance the efficiency in integrated management of chilli anthracnose. Moreover, more research is required to find better alternative methods to control chilli anthracnose by involving vigorous evaluation and identification of resistant cultivars of chilli against this disease.

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Chapter 3

Hot Water Seed Treatment: A Review

Suryapal Singh, Harshita Singh and Narender K. Bharat

Abstract

In present day agriculture, use of chemicals for crop production is discouraged. Hence, other alternative treatments for disease control must be developed, and hot water treatment is one of them. It is a feasible practice, both financially and time wise. Hot water soaking is a very age-old practice, efficient in destroying pathogens borne both outside the testa and inside the seed testa by using temperature hot enough to kill the organism but not quite hot enough to kill the seed. Extensive research work has been reported on hot water treatment in vegetables. Therefore, an attempt has been made to review the information available regarding the effect of hot water treatment on growth, disease incidence and yield of vegetables.

Keywords: hot water treatment, capsicum, seed-borne disease

1. Introduction

In the present age when the crop production is protected by chemicals and threat of disease due to exposure to these chemicals is at a rise, organic farming is getting momentum. This organic farming is regulated by certain certifications including chemical-free state of agriculture farm. In this condition use of chemicals will not help farmers for these certifications; rather, there is rejection of the produce. Here it becomes pertinent to involve those practices which are natural and can help control diseases naturally. Priming and pelleting are commonly used practices for seed treatment to enhance the production of *Capsicum*, but some chemicals are involved in it. *Capsicum* is in great demand as it is a vegetable with very rich contents of vitamins and minerals and is cooked in combination and individually as per the taste of consumers. Rejections of pesticide, insecticide and chemical sprayed crop attracted the attention for the present review where hot water seed treatment is controlling the diseases in bell pepper. Pepper (*Capsicum annuum* L. var. *grossum*) is commonly known as *Capsicum*, *Shimla Mirch*, green pepper, cherry pepper or bell pepper, and it belongs to the solanaceous group of vegetables. *Capsicum* is one of the most important vegetable crops grown extensively throughout the world especially in the temperate countries [1] and has attained a status of high value crop in recent years because of its delicacy and pleasant flavour coupled with rich content of ascorbic acid and other vitamins and minerals [2]. Fruits of sweet pepper are either used as salad or cooked as vegetable or processed and is appreciated worldwide for its flavour, aroma and colour. It contains several bioactive substances like capsaicin, vitamin E, pro-vitamin A, carotenoids, flavonoids and other secondary metabolites with antioxidant properties [3]. In the states of Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Darjeeling District of West Bengal and hills of UP, bell pepper is grown in summer, whereas in the states of Karnataka, Tamil Nadu, Bihar and Maharashtra, it is grown in the autumn season [4]. In India, bell pepper covers an area of 29,800 ha with 171,370 tonnes production and productivity of 5.75 tonnes/ha [5]. Sweet pepper is a warm-season crop, which grows well under an extended frost-free season, with the capability of producing high yields of exceptional quality. The best temperature range for sweet pepper growth is 20–25°C, whereas the best germination temperature is 29°C. A temperature of less than 29°C reduces the growth of seedling, leading to increased exposure of seedlings to insects, diseases or salts, resulting in damaged or dead seedlings [6]. High temperatures adversely affect the productivity of many plant species of which sweet pepper is of no exception.

High humidity in the environment and moist soil together with optimum temperature result in high incidence of various diseases. Diseases like bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), *Cercospora* leaf spot (*C. capsici*), anthracnose (*Colletotrichum capsici*) and virus diseases are seed borne in nature. To avoid the occurrence of such diseases, seed treatment with various chemicals has been recommended from time to time [7]. But in present day agriculture, use of chemicals for crop production is discouraged. Hence, other alternative treatments for disease control must be developed. One such treatment is with hot water which is economically as well as temporally feasible. Farmers, along with a little technical assistance, can easily adopt this treatment. This treatment is also successful for destroying viruses like *mosaic virus* that affect bell pepper. It is very efficient in destroying pathogens borne both outside the testa and inside the seed testa. In this area no such work has been done in the case of bell pepper.

2. Disease of sweet pepper

Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), bacterial wilt (*Pseudomonas solanacearum*), anthracnose/ripe fruit (*Colletotrichum* spp.), *Cercospora* leaf spot/frog eye leaf spot (*C. capsici*), bacterial canker (*Clavibacter michiganensis*) and viruses like Tomato spotted wilt virus (TSWV) are some of the most important diseases of sweet pepper that cause huge economic losses to the farmers. Seed-borne diseases of bell pepper are the most important problems in organic farming systems because of the limitation in chemical control methods. For the successful management of any disease under normal conditions, sanitation, elimination of primary source and chemical protection at initial stages are some of the measures recommended. Various workers are now engaged in developing and testing the non-chemical methods for seed-borne diseases of vegetables including bell pepper.

3. Seed technology

Seed is the primary and essential starting point of a wide range of horticultural crops, including the majority of vegetables. The high-quality seeds of vegetable varieties that display early, consistent, dynamic seedlings and better- and good-quality fruits from individual seed sown at favourable or unfavourable conditions have increased significantly in recent years. Seedling emergence and field stand establishment is one of the problems faced by the growers, especially in early planting where adverse conditions are prevailing (low temperature and high soil moisture). Delayed, erratic germination and emergence, poor stand, slow early seedling growth rate and non-uniform maturity often limit crop production even under optimum environmental conditions [8–10]. Extensive seed germination and seedling appearance has increased the occurrence of pre-damping off mortality caused by soil-borne fungi [11]. This also leads to establishment of weeds in the fields even before the crop seedlings are mature enough to be cultivated, competing with the main crop for nutrients, and moreover, they hinder the processes of fertilisation, chemical application and mechanical harvesting.

4. Hot water treatment

The most appropriate seed treatment with respect to least damage, economy, efficiency and application is hot water soaking. It is an old-age practice based on treatment with hot water whose temperature is high enough to kill the pathogen but not high enough to harm the seed, hence a very good technique to control many seed-borne diseases [12, 13]. Heat treatment may be applied for agricultural commodities by (1) immersion in hot water, (2) exposure to vapour heat, (3) exposure to hot dry air, (4) treatment with infrared radiation or (5) microwave radiation. Hot water treatments of seed and plant material are classical thermophysical methods of plant protection and are more eco-friendly and effective than chemical treatments.

Hot water treatment can be damaging or not practical for seeds of peas, beans, cucumbers, lettuce, sweet corn, beets and some other crops [12, 14, 15], but it is highly recommended for pepper, eggplant, tomato, cucumber, carrot, spinach, lettuce, celery, cabbage, turnip, radish and other crucifers. It may also severely damage old seeds, and therefore, a small sample of any seed lot over 1 year should be first treated and then tested for germination to determine the amount of injury that may occur. Hot water treatment is recommended for seeds with surface or deep-seated infections. Effective treatment temperature and duration have to be found out for every vegetable crop and the relevant pathogens. The principle is to eliminate the pathogens as far as possible without decreasing germination of seeds. For example, just a 5-min difference in treatment time can lead to diverse differences in the germination rate of cabbage seed.

A number of tests and studies of heat treatment must be undertaken to optimise the time and temperature that are most adaptable to the seeds to be treated and the pathogens to be killed before practical application. Susceptibility to heat damage may differ among different varieties of plant species [16, 17]. The time/temperature combination for a given plant seed depends on many factors interacting with the heat susceptibility of the host, viz., conditions of external layers, dormancy, moisture content, age and vigour [18]. Particularly, it has long been known that the smaller the initial water content of seeds is at the time of heating, the greater the resistance to high temperatures [19]. Two major groups of proteins may be activated by the hot water treatments that induce fruit resistance: heat shock proteins (HSPs) and pathogenesis-related (PR) proteins. HSPs are believed to play a major role in thermotolerance [20–22]. Among the PR proteins, most characterised enzymes chitinases and β-1,3-glucanases hydrolyse polymers of fungal cell walls and are, therefore, thought to be involved in the plant defence mechanism against fungal infection [23, 24].

5. Effect of hot water treatment on different vegetables

Nega et al. [15] stated that even with longer treatment times, hot water treatment with a temperature of 40°C had no significant effect on the seed-borne pathogens. However, on all the crops investigated, hot water treatments at temperatures

50 or 53°C for 10–30 min had a good phytosanitary effect. In the majority of cases, these treatment conditions did not affect seed germination. Therefore, to reduce the effect of higher temperature like 53°C on germination, comparatively shorter treatment time must be used, especially on sensitive crops like cabbage, etc. The treatment of 50°C for 30 min is optimal against *Phoma lingam* on cabbage. They also observed that on crops like carrot, cabbage and parsley treatments at temperatures 50–53°C for 10–30 min gave a good elimination of *Alternaria* species.

In the past, Walker [25] observed complete abolition of *P. lingam* from cabbage seeds with treatment at 50°C for 30 min. Clayton [26] recorded similar results for 25 min as well as for 30 min at 50°C. Bant and Storey [27] showed good effects of hot water treatment against *S. apiicola* on celery in field trials. In the view of lack of alternatives, this treatment has proved to be an efficient method on celery and parsley seeds against the *Septoria* species [28] and that too with well-known yield increase. Due to the rapid spread of bacterial diseases like those caused by *Xanthomonas* species [29], hot water treatment has emerged as an important method to control seed-borne bacterial diseases because of lack of chemical or other well-established treatments [30, 31]. The documented efficiency of this treatment on cabbage and cauliflower against *X. campestris* pv. *campestris* is between 25 and 90%. The treatments differ between 50°C for 10–60 min [32–34] and 52°C for 30 min [35].

Melanie et al. [36] demonstrated the efficiency of hot water treatment method in reducing bacterial diseases like bacterial spot and bacterial canker in tomato under greenhouse as well as open-field conditions. They also observed that after treating, seedlings from the same seed lots with hot water did not get diseased in the greenhouse or fields. In plots/fields established from hot water-treated seed, the occurrence of bacterial canker was less extensive, and yields were higher than the plots/fields established from non-treated seeds. Also, fruits from non-treated seeds were considerably smaller than fruits from treated seeds. Reduced infection frequency of bacteria responsible for bacterial canker and bacterial leaf spot was observed in tomato seeds after hot water treatment with increased fruit size and yield.

Hot water treatment of seeds of okra (*Abelmoschus esculentus*) at 52°C for 30 min resulted in the improved crop, both in greenhouse and field conditions. The improvement was with respect to increase in fruit number, leaf number, fruit length and girth, total number of seeds per fruit, seed weight and plant biomass. The hot water treatment of seeds also reduced the frequency of mycoflora infection in the seeds, hence enhancing the vigour index and germination percentage of the seedlings [37]. After soaking carrot seeds in hot water at 52°C for 25 min, the bacterial pathogen (*Xanthomonas hortorum* pv. *carotae*) in and on the carrot was killed [38]. On the other hand, Nandini and Shripad [39], observed that hot water treatment at 52°C for 10 min was effective in controlling the bacterial blight of cowpea with minimum number of infected seedlings and percentage of seedling infection. Germination was also not much affected as compared to control (58 vs. 76.00%).

The effects of hot water treatments of carrot seeds on seed-borne fungi, germination, emergence and yield were studied by Hermansen et al. [40] where the seeds infected with *Alternaria dauci* were hot water treated at temperature ranging from 44 to 59°C at intervals of 5°C for periods of 5–40 min. Treatment of carrot seeds with hot water at 44, 49 and 54°C improved germination rate and reduced the occurrence of *A. dauci*. Hot water treatment at 54°C for 20 min inhibited *A. dauci* without negatively affecting germination rate or yield. Ranganna et al. [41] demonstrated that the potato tubers can safely be stored without sprouting for 12 weeks at 8 or 18°C, if treated with 57.5°C hot water for 20–30 min.

Hot water treatment of seeds was also observed to be helpful in controlling seed-borne pathogens in sweet pepper. Aguilar et al. [42] observed that hot water treatment of bell pepper at 45°C for 15 min or 53°C for 4 min before storing them at 8°C reduced the occurrence of fungal infections. Several hot water treatments of bell pepper seeds resulted in considerable drop-off in seed viability but had no effect on seed vigour [43]. No study can be found in the literature that attempted to arrive at the optimum time-temperature combination for sweet pepper. Therefore, the effect of hot water treatments of sweet pepper seeds on seed viability and seedling vigour needs to be investigated (**Table 1**).

Causal agent	Host	Thermotherapy tested	Result	Ref.
Clavibacter michiganensis subsp. *michiganensis*	Tomato (*Lycopersicon esculentum*)	Hot water treatment at 53, 54 and 55°C for 10–60 min	Germination remained unaffected up to 55°C for 30 min; bacteria were recovered after 30 min at 53 and 54°C and not after 40 min	Bryan [44]
C. m. subsp. *michiganensis*	Tomato	Soaking infected seeds 30 min in hot water at 56°C	Plants develop significantly less disease than when no seed treatment is used; seed germination is slightly reduced	Shoemaker and Echandi [45]
P. s. pv. *phaseolicola*	Bean (*Phaseolus vulgaris*)	Hot water treatments: 50°C for 45–60 min	Reduction of bacterial number by 98–100% but 45% reduction in seed germination for 60 min soaking with naturally infected seeds	Tamietti [46]; Tamietti and Garibaldi [47]
P. s. pv. *pisi*	Pea (*Pisum sativum*)	Dry heat at 65°C for 72 h and soaking in water at 55°C for 15 min	Significant reduction of pathogen contamination but germination lowered 5–20%	Grondeau et al. [48]
P. s. pv. *tomato*	Tomato	Infected seeds are subjected to hot water treatment at 48°C for 60 min	Pathogens are killed, and germination is not affected	Devash et al. [49]
Xanthomonas campestris pv. *campestris*	Cabbage, cauliflower (*Brassica oleracea*)	Hot water treatment at 50°C for 30 min	Pathogens are eliminated successfully by this treatment to prevent seedling infestation	Shekhawat et al. [34]
X. c. pv. *carotae*	Carrot (*Daucus carota*)	Hot water treatment (52°C for 10 min)	Can prevent pathogen infestation	Ark and Gardner [50]
X. c. pv. *cucurbitae*	Pumpkin (*Cucumis pepo*)	Hot water treatment at 54 and 56°C for 30 min	Greatly reduces the level of seed infestation but does not completely eliminate it	Moffett and Wood [51]

Adopted from Grondeau et al. [52].

Table 1.
Thermotherapy to free seeds from pathogenic bacteria.

6. Conclusion

High incidences of disease in consumers leading to fatal diseases like cancer have attracted the attention of researchers. The causative agents were explored, and the focus was indiscriminate use of chemicals starting from seed treatment to crop productions. The residual effects of pesticides, herbicides and other chemicals are long lasting, adversely affecting the health of consumers. Some mechanism and treatments are needed to address these problems. Hot water treatment is one such mechanism which has been reviewed in the chapter to help the consumers taking care of all the public health issues. The present study provides easy to practice technique to farmers without involving cumbersome techniques to farmers to protect the capsicum against bacterial, viral and fungal diseases, leading to optimal productions without harming the interest of consumers.

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Chapter 4

Biological Control in *Capsicum* with Microbial Agents

Lorena Barra-Bucarei and Javiera Ortiz

Abstract

Capsicum annuum L. has great importance worldwide for its nutritional characteristics and its antioxidant content. It is cultivated in different geographical areas, under field and greenhouse conditions, and its production can be used for fresh consumption or processing. During its growth, it can be affected by biotic factors, such as pests and diseases that negatively affect the production and quality of its fruits, thus making adequate control measures necessary to avoid relevant economic losses. The environmental conditions that occur in its production promote the development of pests and diseases that can progress rapidly, making it increasingly difficult to manage populations of *Capsicum*. Traditionally, chemical pesticides have been used to deal with these problems, but their indiscriminate use has had negative consequences on the environment and human health. Biological control, based on the use of microorganisms, is thus presented as an efficient and sustainable alternative for *Capsicum* cultivation and offers a series of additional benefits. This chapter reviews the control alternatives available with microbial agents and their applications in the protection of *Capsicum* plants.

Keywords: beneficial microorganisms, entomopathogenic fungi, *Beauveria bassiana*, *Trichoderma*, endophytes

1. Introduction

The genus *Capsicum* belonging to the family *Solanaceae* consists of approximately 31 species, of which only five have been domesticated: *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens* [1]. *Capsicum* is known by various names including pepper, chile, chili, chilli, aji, and paprika. Throughout the world, *Capsicum annuum L.* is the most commercially important and widely grown species within this genus. The abundant varieties of *C. annuum*, including sweet peppers and chilli peppers, are important horticultural crops produced worldwide, especially in countries such as Spain and Mexico. Their fruits have remarkable sensory attributes in terms of colour, acidity and aroma, as well as an ample diversity of antioxidants, such as phenolic compounds and flavonoids [2]. Moreover, some types present high levels of capsaicin (8-methyl-*n*-vanillyl-6-nonenamide), which provide them with their spicy flavour in addition to therapeutic applications due to their anti-cancer properties [3].

The growth and development of *Capsicum* can be limited by various abiotic and biotic factors that negatively affect its fruit production and quality. It is estimated that losses caused by biotic factors such as invertebrates, pathogens and weeds can vary from 27 to 42%, which would increase to between 48 and 83% if the crops were not protected [4]. Among these, pests and plant diseases seriously affect *Capsicum* crops, in addition to the fact that their production is carried out under limited environmental conditions, which translates into a decrease in yield and quality [5]. For the control of these biotic agents, chemically synthesised pesticides have been traditionally used, which have generated several controversies due to their toxicity in humans and animals, and their damaging effects to the environment. Additionally, they can generate resistance in pathogenic microorganisms [6] and insects [7].

Regarding phytosanitary problems, the high temperatures and high levels of humidity generated in intensive production systems promote the development of fungal diseases that can progress rapidly [8]. Among the diseases that affect *Capsicum*, soil-borne diseases caused by pathogens of the genus *Phytophthora*, *Fusarium*, *Pythium* and *Rhizoctonia* are especially significant. At the fruit level, this crop is affected by pathogens such as *Botrytis cinerea* and *Anthracnose*, the latter caused by a complex of *Colletotrichum* species that is considered a serious problem with heavy losses in fruit yield, exceeding 80% [9]. Another important problem in the cultivation of *Capsicum* is viruses, since there are approximately 70 types that can affect this crop [10], especially the cucumber mosaic virus, pepper mild mottle virus and potato virus Y, among others [11–13]. In general, viruses can interfere with the chlorophyll synthesis of the plant, causing chlorosis and mottling of the foliage (mosaic).

On the other hand, several insect pests affect this crop during the entire growth and production cycle, causing significant yield losses ranging from 50 to 90% [14]. This has resulted in an intensive use of insecticides, mainly chemical. Among the plagues that affect *Capsicum* are: whiteflies, like *Trialeurodes vaporariorum* Westwood (Hemiptera: *Aleyrodidae*) and *Bemisia tabaci* Gennadius (Hemiptera: *Aleyrodidae*); flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: *Thripidae*); aphids *Myzus persicae* Sulzer (Hemiptera: *Aphididae*) and *Aphis gossypii* Glover (Hemiptera: *Aphididae*); worms *Helicoverpa armigera* Hübner (*Lepidoptera: Noctuidae*), *Spodoptera litura* Fabricius and *S. exigua* Hübner (Lepidoptera: *Noctuidae*); and mites *Polyphagotarsonemus latus* Banks (Acari: *Tarsonemidae*), *Tetranychus urticae* Koch, *T. ludeni* Zacher and *T. evansi* Baker and Pritchard (Acari: *Tetranychidae*) [15–17]. Within this group, whiteflies, aphids and thrips are considered pests of economic importance worldwide [18]. They have a wide range of hosts, from agricultural species to ornamental plants and are difficult to control due to their high reproductive rate, short life cycle and cryptic behaviour [19–21].

Finally, phytoparasitic nematodes are also organisms present in the cultivation of *Capsicum* and can cause serious problems affecting its performance [22]. Among the nematodes that affect *Capsicum*, those of the genus *Meloidogyne* are especially significant, such as *M. incognita* (Kofoid and White) Chitwood, *M. arenaria* (Neal) Chitwood, *M. enterolobii* (Yang and Eisenback) and *M. javanica* (Treub) Chitwood, which are responsible for root-knot disease [23–25]. These nematodes are found throughout the world, especially in warm areas and in greenhouses. Other nematodes that also cause economic damage in *Capsicum* include the false root-knot nematode *Nacobbus aberrans* Thorne and Allen [26]. Nematode control strategies are based on the use of chemicals; however, their high cost and principally their toxic effects have led to a search for more sustainable alternatives.

With a growing world population, currently, the main challenge for agriculture is to achieve food security; thus, food production has increased in recent years. However, pests and plant diseases, where *Capsicum* is no exception, have also increased as a result of changing climatic conditions, intensification of production systems and the opening of borders for the free transit of food, including fresh produce. In a conventional way, the high populations of insect pests and plant diseases have been controlled through the use of chemical pesticides. Biological control through the use of antagonistic microorganisms, such as bacteria, fungi and viruses, is presented as an alternative to the use of chemicals. It has received more attention in recent years and arises in response to the search for ways to control pathogens, insects and nematodes in a sustainable manner. However, this type of control must meet some requirements such as being effective against the target organism, not causing problems to the health of people and animals, reaching adequate control levels in the field, the feasibility of being incorporated into integrated management practices and meeting with phytosanitary measures according to the enforced regulations in each country where they are used.

Considering the above, the objective of this chapter is to review the importance of microbial agents in the biological control of pests, plant diseases and nematodes in *Capsicum*.

2. Microbial biological agents

2.1 Biological pest control with microorganisms

In recent years, world markets have expressed increasing concern about the use of agrochemicals. Biological control, which is defined as the reduction of pest populations by natural enemies and usually involves human intervention, is presented as an alternative to the use of chemical pesticides [27]. Biological control agents are classified into predators, parasitoids and pathogens (microorganisms that cause diseases).

After being identified, isolated and reproduced, biocontrol microorganisms are applied in a directed way in dilutions or released on the insect pests and diseases of the crops so that they can carry out their colonising action, produce antagonism, and specific diseases in the agents that require control, with the purpose of reducing the incidence to inoffensive levels [28]. Many microorganisms have been used as biopesticides because they offer a number of additional benefits beyond their target function [29]. An antagonist microorganism used for biological control must meet certain requirements, such as being genetically stable, effective at low concentrations, undemanding in nutrients, adapted to different environmental conditions, effective for a wide range of pathogenic microorganisms, easily grown, easily manipulated, resistant to chemical pesticides, compatible with commercial processes, not phytotoxic and not harmful to humans [30].

Among the microorganisms that have been most studied and reported as antagonists of insect pests and plant pathogens of *Capsicum* are bacteria such as *Bacillus* spp. and *Pseudomonas* spp., and the fungi *Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp., *Trichoderma* spp. and *Clonostachys* spp., while the viruses of the Baculovirus group have proven to be effective insect controllers. The genus *Bacillus* is made up of species that have been widely used to control insect pests and plant diseases due to the morphological and physiological characteristics that allow them to be ubiquitous in nature. It is especially important to note that species of this genus produce metabolites with antimicrobial properties used for the control of plant pathogens. In addition, the species *B. thuringiensis* Berliner has important qualities for the control of nematodes and protozoa [31, 32]. Another important group used in the control of insect pests in *Capsicum* is the entomopathogenic fungi, which have been widely studied. For more than a century, Pasteur predicted the advantages of entomopathogenic fungi because of their role as bioregulators of pests, acting as parasites to insects that are harmful to plants. Currently, more than 700 species of fungi are known to affect insects of various orders and their use as a biopesticide has increased during the last decade [33]. Mazón [34] indicated that the most important group of entomopathogenic fungi, with practical purposes for pest control, is constituted by *Metarhizium anisopliae* and *Beauveria bassiana*.

2.1.1 Entomopathogenic fungi

Entomopathogenic fungi (EPF) parasitize insects causing serious damage that can even lead to their death. The traditional mode of infecting insects with EPF involves the inoculation of conidia into the cuticle, followed by the formation of a germination tube and an appressorium which, through mechanical and enzymatic action, penetrates the cuticle and reaches the hemocele [35]. After the invasion of the hemocele, these fungi have the ability to re-cross the cuticle of the host and go outside, where they can continue to develop saprophytically on the insect, sporulating and turning them into new foci of dissemination of conidia [36].

EPF have potential for the control of whitefly in *C. annuum* cultivation. The effect of the *B. bassiana* strain M130 on *T. vaporariorum* adults was evaluated under greenhouse conditions reaching control levels of 45.3% in conidia applications to the foliage [14]. Its control of *B. tabaci* was also evaluated; applications of conidia in different concentrations applied to foliage had an effect on the mortality of eggs and nymphs of 37.8–59.04% and 38–75.9%, respectively. The highest percentages were reached with the highest concentrations (2×10^8 conidia/mL) of the Bb01 strain [37]. Flower thrips are an important pest in *Capsicum*; they affect its leaves, flowers and fruits, besides transmitting several viruses, as do white flies. For the control of flower thrips, several EPF have been evaluated as applications of *M. anisopliae* (Met52—Bioglobal Company) by spraying the foliage (flowers and leaves) proved to have the same efficacy on the number of adults of *F. occidentalis* as chemical insecticides [38]. The EPF *Fusarium subglutinans* (strain 12A), applied at concentrations of 1×10^6 conidia/mL, has proven to have the greatest lethal effect (58%) on the second instar nymphs [39]. Other EPF, applied individually or in combinations, have been evaluated in the control of this insect with different levels of efficacy [40]. These fungi have also presented biocontrol action against several aphids. Trials carried out by Curtis et al. [41], where the use of different additives in the formulation of the EPF *Verticillium lecanii* (Zimmerman), now classified as *Lecanicillium lecanii*, was evaluated in applications to pepper foliage for the control of *Myzus persicae*, determined that the formulations did not manage to increase the efficacy of the fungus since the applications of conidia in water suspensions reached the lowest infection rates (<5.0%). The controlling effect of *Lecanicillium attenuatum* (Petch) (CS625) on *Aphis gossypii* has also been evaluated in laboratory trials. The evaluated strain negatively affected aphid populations by decreasing their life expectancy and total fertility, in addition to the direct effect on nymphs [42]. Moreover, these fungi have also proven to be effective in controlling mites. Nugroho and Bin Ibrahim [43] determined that under field conditions, *B. bassiana* was the most effective in suppressing *P. latus* populations (eggs per outbreak) in chilli peppers, in comparison with strains of *M. anisopliae* and *P. fumosoroseus*.

2.1.2 Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are widely distributed throughout the world and have a large number of host insects [44]. They are soft-bodied, non-segmented vermiform organisms that are sometimes forced or opt to parasitize insects. They use insects as feeding, dispersal and propagation substrates, characteristics that have allowed them to be used for the biological control of insect pests. Naturally found in the soil, they are able to pursue and locate their host insect, responding to carbon dioxide emissions, vibrations and chemical signals. They have potential for use in crop protection because they are easy to mass produce and harmless to vertebrates and plants [45, 46]. The genera most used in the biological control of insect pests are *Steinernema* spp. and *Heterorhabditis* spp. These nematodes establish a mutualistic association with bacteria of the family Enterobacteriaceae, which are Gram-negative and facultatively anaerobic. Nematodes of the genus *Steinernema* are associated with bacteria of the genus *Xenorhabdus*, while those of the genus *Heterorhabditis* are associated with *Photorhabdus* [47].

Studies by Uhan [48] showed that nematodes of the genus *Steinernema* caused between 23.9 and 78.3% mortality of *S. litura* in pepper plants. The effect of native strains of *Steinernema feltiae* (Filipjev) (Rhabditida: *Steinernematidae*) and *S. carpocapsae* (Weiser) (Rhabditida: *Steinernematidae*) against *S. littoralis* and *Agriotes sordidus* Illiger (Coleoptera: *Elateridae*) larvae and *Ceratitis capitata* (Wiedemann) (Diptera: *Tephritidae*) pupae has also been studied at laboratory and greenhouse levels using pepper plants, with interesting levels of control that varied depending on the strain and soil texture [49]. Although EPNs have been used in *Capsicum* mainly for insects that have larval stages in the soil, they have also been evaluated against pests that spend their entire life cycle on the aerial part of the plant. Studies carried out by Rezaei et al. [50] on pepper plants determined that strains of *S. feltiae* and *H. bacteriophora* (Koppert Biological Systems) had a biocontrol effect on nymphs (second stage) and adults of *T. vaporariorum* in foliar applications.

2.1.3 Entomopathogenic bacteria

One of the most widely used bacteria for insect control is *Bacillus thuringiensis*, a Gram-positive bacterium, which has the ability to form spores as well as synthesise crystalline inclusions containing one or more proteins, some of which are toxic to a significant number of insect pests of the orders Lepidoptera, Diptera, Coleoptera and Hemiptera [51–53]. Evaluations conducted under laboratory conditions determined that 17 of 40 *B. thuringiensis* strains achieved mortality rates of *M. persicae* ranging from 64.4 to 88.9% [54]. On the other hand, some researchers have evaluated the effect of *B. subtilis* strains on this insect; however, no evidence was found of the direct effect of the biocontrol, rather the bacteria was found to promote plant growth by indirectly mitigating the damage caused by the insect [55, 56].

The Gram-negative bacterium *Chromobacterium* subtsugae presents insecticide activity against several orders, highlighting its biocontrol effect on *B. tabaci*, an important pest of *Capsicum*. Its broad-spectrum activity in insect control is related to multiple modes of action that probably involve different chemical compounds [57]. This bacterium could also control populations of *F. occidentalis* [58]. Recently, other bacteria with entomopathogenic action have been studied, such as *Serratia marcescens*. This bacterium as well as *B. cereus*, *B. pumilus* and some entomopathogenic fungi produce the enzyme chitinase, which has an important action in the pathogenicity of insects. Studies carried out by Aggarwal et al. [59] showed that *S. marcescens* (SEN strain) has an important insecticide activity against all stages of development of *S. litura* larvae; the ingestion of this bacterium produced larvae and pupae with less weight, as well as negatively affecting the fertility of the eggs.

Bacteria have also been evaluated for the control of mites. Aksoy et al. [60] investigated the effect of the biocontrol *Pseudomonas putida* (Biotype B) on *T. urticae* at the laboratory level. The bacterium was applied by spraying and dipping newly emerged females, achieving a decrease in the total number of eggs and hatching, compared to their respective controls. Spraying was the most effective method of application. On the other hand, *Burkholderia* spp. (strain A396), after a heat treatment, showed oral toxicity and contact effects against *S. exigua* and *T. urticae*;

its insecticidal action could be related to the production of different bacterial metabolites [61]. There is another group of entomopathogenic bacteria that includes the endosymbionts of the entomopathogenic nematodes, of which the genera *Xenorhabdus* and *Photorhabdus* are especially important [62]. These bacteria can produce metabolites that allow them to colonise and reproduce inside insects.

Although entomopathogenic bacteria have great potential to control insects affecting the cultivation of *C. annuum*, studies with this species are still scarce.

2.2 Disease control with microorganisms

One of the main problems that negatively affect the cultivation of *Capsicum* is the presence of a phytopathogenic complex formed by the genus *Fusarium*, together with others, such as *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia* spp., which are agents that cause seedlings to fall. Together, these can cause losses of between 60 and 100% in production [63]. Nonetheless, conventional control methods have been insufficient and difficult to apply, in addition to the problems associated with soil contamination, phytotoxicity and resistance production in the target pathogen [64]. Considering the above, the use of beneficial microorganisms is presented as an alternative for the control of these pathogens, not only for soil-borne diseases but also for pathogens that can proliferate in the aerial part of the plant and can also compensate for the negative environmental impacts caused by chemical pesticides [65]. Studies on the biological control of diseases through the use of microorganisms have recently increased, and the role of the interactions of beneficial microorganisms with the plant and/or the pathogen is receiving more and more attention, not only because of their antagonistic action but also because of their potential to promote plant growth, thus contributing to a more sustainable production over time [66].

Among the most commonly used microorganisms for the control of pathogens are fungi of the genera *Trichoderma*, *Clonostachys*, and *Penicillium*; and the bacteria *Pseudomonas* and *Bacillus*. In relation to their antagonistic activity, these present diverse mechanisms of action that can act independently or jointly. If the antagonist can express different mechanisms of action, the likelihood of developing resistance in the pathogen is reduced. The mechanisms of action described include competition for nutrients and space, antibiosis, parasitism (considered as an antagonistic symbiosis between organisms) and activation of systemic resistance. The use of these control agents is projected as a more efficient control alternative; however, there are several aspects that should be studied so that they can express their full potential, such as the form of application, the combined use of antagonists, formulation and conditions during application [67]. Moreover, the possibility that beneficial microorganisms may persist in the soil could be an important advantage over the use of pesticides. An additional advantage is that if they remain in the rhizosphere they are in the first line of defence against attack by soil pathogens.

2.2.1 Antagonistic fungi

A diverse group of fungi have been shown to antagonise a significant number of pathogens affecting *Capsicum,* such as *Phytophthora* spp., *Colletotrichum* spp., *Rhizoctonia* spp., *Fusarium* spp., and *Botrytis cinerea*, among others [68–72]. One of the most cited examples in the literature are the fungi of the genus *Trichoderma*, which due to their diversity, versatility, adaptability and easy handling have proven to be good candidates for commercial development as a biofungicide. Moreover, these fungi produce three types of propagules, hyphae, chlamydospores and conidia, all of which are active against pathogens in different phases of their life cycle, along with presenting an interesting activity promoting plant growth.

Studies carried out by Ahmed [68] indicate that *T. harzianum* presents an antagonistic effect against the pathogen *P. capsici* because it significantly reduces stem necrosis compared to values obtained in plants inoculated only with the pathogen. Applications of *Trichoderma* spp. in jalapeño peppers were able to decrease the growth rate of *P. capsici* in addition to increasing plant growth [73]. Another soil fungus controlled by *Trichoderma* is *Fusarium* spp., which was confirmed in studies carried out by Sinha et al. [74] on chilli peppers under greenhouse conditions, which showed that strains of *T. viride* and *T. harzianum* reduced the incidence of *F. oxysporum* f. sp. *capsici* by 83.93–87.5%. *Trichoderma* has also been used to control anthracnose, which is caused by several species of *Colletotrichum*. Applications of strains of *T. harzianum* (BHUF4) and *T. asperellum* (T16A) promote the accumulation of the phenol that stimulates the expression of the defence gene, both of which have proven to induce and acquire systemic resistance, providing a solid protection against *C. truncatum* [72]. Studies carried out by Rahman et al. [71] also determined that *Trichoderma* spp. had a biocontrol effect on *C. capsici* in chilli peppers since they managed to decrease the percentage of infection in fruit, although the strain *T*. *harzianum* (IMI-392433) presented the best antagonistic effect against this pathogen, all the strains (*T. harzianum, T. viride* and *T. pseudokoningii*) presented a promoter effect in plant growth and higher fruit yield.

The *Clonostachys* fungus also presents important characteristics in the biological control of pathogenic fungi. Nobre et al. [69] determined that strains of *C. rosea* were able to suppress more than 80% of the sporulation of *B. cinerea* in chilli peppers and tomatoes. Another fungus studied for disease control in *Capsicum* is *Xylaria poitei*. The antagonistic activity *in vivo* and the protection that *Xylaria poitei* provided chilli pepper seedlings against the pathogen *P. capsici* were evaluated. This antagonist allowed the survival of 58.3% of the seedlings, while seedlings inoculated only with the pathogen showed 100% mortality [75].

2.2.2 Antagonistic bacteria

Several species of *Bacillus* are known for their antagonistic capacity against pathogens that cause disease in plants; many of these are also considered because they could have a direct action in disease control as a result of the production of metabolites or indirectly by promoting growth. These bacteria act not only on soil-borne diseases but also on leaf and fruit pathogens [76–78]. Diverse studies have provided evidence that plant growth-promoting bacteria have the ability to activate systemic resistance induced by the activation of physical or chemical defences of the host plant by an inducing agent [79].

Studies carried out by Yu et al. [77] determined that applications of *B. subtilis* (CAS15 strain) significantly suppressed spore germination of *F. oxysporum* f. sp. *capsici* by 8–64%. In addition, the incidence of the disease was much lower in plants treated with the bacteria compared to the control (12.5–56.9%); these results are attributed to the induction of systemic resistance. This bacterium has also been used in *Capsicum* for the control of the anthracnose disease in chilli plants caused by *Colletotrichum gloeosporioides* (OGC1 strain). Seeds treated with the bacterium showed a 65% decrease in disease incidence compared to seeds treated with the pathogen alone [78]. Some *Bacillus* species have also been evaluated in the control of diseases caused by pathogenic bacteria, such as pepper bacterial wilt caused by *Ralstonia solanacearum*, an important *Capsicum* disease [79]. The application of *B. amyloliquefaciens* (strain Bg-C31) and its metabolites significantly reduced the percentage of plants affected by *R. solanacearum* at the field level and in pepper pots [80].

Pseudomonas is another group of bacteria used for disease control in *Capsicum*; among these, the most reported is *P. fluorescens* which has been shown to have a biocontrol effect on pathogens and also some phytoparasitic nematodes [81, 82]. Basu [83] evaluated the effect of *P. fluorescens* on *Ralstonia solanacearum* in chilli pepper plants and determined that combined applications of the antagonist to the seed and the soil resulted in the lowest percentages of disease incidence (0.83–10.82%), compared to the control which was inoculated only with the pathogen (9.99–44.96%).

Many bacteria have demonstrated an important potential for the control of diseases caused by fungi and bacteria; nevertheless, many of them have been evaluated under controlled conditions, which makes it necessary to concentrate efforts to increase the number of field level trials, and thus determine the real potential of being incorporated into the cultivation of *Capsicum*.

2.3 Nematodes with microorganisms

Infestation of peppers by nematodes, such as *Meloidogyne* spp., is one of the major problems for pepper production worldwide [84]. They attack the roots, leading to root system dysfunction, reduced rooting volume, inefficient use of water and nutrients, reduced crop and plant growth and yield [85]. Another nematode that causes damage in *Capsicum* is the false root-knot nematode, *Nacobbus aberrans*, which is associated with *F. oxysporum* and *P. capsici*, and can cause up to 100% crop loss [86]. For its control, chemical nematicides are applied; however, in the cultivation of *C. annuum*, its use has been restricted due to its toxicity levels, residual elements in the environment and the selection of resistant nematode populations [87].

An alternative for the biological control of phytoparasitic nematodes is nematophagous fungi. These ubiquitous microorganisms are capable of modifying their saprophytic behaviour and can feed on nematodes in unfavourable nutritional conditions. They are natural enemies of nematodes and have developed highly sophisticated infection strategies [88]. They are classified into three groups according to their predation characteristics. First are the nematode traps or predators (their modified hyphae form traps and through a chemical and mechanical process they digest the nematode), second are the opportunists or ovicides (they form traps to catch eggs, cysts and females) and finally the endoparasites (forced parasites of the nematodes that use their spores as structures of infection, which can adhere to the cuticle of the nematode or be ingested) [89, 90].

Paecilomyces lilacinus have a negative effect on the root nodulation produced by *M. incognita* in peppers [91]. Another trial used *B. bassiana* strains and determined that applications of this fungus to the substrate in pepper plants decrease the number of root nodules caused by *M. incognita* [92]. The combination of nematophagous fungi has also been evaluated on *M. incognita*. Studies carried out by Requena Candela [93] demonstrated the antagonistic effect of the combination of *P. lilacinus* and *T. harzianum* by reaching decreases in the incidence of nematodes close to 70%. Pérez-Rodríguez et al. [94] evaluated the nematophagous action of the fungus *Pochonia chlamydosporia* for the control of *N. aberrans* nematodes in broad chilli peppers and determined that the combination of vermicompost with the nematophagous achieves a reduction in the number of juvenile nematodes, the number of eggs and females per gram of roots, as well as increasing the dry matter of the plants.

2.4 Endophytic microorganisms for the control of insect pests and plant diseases

Endophytes are defined as microorganisms that spend most or all of their life cycle colonising host plant tissues without causing obvious damage [95].

Endophytes are associated with most plant species, are naturally found in the ecosystem and are considered an extremely important partner for plants [96, 97]. These microorganisms have been of interest for study during the last few years due to the beneficial characteristics they are able to bestow onto their hosts [98], among which are the promotion of plant growth, inhibition of pathogenic organisms, control of insect pests, removal of contaminants from the soil and increased tolerance to extreme conditions of temperature, water availability and salinity [99–101]. Consequently, they have an important future in agrifood production.

Currently, several studies have shown that endophytic fungi can protect host plants against pathogens and herbivores [102, 103]. Some endophytes, when artificially inoculated, can confer the beneficial characteristics mentioned above to their hosts, as they can influence key aspects of physiology. The host plant receives multiple benefits from its interaction with the endophyte in exchange for carbon-based resources [104]. The type of interaction used by endophyte biological controllers is not yet clear [105], but it is believed that this type of interaction is established through a process of co-evolution [106].

These fungi generate interspecific interactions and protection against the incidence of pathogens produced by direct mechanisms such as competition, parasitism, antibiosis and indirect mechanisms such as induction of resistance [97, 104]. The activation of systemic resistance in the plant may be due to the presence of endophytic fungi, a mechanism that has already been shown to be effective against other fungal pathogens [107]. On the other hand, among the most important reported mechanisms for pest control are parasitism by ingestion [108], antagonism by the action of metabolites [109, 110], systemic resistance [111] and a tritrophic action [112].

Works carried out by Barra-Bucarei et al. [113] present the first report of the endophyte *B. bassiana* with antagonistic action against *Botrytis cinerea* in chilli pepper plants. Five native strains were evaluated, and the percentage of leaf area affected by the pathogen (PSAP) was determined. Plants inoculated with endophytes showed 2 to 18% of PSAP compared with plants treated only with the pathogen, which exhibited early symptoms of the disease with a PSAP of 63%. Another study conducted by the same authors (unpublished data) demonstrated that the same strains are endophytic (**Figure 1**) and reduced the symptoms caused

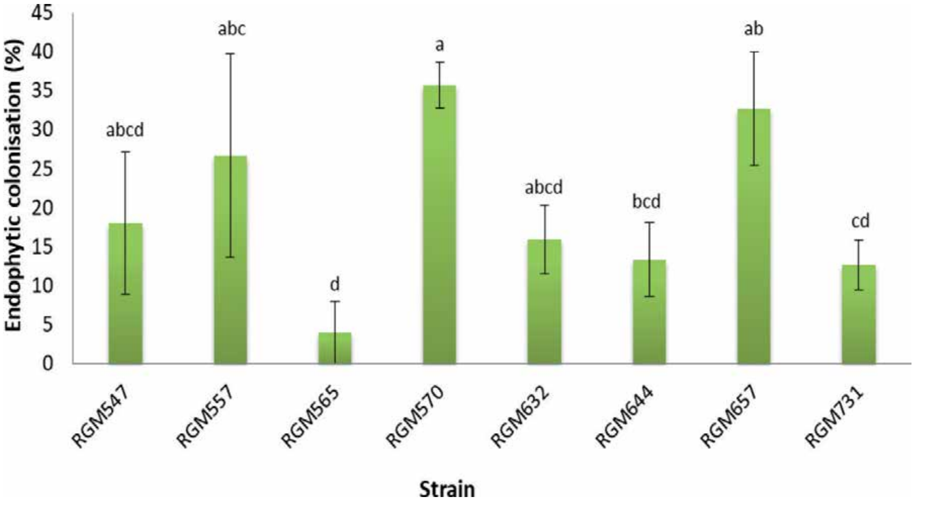

Figure 1.
Endophytic colonisation of B. bassiana *strains in chilli pepper plants (leaves, stems and roots), 30 days after inoculation (n = 5). Data represent the mean standard error. Different letters over the bars represent significant differences among the treatments according to the Fisher's LSD test (p < 0.05).*

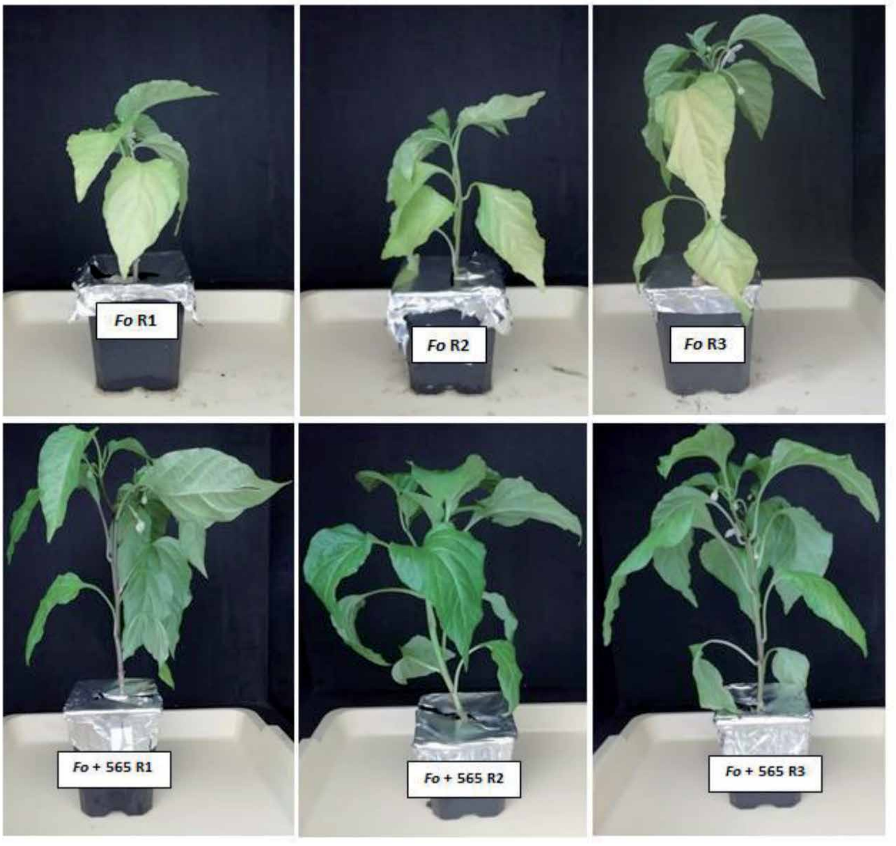

Figure 2.
In vivo *antagonism of* B. bassiana *(565) against* F. oxysporum *(*Fo*). Plants inoculated with* F. oxysporum *(*Fo*) showed chlorosis and plants inoculated with* B. bassiana *RGM565 +* Fo *showed no symptoms, 45 days after inoculation (n = 10).*

by *F. oxysporum* in chilli pepper plants, increasing dry matter and decreasing the level of leaf chlorosis in adult plants, making the plants more resistant to the attack of this pathogen (**Figure 2**). These studies coincide with research conducted by Jaber and Alananbeh [103], in pepper plants which provided evidence that commercial strains of *B. bassiana* (Naturalis) and *M. brunneum* (Bipesco5) can inhibit the growth of several species of *Fusarium* (*F. oxysporum*, *F. culmorum* and *F. moniliforme*) *in vitro* and in potting trials.

3. Conclusion

With the increase of the world population, it is urgent for the development of sustainable strategies to improve food availability. *Capsicum annuum* is an excellent source of natural health-related compounds, such as micronutrients and antioxidants; its fruits have been used for fresh and cooked consumption. The crop of this vegetable can be negatively affected by biotic factors, provoking decreased yields. In this chapter reviewed, we have provided evidence of the potential that microorganisms present for the control of insect pests, plant diseases, and nematodes in the *Capsicum* crop. There are a significant number of investigations carried out under laboratory conditions with good biocontrol results; however, it is necessary to increase the number of field studies and application methods, since environmental conditions could condition the behaviour of microorganisms and not reach the same levels of efficacy obtained under controlled conditions. In addition, it is necessary to study the ecological behaviour of beneficial microorganisms and their interaction with others. The biocontrol microbial agents can fulfil diverse functions in plants and give an eco-friendly approach to promoting sustainable agriculture.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Management of Viruses and Viral Diseases of Pepper (*Capsicum* spp.) in Africa

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Abstract

Increasing outbreaks of virus species infecting pepper (*Capsicum* spp.) is a major problem for growers in Africa due to a combination of factors, including expansion of pepper cultivation, abundance of insect vectors and climate change. More than 45 viruses have been identified to infect pepper crops causing economic loss in terms of reduced quality and marketable yield, sometimes up to 100%. The *Pepper veinal mottle virus* (PVMV), *Potato virus Y* (PVY) and *Cucumber mosaic virus* (CMV) are endemic in many countries including Uganda, Mali, Cameroon, Morocco and Nigeria. Current management options for virus infection in *Capsicum* spp. is by the integration of several approaches. More importantly, eradication of infected plants, cultivation of disease resistant varieties, improved cultural practices and judicious use of insecticides especially when plants are young and easily colonized by vectors. In recent years, eco-friendly control measures are needful to reduce occurrence of virus diseases in *Capsicum* spp.

Keywords: climate change, economic loss, outbreaks, management options, virus infection

1. Introduction

Peppers (*Capsicum* spp.) are one of the most important spices and vegetable crops in the economic and social life of people living worldwide [1]. Viruses are among the most important factors threatening *Capsicum* spp. production in several regions like Australia [2], Europe [3], Asia [4] and Africa [5]. They cause diseases that not only reduce yield and quality of fruits, but also increase the cost of preventive measures and cost of producing clean planting materials. In addition, the high genetic diversity of virus strains and their accumulation in propagation materials makes them easily spread into unaffected areas [6].

In Africa, more than 45 viruses (grouped into eight genera namely, *Potyvirus*, *Tospovirus*, *Begomovirus*, *Cucumovirus*, *Tobamovirus*, *Polerovirus*, *Alfamovirus* and *Potexvirus*) have been identified to infect pepper crops causing reduced quality and marketable yield, sometimes up to 100% [7]. The major viruses of *Capsicum* spp. include *Pepper veinal mottle virus* (PVMV), *Chilli veinal mottle virus* (ChiVMV), *Potato virus Y* (PVY), *Tobacco etch virus* (TEV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Pepper mild mottle virus* (PMMoV), *Pepper leaf curl virus*

(PepLCV), *Tomato yellow leaf curl virus* (TYLCV), *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV) and *Tomato spotted wilt virus* (TSWV) while other viruses of minor importance include *Pepper vein yellows virus* (PeVYV) and *Potato virus X* (PVX) [8]. These viruses are mainly vectored by aphids, whitefly or thrips during feeding probes. However, mechanical transmission and seed transmission are efficient means for the spread of infection. Typical symptoms induced by viruses infecting *Capsicum* spp. include stunting, curling and mottling of foliage and fruits. Symptoms can vary significantly with cultivar, plant age, virus isolate and environmental condition. In recent years, co-infection of previously non-existent virus strains is most devastating [7].

The increasing outbreaks of virus species infecting *Capsicum* spp. have become a major problem for growers in many countries including Ghana, Uganda, Mali, Cameroon, Morocco and Nigeria [9, 10]. This is due to a combination of factors, including expansion and intensification of pepper cultivation, availability of volunteer hosts, abundance of insect vectors and climate change [6, 11]. Many techniques are used to minimize viral infections in *Capsicum* spp., but are successful when they begin prior to planting of the crop. Current management options for virus infection in *Capsicum* spp. is by the integration of several approaches. These include use of protected nurseries, cultivation of disease resistant varieties and ensuring adequate phyto-sanitary conditions after transplanting.

Generally, eco-friendly management measures are needful to reduce occurrence of virus diseases in *Capsicum* spp. and decrease the rate of spread of same into unaffected regions. This is important because of the need for increased international movement and exchange of pepper germplasm. This will also facilitate the selection and breeding for improved *Capsicum* spp. adapted to local conditions and uses. Therefore, there is need for a review of viruses infecting *Capsicum* spp., their symptoms, mode of transmission and management options.

2. Viruses of pepper

2.1 Genus: *Potyvirus*

The genus *Potyvirus* consists of a large group of plant viruses that are widespread, infecting economically important crops like tomato, potato, eggplant and pepper [12]. Members of this genus have non-enveloped flexuous filamentous single-stranded RNA (ssRNA) particles about 680–900 nm long and 11–15 nm wide [13]. The symptoms induced by potyviruses include mottling, mosaic, curling, vein banding or clearing, chlorosis, blistering and severe stunting of the whole plant. Severity of these symptoms depends on the *Capsicum* variety, virus strain and presence of other viruses. The major potyviruses infecting *Capsicum* spp. in Africa include PVMV, ChiVMV, PepMoV, PVY and TEV [12].

2.1.1 Pepper veinal mottle virus *(PVMV)*

Pepper veinal mottle virus (PVMV) was first reported in Ghana in 1971 and has since then spread to other regions in Africa including Ethiopia, Kenya, Tanzania, Uganda and Rwanda [14] causing yield loss ranging from 70 to 100 % [15]. Infection of PVMV also occurs in other solanaceous crops, including eggplant and tomato [16]. Eight species of aphids have been shown to transmit the virus in a non-persistent manner, of which *Myzus persicae*, *Aphis craccivora*, *A. gossypii* and *A. spiraecola* are rated as efficient vectors [17]. Mechanical transmission also occurs through contact with infected sap, but not via seeds. Several symptoms associated with

PVMV include leaf mottle, leaf mosaic, leaf curl, vein banding, leaf ring spots, leaf deformation, leaf chlorosis, blistering and severe stunting of the whole plant [18].

2.1.1.1 Management of PVMV

Plastic mulches have been reported to reduce insect infestation and the concurrent damage associated with the insect transmission of viruses [10]. Intercropping *Capsicum* spp. with maize can also reduce the incidence of the disease compared to sole cropping [17]. The use of resistant varieties, raising of seedlings in insect-proof nursery and roguing of infected plants once detected are also crucial to reduce PVMV incidence and spread in the field. These are recommended as integrated ways for managing PVMV infection in the field [7].

2.1.2 Chilli veinal mottle virus *(ChiVMV)*

Chilli veinal mottle virus (ChiVMV) has been reported in various countries of West and East Africa including Ethiopia, Uganda and Tanzania [19]. The virus is not transmitted through seeds but is acquired mechanically and is transmitted by several species of aphids in a non-persistent manner. These vectors (*Myzus persicae*, *Aphis gossypii*, *Aphis craccivora*, *Aphis spiraecola*, *Rhopalosiphum maidis*, *Toxoptera citricida* and *Hysteroneura setariae*) retain the virus for not more than1 hour after virus acquisition [20]. The winged aphids are generally the most efficient vectors to transmit the virus from field to field and are the most difficult to control [21]. Typical symptoms caused by ChiVMV include leaf mottle and dark green vein-banding.

2.1.2.1 Management of ChiVMV

The effective management strategies for ChiVMV include early identification and timely disposal of infected seedlings, application of mineral oil-based insecticides in controlling aphid populations early enough during planting, careful handling of diseased-free seedlings and use of resistant varieties. In addition, removal of solanaceous weeds, which serve as alternate hosts, can reduce incidence of ChiVMV in pepper gardens [21].

2.1.3 Potato virus Y *(PVY)*

Potato virus Y (PVY) is common among solanaceous crops including pepper, potato, tomato, tobacco and many weeds [22]. It has been reported in many African countries including Zimbabwe, Zambia, Kenya, Tanzania, Malawi, Madagascar, Ethiopia and Nigeria [23]. Isolates of PVY from pepper do not infect potato and vice versa [24]. Strains of PVY infecting sweet pepper are classified by their ability to overcome resistance genes based on differential host reactions. They cause yield reductions of 20–70% in pepper production [25]. The virus is transmitted in a non-persistent manner through several species of aphids, but the green peach aphid (*Myzus persicae*) is considered generally to be the most important vector. Infection of PVY can spread during grafting, handling of plant and use of unsterilized farming equipment. The symptoms of PVY include stunting or dwarfing of plant, systemic vein clearing and banding, leaf mosaic and small deformed fruit with a mosaic pattern making them unmarketable [26].

2.1.3.1 Management of PVY

Management of PVY is successful through the use of resistant varieties, elimination of solanaceous weeds and adjoining volunteer host plants, scheduling the time of planting to conform with period of least aphid population, careful handling of plant seedlings especially during nursery and farming operations. Early detection and roguing of infected plants are the effective measures to reduce incidence of PVY. Additionally, intercropping pepper with maize has been found helpful in the management of potyviruses in pepper fields [27].

2.1.4 Tobacco etch virus *(TEV)*

Tobacco etch virus (TEV) naturally occurs in co-infection with PVY [13]. In Africa, TEV is endemic and widespread in Madagascar and occasionally in South Africa. The virus is transmitted by several species of aphids in a non-persistent manner. Infected plants are lighter in colour and have mosaic patterns on their leaves, sometimes with dark-green banding along the leaf veins. Plants may also be stunted, showing leaf curl and fruit distortion.

2.1.4.1 Management of TEV

The use of cultivars with PVY resistance helps manage TEV because resistance to the two viruses is closely-linked. Additionally, pepper seedlings should be produced in protected culture system equipped with nets or screens to exclude aphids. Scheduling planting dates to avoid periods of high aphid activity early in the season and controlling weeds, volunteer host plants in and around seedlings will lower the amount of virus inoculum introduced into the crop. Application of mineral oil has been reported to reduce incidence of TEV in pepper [10].

2.2 Genus: *Tobamovirus*

The viruses in the genus *Tobamovirus* are especially important because they do not require biological vectors for transmission. Members of this genus have flexuous filamentous ssRNA particles approximately 300 nm in length and 18 nm in diameter that can persist for years and survive many adverse environmental conditions because of their strong structural coat protein. Due to their high stability, tobamoviruses remain infectious in contaminated plant debris, compost, soil and irrigation water. Viruses in this genus include TMV, ToMV and PMMoV [13].

2.2.1 Tobacco mosaic virus *(TMV)*

Tobacco mosaic virus (TMV) is the first ever virus to be identified. The virus infects more than 350 plant species, including at least 125 crop species such as tobacco, tomato, pepper, eggplant, potato and cucumber [28]. *Tobacco mosaic virus* has been found to infect pepper in Uganda, Tanzania, Zimbabwe, Sudan, Zambia (East Africa) and (Nigeria, Ghana) West Africa [29, 30]. The virus survives in infected plant materials for months or years and multiplies in living plant tissue but can remain dormant in dead plant tissue, retaining its infectiveness [31]. Tobacco mosaic virus spreads mainly through contact between plants, infected seeds and by mechanical means but not insect vectors. Typical symptoms of TMV infection include chlorotic leaves, mosaic patterns on leaves, leaf distortion and stunted growth usually associated with reduced fruit size [32].

2.2.1.1 Management of TMV

Management of TMV can be achieved through careful handling of plants, disinfection of farm tools, eradication of infected plants and treatment of seeds with 10% trisodium phosphate (TSP). The use of resistant cultivar to TMV infection has been demonstrated in Nigeria [33]. Also, healthy seed-testing and 2-year minimum crop rotation is advisable. *Capsicum* spp. should not be planted alongside other susceptible crops such as tomato and potato.

2.2.2 Tomato mosaic virus *(ToMV)*

Tomato mosaic virus (ToMV) has a worldwide distribution and is often endemic in African countries such as Uganda, Zambia and Cameroon [29]. The virus is one of the most resilient viruses in terms of its ability to survive outside plant cells and dead tissues. *Tomato mosaic virus* is known to infect more than 150 economically important crop species, including vegetables and ornamental plants. Natural mode of spread of ToMV is by contact with infected sap but it is also being transmitted mechanically during farming operations [6]. The occurrence of ToMV is more predominant in pepper than TMV even though both virus species produce similar symptoms on pepper. Typical symptoms include severe stunting of plants with chlorotic mosaic patterns on leaves and fruits [34]. Additional symptoms may vary depending on the plant age, virus strain and environmental condition. These include distorted leaves, which often occur with premature defoliation and necrotic (brown) patches on leaves and fruits [35].

2.2.2.1 Management of ToMV

Management options for ToMV include rotation to non-host plants after an infection outbreak. The use of resistant varieties coupled with improved cultural practices can help to improve production especially in endemic areas. Disinfection of screen-house soils, planting tools and containers ensures clean planting materials in the nursery. After transplanting, careful handling of healthy seedlings while removing symptomatic ones can reduce spread of the virus. Sterilizing seeds with 10% TSP can help remove ToMV present on the seed coat [36].

2.2.3 Pepper mild mottle virus *(PMMoV)*

Pepper mild mottle virus (PMMoV) has been widely reported in some African countries including Uganda, Zambia, Tanzania, Nigeria and Ghana. It has been shown to infect up to 24 other plant species including *Solanaceae*, *Chenopodiaceae*, *Cucurbitaceae*, *Labiatae* and *Plantaginaceae* [29, 23]. In cultivated pepper plants, PMMoV can be transmitted through seed and contact with infected plant sap [37]. Seedlings can also be infected by mechanical contamination during transplanting or other cultural routine. *Pepper mild mottle virus* persists in soil and on infected debris, which serve as primary source of inoculum for subsequent planting. Symptoms of PMMoV include various degrees of leaf mottling, leaf mosaic, leaf chlorosis, necrosis, leaf curl and growth decline. These symptoms are more pronounced in young plants than in older plants [38].

2.2.3.1 Management of PMMoV

Treatment of *Capsicum* spp. seeds with 10% TSP for 2.5 hours (h) reduces the incidence of PMMoV. Other management options include sterilization of soil before planting, especially in screen-houses and ensuring good field hygiene after transplanting [13].

2.3 Genus: *Begomovirus*

The genus *Begomovirus* comprises monopartite or bipartite plant viruses that infect a wide range of crops throughout the world. Viruses in this genus are exclusively transmitted by the whitefly (*Bemisia tabaci*) in a persistent and circulative manner. These virus species have circular single-stranded DNA (ssDNAs) particles approximately 30 nm in length and 18 nm in diameter. Examples of important begomoviruses affecting pepper include PepLCV and TYLCV.

2.3.1 Pepper leaf curl virus *(PepLCV)*

Pepper leaf curl virus (PepLCV) has been found in different countries of Africa and is responsible for several epidemics causing severe economic losses. Transmission of PepLCV is most damaging when plants are infected at early growth stage preventing the proper formation of flowers resulting in low fruit production. Symptoms of PepLCV include severe stunting, flower bud abscission, reduced pollen production, upward curling of leaves, leaf chlorosis and elimination of fruit production. Yield losses may be between 90 and 100% [39].

2.3.1.1 Management of PepLCV

The most widely used treatments include use of insecticides and other cultural methods to control vector population. Other methods to control the spread of PepLCV include planting resistant or tolerant plants, crop rotation and border planting, and plastic mulching [40].

2.3.2 Tomato yellow leaf curl virus *(TYLCV)*

Tomato yellow leaf curl virus (TYLCV) is *one* of the most destructive viruses affecting a wide host range of vegetable crops, including Okra (*Abelmoschus esculentus*), tomato (*Solanum lycopersicum*), sweet and chilli pepper (*Capsicum* spp.), tobacco (*Nicotiana tabacum*), common bean (*Phaseolus vulgaris*) and some weeds [41]. The virus has been reported in *C. annuum* in Tunisia [42]. However, its distribution spreads across East, West and Central Africa. Movement of infected plants or virus-carrying whiteflies aids the spread of the virus. Typical symptoms of TYLCV in infected pepper plants include interveinal yellowing, leaf curling and stunting which cause critical production losses.

2.3.2.1 Management of TYLCV

Plastic mulching has been effective in the control of whitefly populations in pepper fields. Other strategies for managing TYLCV include raising of seedlings in pest-proof nurseries, adoption of crop rotation, use of insecticides, selection of resistant varieties for planting, improved cultural practices to remove weeds and alternative host plants [43].

2.4 Genus: *Cucumovirus*

The genus *Cucumovirus* comprises tripartite ssRNAs encapsidated in small icosahedral particles approximately 29 nm in diameter. Viruses in this genus infect over 1200 plant species worldwide [44], including weeds and wild species.

2.4.1 Cucumber mosaic virus *(CMV)*

Cucumber mosaic virus (CMV) is an important viral disease of pepper with a worldwide distribution. In Africa, the virus has been reported in Uganda, Zambia, Tanzania, Ethiopia, Zimbabwe, Kenya, Malawi, Madagascar, Sudan, Rwanda, Ghana and Nigeria [14, 45]. Although the virus exists as a number of strains, all are apparently capable of infecting pepper and differ only in their symptom expression. The age of a plant at the time of infection strongly influences what type of symptoms will be manifested. Symptoms of naturally affected pepper plants vary and most prominent ones are mild mosaic and dull-coloured leaves, mottling, shoe string, fern leaf, vein banding, vein clearing, leaf deformation, stunted growth and reduced fruit size [46, 47]. More than 80 species of aphids transmit the virus in a non-persistent way but *Aphis gossypii* and *Myzus persicae* are the most efficient [48]. Additionally, transmission can be through seeds, parasitic weeds and mechanically [26, 49].

2.4.1.1 Management of CMV

Strategies to delay early infection can be used to enhance yield. Isolation of pepper from weedy border areas or growing them next to taller border plants such as maize, which can function as a non-susceptible host, use of certified seeds and plants, screening and disinfection of infected mother stock, washing and disinfecting of hands and tools, and planting of resistant pepper genotypes constitute the effective means of managing CMV [47].

2.5 Genus: *Alfamovirus*

The genus *Alfamovirus* consists of ssRNA viral particles. A typical member of this genus is *Alfalfa mosaic virus* (AMV) having natural host range including over 250 plant species and is closely related to CMV [13].

2.5.1 Alfalfa mosaic virus *(AMV)*

Alfalfa mosaic virus (AMV) is distributed worldwide, having a wide host range. In Africa, it has been reported in Zambia [50]. The virus is transmitted mechanically and in a non-persistent manner by numerous aphid species. Transmission of AMV also occurs through pepper seeds or pollen [9]. Typical symptoms associated with AMV in pepper include bright yellow or blotchy white mosaic patterns on pepper leaves [6]. Additional symptoms include stunted growth with deformed and blotchy fruits, especially if plants are infected at young stage. *Alfalfa mosaic virus* can cause important yield losses and increased susceptibility of pepper to other pathogens.

2.5.1.1 Management of AMV

Alfalfa mosaic virus can be successfully managed by reducing aphid population by use of insecticides. The use of resistant varieties and regular rouging of weeds from the fields are effective means to manage the virus [51].

2.6 Genus: *Tospovirus*

The genus *Tospovirus* consists of important species of plant viruses that cause great losses in many economically important crops. Members of this genus have tripartite ssRNA particles about 80–120 nm in size [42, 52]. The major virus species in the genus *Tospovirus* is *Tomato spotted wilt virus* (TSWV), which infects various *Capsicum* spp. in Africa [13].

2.6.1 Tomato spotted wilt virus *(TSWV)*

Tomato spotted wilt virus (TSWV) causes serious crop losses in many economically important crops, including vegetables and ornamental crops. It has been reported to affect pepper in Zimbabwe in Africa [53]. The virus is transmitted in a persistent and propagative manner by several species of thrips. The western flower thrip (*Frankliniella occidentalis*) is the most efficient [54]. However, only the adult thrips that feed on infected plants transmit the virus after inoculation period of less than 48 h. *Tomato spotted wilt virus* is known to infect more than 1000 different plant species from about 80 botanical families. Symptoms of the virus can be very host-specific. Typical symptoms observed in pepper include yellowing or browning of leaves, chlorotic or necrotic ringspots on leaves and fruits, necrotic streaks on stems with terminated shoots and fruits [6].

2.6.1.1 Management of TSWV

Early detection and roguing of infected plants is important for reducing TSWV incidence in the field. Intercropping pepper with companion crops like ginger (*Zingiber officinale*) can reduce the vector from reaching its host. Additionally, use of insecticide is a critical measure in management of TSWV [9].

2.7 Genus: *Polerovirus*

2.7.1 Pepper vein yellows virus *(PeVYV)*

Pepper vein yellows virus (PeVYV) has been reported in Ivory Coast, Mali, Republic of Benin, Sudan and Tunisia in Africa with infection rates of up to 100%. The virus is spread in circulative and non-propagative manner by *A. gossypii* and *M. persicae* [55]. Major host plants include *Capsicum* spp. and *Solanum nigrum* [56]; however, some alternate hosts such as *Chenopodium amaranticolor*, *Curcubrita pepo*, *Datura stramonium*, *Gomphrena globose* and *Nicotiana* spp. have been reported [56]. Symptoms on cultivated pepper plants include leaf curling, deformation, reduced leaf size, puckering, interveinal yellowing, vein clearing and yellow patches on leaves [57].

2.7.1.1 Management of PeVYV

The most effective means of controlling PeVYV is preventing its introduction. Careful sourcing of plants, keeping aphid population low, raising awareness of its symptoms will assist in preventing the establishment of the virus [56].

2.8 Genus: *Potexvirus*

2.8.1 Potato virus X *(PVX)*

Potato virus X (PVX) has been reported in Ethiopia, Zambia and Zimbabwe to infect a wide range of solanaceous crops including pepper, tomato, potato and tobacco. The virus causes a range of symptoms including mottling, severe necrosis of leaves and stems and sometimes, defoliation of some cultivars. Symptoms of PVX are worsened in co-infection with other viruses, especially *Potato virus Y* [58, 59]. *Potato virus X* is transmitted mechanically and by contact between plants, but not through seeds [60]. PVX is of minor importance in pepper production [60].

2.8.1.1 Management of PVX

Planting resistant cultivar is the most economic and effective way of managing the virus [61].

3. Conclusion

Viruses remain a primary constraint to production of high-quality *Capsicum* spp. worldwide, especially in the developing regions [1]. In Africa, weeds often become reservoir hosts for vectors and virus species that attack pepper in the field [47]. Currently, virus species in the genus *Potyvirus*, *Cucumovirus*, *Begomovirus* and *Tobamovirus* are major concern for many growers because of their effects on yield [7, 62]. The severity of infection depends on environmental conditions, host varieties and individual virus infection (**Figure 1**). Generally, these virus infections cannot be totally eradicated in many plantations where they occurred [10]. However, prompt action against the damage caused by viruses is with the use of resistant varieties and ensuring adequate phyto-sanitary conditions within the field. Therefore, awareness of local farmers on the impact of field hygiene must be improved as adjunct to using tolerant varieties. Additionally, screening of young seedlings for infection before they ever reach the field is crucial to reduce virus occurrence. Ultimately, the development of eco-friendly ways of virus disease management will help to improve yield in the pepper industry.

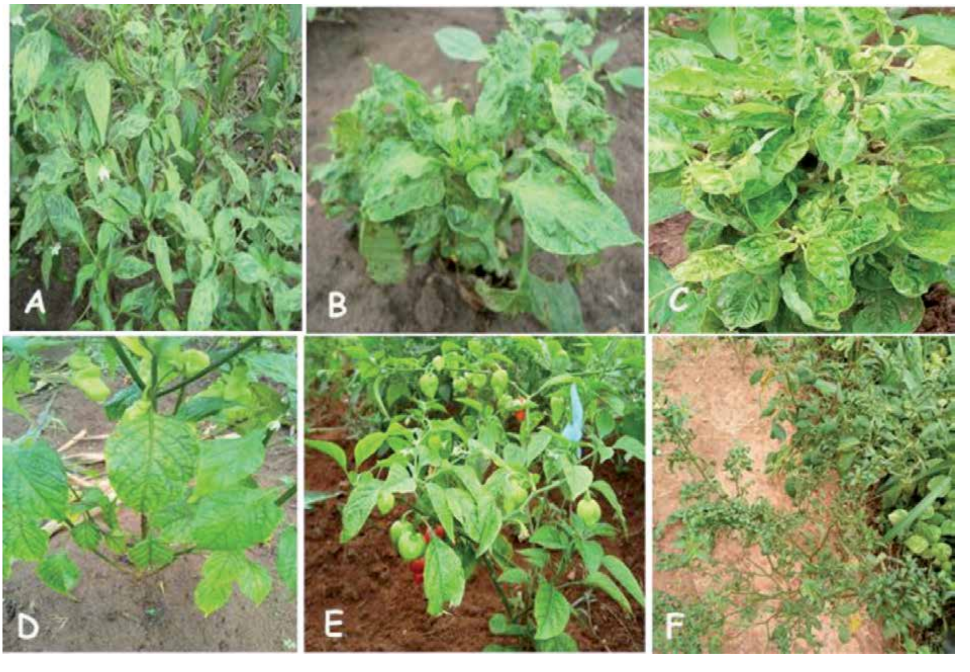

Figure 1.
Possible symptoms that can be observe on pepper in the field. (A) Mosaic pattern on leaves, (B) leaf mottling, (C) leaf curl, (D) vein banding, (E) vein yellowing and (F) leaf reduction.

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Section 3

Bio-active Constituents of *Capsicum*

Chapter 6

Hot Pepper (*Capsicum annuum* L.): An Alternative Food to Reduce Micronutrient Deficiencies in Human

S.R. Krishna Motukuri and Nallamothu Jaswanthi

Abstract

Hot peppers are good source of bioactive compounds particularly phytochemicals, capsaicin, oleoresins, and rich organic micronutrients with potential health benefits. It plays an important role in diets and possesses micronutrients. Micronutrient dietary deficiency remains a massive problem in the world which may cause several chronic health diseases. More than 2 billion individuals are facing micronutrient deficiencies, viz., zinc, iodine, and iron, followed by vitamins. Among various approaches to overcome human nutrition deficiencies, a diet with fruits and vegetables that are rich in micronutrients is considered as the best solution. Hot pepper consumption worldwide is well known for its high nutritional content which in turn reduces human micronutrient deficiencies. Thus sufficient amounts of micronutrients can be provided by incorporating the nutrient-rich pepper in diet that could beneficially help in combating nutrient deficiencies. Thus, in the present chapter, an overview of the micronutrient deficiency is described, and the nutrition status of hot pepper that utilized in eradicating human micronutrient dietary deficiencies is also discussed.

Keywords: *Capsicum*, hot pepper, micronutrients, human dietary deficiency, advanced approaches

1. Introduction

Micronutrients (minerals and vitamins) are obtained from our diet; though required in small amounts, they are necessary for good growth and development [1]. Micronutrients, namely, iron, cobalt, manganese, boron, molybdenum, chlorine, nickel, zinc, copper, iodine, fluorine, and chromium, are essential for human health. Many studies showed that nutrition plays a vital role in providing the substances essential for the formation of the early brain structure and also supporting for its good functioning [2]. Micronutrients play a unique metabolic role as cofactors in the absorption and digestion of carbohydrates, proteins, amino acids, and lipids to release energy [3]. Micronutrients play a very important role in all the metabolic activities like cell signaling, motility, cell division, and cell differentiation and death and, in turn, regulate the tissue development [4].

Micronutrient deficiencies can badly affect the health of individuals, which influence the world's morbidity and mortality [5]. These deficiencies are referred to as "hidden hunger" which affects about 2 billion people [6] and may lead to high rates of illness, obesity, underdevelopment, and even deaths in children of age below 5 years [7]. Among various vegetables and fruits that are rich in micronutrients, chilies have various essential nutrients, minerals, vitamins (A, C, E), and other important phytochemical compounds [8]. They also pose some human health benefits like preventing diseases such as obesity, heart diseases, and different cancers [9]. An alkaloid, namely, capsaicin, is present in pepper which has antimicrobial, anti-inflammatory, and anticancer effects on the digestive system and is used in relieving pain and also to lose weight [10]. Thus providing diet that is rich in pepper can be supportive in an ongoing quest to ease micronutrient deficiencies.

2. Micronutrient deficiency in humans

Some studies reveal the importance of micronutrients in pregnant women and generally given as supplements, which include vitamins, viz., A, cobalamin, pyridoxine, C, D, and E, iron, zinc, iodine, copper, and selenium. Micronutrient deficiencies may mimic radiation or some chemicals which damage the nucleic acids and lead to cancer [11]. Some studies reveal that these deficiencies are related with the danger of HIV infection progression and deaths [12]. Deficiency of vitamin A may cause low serum level of retinol which affects nearly 15% of pregnant women and which leads to night blindness in 8% of them [4]. The reduced form of vitamin C [ascorbic acid (AA)] is the principal chemical structure that appears as an important micronutrient that involves in various physiological functions. Ascorbic acid plays a vital role in reducing the antioxidants and enhances the production of ROS to prevent cancer [13]. Vitamin D is generally known as fat-soluble compound with antiproliferative effect and also involved in the development of bone and immune system [14]. Some studies reported that vitamin D metabolites help in protection against cancer [15].

Vitamin E deficiency can lead to enhanced peroxidation which leads to symptoms like walking difficulties and severe development of speech, reduced fat absorption syndrome, and lipoprotein abnormalities [16]. Deficiencies of iron may affect the growth and mental development and also decreases the capability to do physical work [3]. Some studies reported that the supplementation of zinc along with other micronutrients can reduce the severity of diarrhea diseases and respiratory infections [17]. These deficiencies occur in individuals who do not consume food that provides micronutrients sufficiently like fortified foods, fruits, and animal products. This is usually due to its excessive cost or may domestically unreachable. Among the various vegetables available, chili (*Capsicum annuum*), which are available to common man at affordable price and provide various vitamins like E, C, B6, B12, and provitamin A and some minerals, possibly will give rise to significantly enhanced nutrition [8].

3. Role of hot pepper in micronutrient deficiencies

To enhance the micronutrient status in humans, nutritionists suggest integrating foods, which are rich in micronutrients, in diet. Among various vegetables, chili fruits are rich in capsaicinoids, carotenoids, tocopherols, provitamin A, ascorbic acid, and several antioxidants. Chilies are also an excellent source of xanthophylls and vitamins B1 (thiamine), B3 (niacin), and P (citrin) [18]. Hot pepper contains proteins (1.9 g), sugars (5.3 g), fiber (1.5 g), fat (0.4 g), energy (8.8 g), ascorbic acid (240%), pyridoxine (39%), vitamin A (32%), copper (14%), iron (13%), potassium (7%), and magnesium (6%) [19]. Nutrient constituents and their composition present in chili species in different countries are represented in **Table 1**. Red sweet

So. no	Constituent	Name of the country	Fruit species	Composition	References
1	Vitamin C	United States	*C. Francisca*	122.0 mg/100 g DW	[20]
		Brazil	*C. chinense*	125 mg/100 g DW	[21]
		India	*Capsicum chinense Jacquin*	109.36 mg/100 g DW	[22]
		Ethiopia	*C. annuum*	84.011–89.011 mg/100 g DW	[23]
2	Capsaicin	Brazil	*C. chinense*	14.0 mg/g DW	[24]
		Central America	*C. annuum*	0.042 mg/g DW	[25]
		India	*C. frutescens*	4.45 mg/g DW	[26]
		Ethiopia	*C. annuum (Marako fana)*	5.5 mg/g DW	[27]
3	Crude protein	America	*C. annuum*	2.9 g/100 g DW	[28]
		India	*C. chinense*	17.5 g/100 g DW	[22]
		Ethiopia	*C. annum (Marako fana)*	118.09 g/100 g DW	[29]
		Brazil	*C. chinense*	0.08–4.5 g/100 g DW	[21]
4	Carbohydrates	America	*C. annuum*	3.0 g/100 g DW	[28]
		India	*C. chinense*	78.1 g/100 g DW	[22]
		Brazil	*C. chinense*	1.8–10.8 g/100 g DW	[21]
		Ethiopia	*C. annum (Marako fana)*	35.3 ± 0.6 g/100 g DW	[29]

Table 1.
Nutrient constituents and their composition in chili species in different countries.

pepper consists of twice the provitamin A than in carrot and double the amount of vitamin C of green pepper, and it also acts as antioxidant and anti-inflammatory phytonutrient [30]. Sweet pepper is fibrous in nature and is the sources of dietary fiber which could help in fighting blood cholesterol level and diabetes better from the body and bloodstream, respectively [31].

4. Medicinal uses of hot pepper

Capsaicin is a pungent alkaloid present in hot pepper species, and it is the principal capsaicinoid that accounts for about 71% of the pungent types, followed by dihydrocapsaicin [32]. Structures of capsaicin and dihydrocapsaicin are in **Figure 1**. The percentage of pungency varies among species by varying the capsaicin and dihydrocapsaicin contents which are due to influence of factors like the developmental stages of fruit and the environmental conditions [33]. Capsaicin possesses good medicinal properties like antimicrobial, anticancer, antidiabetic, and analgesic properties. Some studies reveal that fruits having higher amounts

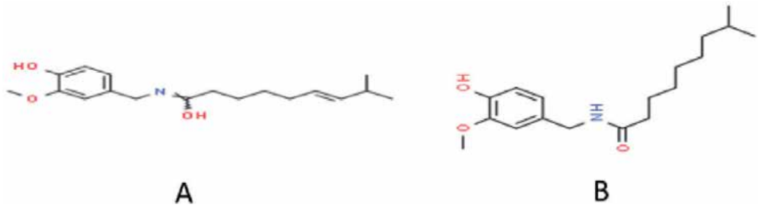

Figure 1.
(A) Capsaicin. (B) Dihydrocapsaicin.

of capsaicin show high antioxidant level [32]. Besides the nutritional benefits, it is also responsible for medical therapeutic and pharmacological uses [34]. Capsaicin has an important role in relieving sore throats, fever, and cold symptoms and also enhances the circulation of blood and strengthens the arteries, thus reducing the risk of heart attacks [3].

Capsaicin is also used as an anti-irritant balm for external use and as painkiller in some topical ointments, nasal sprays, and skin patches and also used in the form of cream for short-term relaxation of muscles and pains related to arthritis, back pain, and other stresses [35]. It also has an antidiabetic activity by enhancing the insulin secretion and releasing bound insulin. Capsaicin helps in producing heat within the body and consumes oxygen which simultaneously burns calories in the body and helps in losing weight [30]. Thus, incorporating pepper-rich food in everyday diet can be useful in enduring pursuit of eliminating micronutrient deficiency. Hot pepper fruits were enriched with antioxidants which protect the human body from harmful effects of free radicals, and it develops resistance against different diseases.

5. Conclusion

Advanced technologies are being used to combat human micronutrient deficiency in developing countries, where poor people are mostly at this risk. In tropical countries, rural farmers traditionally cultivate wide range of vegetables; among them chili is grown globally, and India is the most important producer and exporter of hot pepper which consists of wide range of micro- and macronutrients. In this chapter, it has been concluded that the chilies are cost-effective vegetables that are significantly rich in essential micro- and macronutrients and also possess healthy components to support human health conditions. The presence of antioxidants and phytochemicals in chili increases its importance in controlling the diseases. Integrating hot pepper in our regular diet can be supportive to alleviate nutrient deficiency in humans.

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Conflict of interest

The authors declare that there is no conflict of interest on this book chapter.

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Chapter 7

Capsicum: Chemistry and Medicinal Properties of Indigenous Indian Varieties

Mithun Rudrapal and Khomendra Kumar Sarwa

Abstract

Capsicum pods (or chili pepper) constitute the world's second most consumed vegetable crop and spice after tomato in our daily culinary practice. Five indigenous species that are widely domesticated in various parts of India include *Capsicum annuum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., *Capsicum baccatum* L., and *Capsicum pubescens* L. The chili pods of *C. chinense* Jacq (locally known as *Bhut Jolokia* in the Assam state of India) was officially recognized as the world's hottest *Capsicum* variety according to the Guinness Book of World Records. Capsaicinoids, a group of chemical principles present in matured *capsicum* pods, are responsible for the pungency as well as pharmacological/medicinal properties of capsicum. Some important capsaicinoids include capsaicin, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. Traditional practices of capsicum pods of *Bhut Jolokia* have been well documented for the management of various human disorders/ailments, particularly in the north-eastern region of India. In modern medicine, *Bhut Jolokia* pods have been used in the treatment of arthritis, gastritis, toothache, and musculoskeletal and neuropathic pain including other pharmacological disorders and microbial infections. Capsaicinoids have been reported to exhibit a diverse range of biological effectiveness such as antioxidant, analgesic, and anti-inflammatory, anticarcinogenic effects.

Keywords: *capsicum*, capsaicinoids, capsaicin, *Bhut Jolokia*, *Dhan Jolokia*, indigenous, Indian varieties, pungency, traditional uses, medicinal properties

1. Introduction

Capsicum (also called as Peeper or Chili pepper) is an important vegetable crop widely cultivated in tropical and subtropical regions of the world. This spice crop has been well known since past 9500 years. *Capsicum* pods or chili pepper constitute the world's second most consumed vegetable crop and spice after tomato in our daily culinary practice [1].

India is the largest producer, consumer, and exporter of *Capsicum* in the world. It contributes about 36% to global production of *Capsicum* and exports about 20% of its total production [2]. The production of *Capsicum* in India is dominated by the state of Andhra Pradesh, which contributes 53% of the total production followed by Karnataka (9%), Odisha (6%), West Bengal (6%), Maharashtra (5%), and Madhya Pradesh (4%). *Capsicum* is also cultivated in other parts of India including the north-eastern states [3].

Several indigenous varieties of *Capsicum* are known to be cultivated in the north-eastern region of India. Some of such indigenous varieties have been well documented to be the hottest *Capsicum* varieties in the world [4]. These indigenous varieties are popularly known by different local names among various communities of the north-eastern states of India. These are namely, *Bih Jolokia* (*Bih* means poison, *Jolokia* means pepper), *Bhut Jolokia* (Bhut means ghost) in Assamese language, *Oo-Morok* (*Oo* means Tree, *Morok* means Chili) in Manipuri language, and *Naga Jolokia* (or *Naga Morich*) in Nagamese language and *Raja Chili* (King of Chili). The word *Jolokia* usually refers to the vernacular name of *Capsicum* pod or chili pepper in Assamese language of India [5–7].

2. Indigenous *Capsicum* varieties of India

The plant of *Capsicum* belongs to the genus *Capsicum*, which is a member of the family Solanaceae [8]. The genus *Capsicum* has approximately 27 species, out of which 5 species are widely domesticated in various parts of India. These are namely, *Capsicum annuum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., *Capsicum baccatum* L., and *Capsicum pubescens* L. [9]. The images of indigenous *Capsicum* varieties of India are presented in **Figure 1**.

Capsicum annuum is the most cultivated species across different parts of India. *Capsicum chinense* and *Capsicum frutescens* are also the most common cultivated species, particularly in the north-eastern region of India. Other three species including *Capsicum baccatum* and *Capsicum pubescens* are also found to be cultivated in the north-eastern states of India [10]. **Table 1** describes five indigenous *Capsicum* varieties of India.

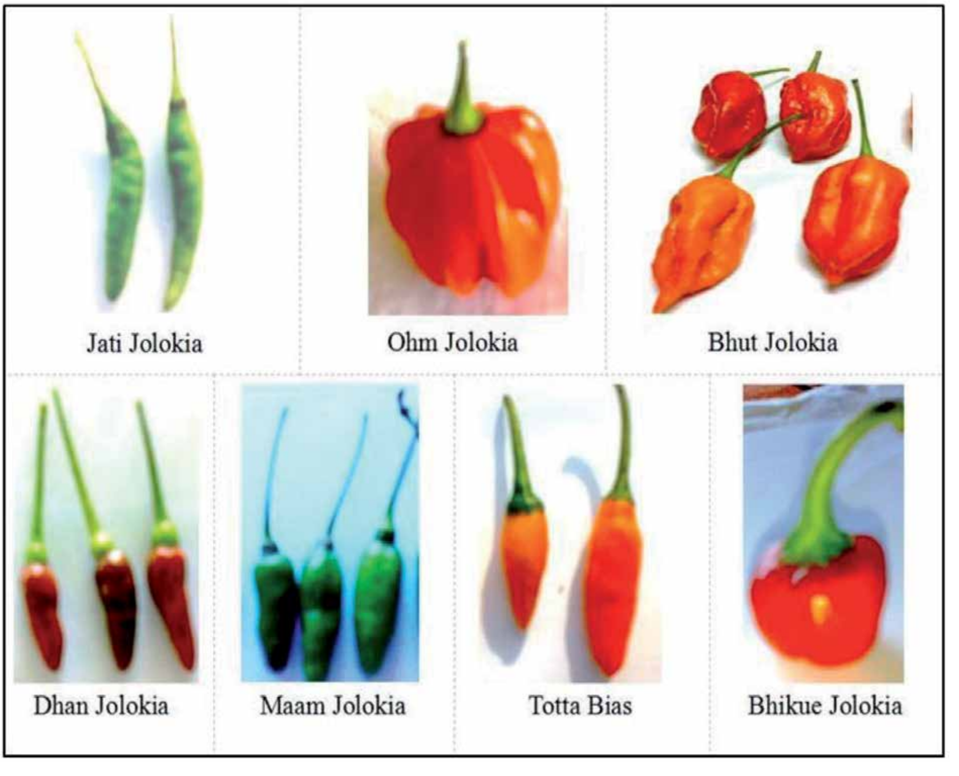

Figure 1.
Photographs showing indigenous Capsicum *varieties of India.*

Species/botanical name	*Capsicum* variety/ vernacular name	Description
Capsicum annum	*Jati Jolokia*	Elongated, 2.5–3.0 cm long, width 0.8–1.0 cm, smooth surface, light red, characteristic odor
Capsicum chinense	*Bhut Jolokia*	Elongated, 5.0–7.0 cm long, 2.53.0 cm width, undulating rough surface, dark red, characteristic aroma
Capsicum frutescens	*Dhan Jolokia*	Tiny in size, elongated, 1.0–1.5 cm long, 0.5 cm wide, smooth surface, light red, characteristic aroma
	Maam Jolokia	Elongated, 1.5–1.75 cm long, width 0.5–0.75 cm, smooth surface, light red, characteristic aroma
	Totta Bias	Elongated, 4.0–5.0 cm long, 1.0 cm wide, smooth surface, orange to light red in color
Capsicum baccatum	*Ohm Jolokia*	Rough shape with a flat base, 2.0–3.0 cm diameter, 2.0–2.5 height, and base divided into three lobes, each having 3–4 grooves, dark red
Capsicum pubescens	*Bhikue Jolokia*	Bell shaped like a tomato with a flat base, 2.0–2.2 cm diameter, 1.5 cm height, smooth surface, dark red, characteristic aroma

Table 1.
Indigenous Capsicum *varieties of India [10, 11].*

3. Chemistry and pungency

The pungency of *Capsicum* pods is mainly attributed to the presence of a group of closely related compounds called capsaicinoids. Capsaicinoids refer to a group of pungent alkaloids that accumulate in the placenta of matured *Capsicum* pods [12]. Chemically, these are acid amides of vanillylamine with C9–C12 branched-chain fatty acid. All capsaicinoids possess a common 3-hydroxy-4-methoxybenzylamide (vanilloid) skeleton, but differ in their hydrophobic alkyl side chain. Differences in the side chain include saturation of the carbon carbon double bond, absence of a methyl group and also changes in the length of hydrocarbon chain [13]. Some important capsaicinoids are capsaicin, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. Capsaicin and dihydrocapsaicin together account for approximately 80% of total capsaicinoids content of *Capsicum* pods. These two compounds are the most important and potent members of capsaicinoids family [14]. The chemical structures of capsaicinoids are displayed in **Figure 2**.

Most of the *Capsicum* species cultivated in India contain around 1% of capsaicinoids, but *Capsicum chinense* L. (*Bhut Jolokia*) and *Capsicum frutescens* (*Dhan Jolokia*) possess around 2–4% of capsaicinoids [15]. However, though seven different indigenous varieties of *Capsicum* are found to be cultivated in the Assam state of India, the *Bhut Jolokia* and *Dhan Jolokia* have been documented to be the most potential *Capsicum* varieties in terms of their capsaicinoids content and level of pungency [16]. The capsaicinoids content (%) of indigenous *Capsicum* varieties of India is depicted in **Table 2**.

It is noteworthy that the capsaicinoids content is a measure of the degree of hotness or the level of pungency of *Capsicum* pods. However, it is also responsible for medicinal properties as well as for the nutritional value of *Capsicum* spice. The Defense Research Laboratory, Tezpur, India reported that the potential of the *Bhut*

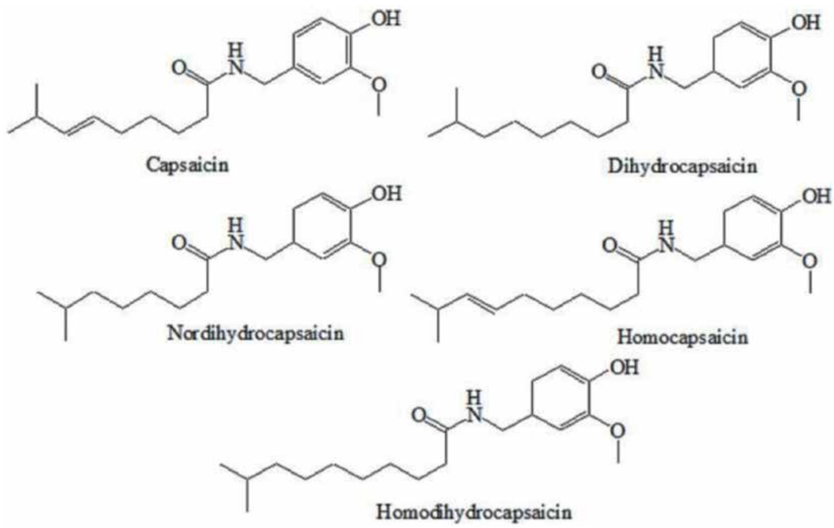

Figure 2.
Chemical structures of capsaicinoids.

Species	*Capsicum* variety	Capsaicinoids content (%)
Capsicum annum	*Jati Jolokia*	0.51
Capsicum chinense	*Bhut Jolokia*	2.45
Capsicum frutescens	*Dhan Jolokia*	2.14
	Maam Jolokia	1.38
	Totta Bias	0.25
Capsicum baccatum	*Ohm Jolokia*	0.67
Capsicum pubescens	*Bhikue Jolokia*	0.92

Table 2.
Capsaicinoids content (%) of indigenous Capsicum *varieties of India [15, 16].*

Jolokia is comparable with that of the Red Savina Habanero of Mexico in terms of Scoville Heat Units (SHUs) [17]. The pungency of *Bhut Jolokia* was recorded 855,000 SHU, while it was 577,000 SHU for Red Savina Habanero. Studies at the New Mexico State University also reported that *Bhut Jolokia* possessed 1,001,304 SHUs, whereas Red Savina had 248,556 SHUs [18]. In September 2006, the *Bhut Jolokia* was officially recognized as the world's hottest *Capsicum* variety measuring over 1,000,000 SHUs according to the Guinness Book of World Records [19].

4. Medicinal properties

Apart from wide commercial applications of *Capsicum* pods or chili pepper as culinary spice and also their use in various food products such as seasoning blends and in the canning industry, *Capsicum* also possess a diverse range of medicinal and/or pharmacological potential. In medicine, capsaicinoids, the active ingredients of *Capsicum* pods, have been used for the treatment of gastritis, arthritis, toothache, musculoskeletal and neuropathic pain, chronic indigestion, other pharmacological disorders and also microbial infections [20–22]. Capsaicinoids have been reported to exhibit a diverse range of biological effectiveness such as antioxidant, analgesic, anti-inflammatory, anticarcinogenic, promotion of energy metabolism, and suppression of fat accumulation [23–26].

Traditional practices of *Bhut Jolokia* have been well documented for the management of various human disorders/ailments, particularly in the north-eastern region of India. It has also been found to be effective in tuning up body muscles and in the treatment of wrist pain after a strenuous physical exercise [5]. The treatment of toothache and muscle pain using the hot infusion of *C. chinense* pods has also been reported in other parts of India [27]. Traditional uses of *C. chinense* leaves in several human aliments such as boil, headache, and night blindness have been well documented, for example, the use of leaf paste in the treatment of boils [28]. The use of *Bhut Jolokia* has also been found effective in the symptomatic relief of asthma because of its bronchodilation effect. Regular consumption of small quantities of *Capsicum* pods of *Bhut Jolokia* is highly beneficial in certain gastrointestinal abnormalities [5, 29].

Researchers have reported that the capsaicinoids content might be responsible for the pharmacological and/or biological potential of *Capsicum* pods. In modern medicine, the capsaicin has been considered to be an effective yet safe topical analgesic as antiarthritic, antioxidant, and anticancer agent. The antiviral efficacy of capsaicin has been reported in the treatment of herpes zoster infection [30]. The Osteoarthritis Research International (ORI) recommended the topical use of capsaicin as an effective adjunctive or alternative medicament to oral analgesic/anti-inflammatory agent for the treatment of moderate to severe pain and inflammation in case where conventional oral analgesic/anti-inflammatory agents generally do not respond [31].

As mentioned above, capsaicin has, therefore, been included in topical therapy for the relief of different neuropathic pain, although it could produce skin irritation. Capsaicin and its analogues have been used in certain topical dosage forms such as creams and patches in order to treat chronic pain syndromes such as post-herpetic neuralgia, musculoskeletal pain, diabetic neuropathy, osteoarthritis, and rheumatoid arthritis [32, 33]. It has also been found beneficial in relieving pain due to rashes, psoriasis, mastectomy, and bladder disorders. Adverse effects (burning, stinging and erythema) are normally limited to the site of application; however, respiratory irritations and occasional systemic effects arising from the inhalation of cream have been reported [34].

5. Mechanism of action

The mechanism of analgesia that capsaicinoids produce is probably due to their counter-intuitive effect [35]. Capsaicinoids applied directly to the skin or injected by intradermal route induce both hyperalgesia and allodynia. Capsaicin causes excitation of certain subsets of dorsal root ganglia giving rise to the stimulation of thin myelinated A-delta and unmyelinated C fibers. These nerve fibers are specific for the transmission of pain signals and therefore seem to be sensitive to vanilloids such as capsaicin, which blocks particularly the transmission of noxious stimuli [36]. Due to the blockade of terminal peripheral nerves, capsaicin inhibits the release of several pro-inflammatory neuropeptides such as Substance P, calcitonin gene-related peptide, and somatostatin [37]. The prolonged stimulation of neurons by capsaicin may deplete the nerve's ability to release the presynaptic neuropeptides, particularly Substance P. The ability of capsaicin to desensitize nociceptors is the main reason behind its therapeutic efficacy [38]. It has also been attributed that capsaicin could modulate the amount of specific neurotransmitter called Substance P, associated with the feeling of pain and thus helps alleviate pain externally [39]. Research findings claim that pure capsaicin could possess similar efficacy in treating joint and muscle pain induced in rheumatism, arthritis, ankylosing spondylitis, and fibromyalgia. Further, clinical reports suggest that capsaicin increases not only the secretion of saliva and gastric juice, but also improves the blood flow in the lining of the gastrointestinal tract, which might be beneficial in gastrointestinal abnormalities [40, 41].

6. Conclusion

Based upon ethnomedicinal potential and scientific evidences of possessing extreme pungency, *Capsicum* chili pod of *Bhut Jolokia* (*Capsicum chinense* Jacq.) is an important economically viable crop of north-eastern region of India. Further, it is believed that favorable geographical and agroclimatic factors are primarily responsible for the higher capsaicinoids content of *Capsicum*. The functional quality depends primarily on the capsaicinoids content, which determines the medicinal potential and/or biological efficacy of *Capsicum* pods. However, *Capsicum* chili pods of *Bhut Jolokia* may be studied for further scientific investigation toward their possible development into topically effective analgesic drugs and/or formulations for the treatment of pain-related disorders and inflammatory illness. In addition to *Bhut Jolokia,* another indigenous variety of *Capsicum*, that is, *Dhan Jolokia* (*Capsicum frutescens)* may also be studied for further scientific investigations.

Conflict of interest

Authors declare that there is no conflict of interest.

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Chapter 8

Anticancer Effect of Capsaicin and Its Analogues

Balasubramanian Arul and Ramalingam Kothai

Abstract

Potent biomolecules from natural products from plants, animals, and minerals are the fundamental basis of the ailment of mankind. *Capsicum* or red pepper plants were grouped under the kingdom Plantae and family Solanaceae. It is used widely throughout the world in foods for their pungent flavor and aroma, and to prolong food spoilage. This chapter presents a frame of a concise compilation of the anticancer and cytotoxic potentials of *Capsicum*, its analogs, and related compounds. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the most predominant and naturally occurring alkaloid from the *Capsicum* species. It also details the anticancer efficacy of capsaicin and its analogs like capsaicinoids and capsiates which possess antioxidants and targets multiple signaling pathways, ontogenesis, and tumor-suppressor genes in various types of cancer models. Capsaicin is a major ingredient and has been linked to suppression of growth in various cancer cells. The data available strongly indicate the significant anticancer benefits of capsaicin and its potent analog molecules. It shows a significant effect on cancer cell proliferation, apoptosis, cancer cell surveillance, growth arrest, and metastasis. This chapter also predominantly focuses on the combinational use of capsaicin with other natural dietary compounds as a measure of synergistic anticancer activities.

Keywords: *Capsicum*, capsaicin, apoptosis, angiogenesis, metastasis, anticancer

1. Introduction

Capsicum is one of the most important genera in the Solanaceae family and consists of almost 30 species [1]. Their fruits were commonly referred to as peppers or chili peppers, having a bell shape that appears in different colors such as red, green, orange, and yellow. These have been commonly used in their diet by human society from ancient times as herb and spice. *Capsicum* is widely used as an essential ingredient in kitchens worldwide for their pungent flavor and scent and to prevent food contamination. It is known under many names, such as red pepper, chili pepper, paprika, tabasco, aji, cayenne, and tabasco jalapeno, because of its global consumption [2]. The genus *Capsicum* is comprised of various numbers of wild and few domestic species. The species domesticated are *Capsicum annum, C. baccalaureus, C. honey, C. frutescens, C. pudders,* in which *C. chinense* is the fruit with the highest pungency [3]. *Capsicum* has been documented for its wide range of activities, such as pain killers, cardiovascular protection, a cancer chemopreventive, and antioxidant effects. This chapter provides a comprehensive description of the capsaicin and its analogs as well as, more specifically, look into its therapeutic potential in various human cancer and future therapeutic directions of capsaicin.

2. Capsaicinoids

The chili-fruit pungent principles are called capsaicin. Capsaicin and many related compounds are called capsaicinoids. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is a crystalline, lipophilic, colorless, and odorless alkaloid that is soluble in fat, alcohol, and oil. The major capsaicinoids or the analogs from *Capsicum annum* species are Capsaicin and 6,7-dihydrocapsaicin. The smaller capsaicinoids are nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin. Capsaicin and their analogs differ only in acyl group saturation [4, 5]. These alkaloids are produced solely by the genus *Capsicum*, and they are used as a potent analgesic for the treatment of pain and inflammation related to a number of diseases [6, 7].

Although capsaicin traditionally is associated with the analgesic activity, its unpleasant side effects, such as gastric irritation, stomach cramps, and burning sensation, limit capsaicin's applications as a clinically viable drug. This has led to extensive research focusing on the discovery and rational design of capsaicin analogs of the second generation, which have greater bioactivity than capsaicin. Modifications in the acid portion of capsaicin were found to generate analogs with different degrees of pungency. Three capsaicin analogs of two pungent and one with very low pungency were obtained by using multiple lengths of the acyl chain and chemical replacements in the aromatic ring [8]. The chemical structure of capsaicin and its analogs [9, 10] were shown in **Figure 1**.

Capsaicin (**Figure 1(1)**) and its analogs such as capsanthin (**Figure 1(2)**), capsanthin 3′-ester (**Figure 1(3)**), capsanthin 3′,3-diester (**Figure 1(4)**), capsorubin (**Figure 1(5)**), capsorubin 3′-ester (**Figure 1(6)**), and capsorubin—3,6-epoxide (**Figure 1(7)**) containing pharmaceutical products have been marketed under the trade name such as Menthacin, Zostrix, and Capzasin-P for topical applications. In 2009, the European Union (EU) and the USA Food and Drug Administration (FDA)

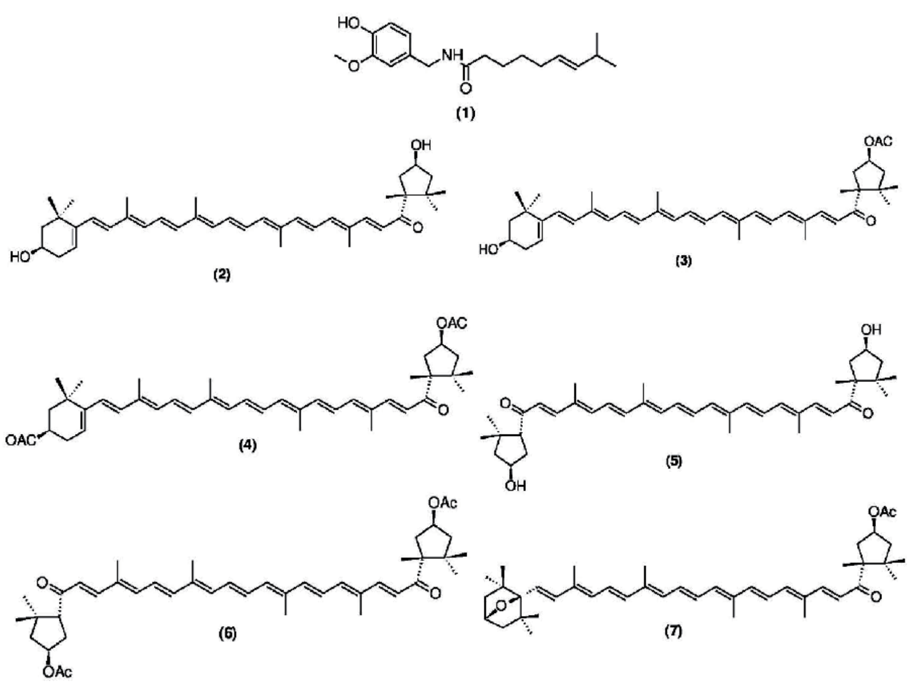

Figure 1.
Capsaicin and its analogs: (1) capsaicin; (2) capsanthin; (3) capsanthin 3′-ester; (4) capsanthin 3′,3-diester; (5) capsorubin; (6) capsorubin 3′-ester; (7) capsorubin—3,6-epoxide.

approved the use of capsaicin 8% patch (Qutenza or NGX-4010) for the treatment of post-herpetic neuralgia (PHN). Resiniferatoxin (RTX) is regarded as an ultra-potent analog of capsaicin that acts as an agonist of Transient receptor potential cation channel subfamily V member 1 (TRPV1) and displays several thousand-fold more potencies than capsaicin [11]. Zucapsaicin, the cis-isomer of capsaicin, shows potent efficacy against episodic cluster migraine prophylaxis, episodic cluster headache, and alleviates neuropathic pain [12]. Thus, capsaicin and its analogs have been used medicinally for centuries, but recently, it has been studied extensively for its analgesic, antioxidant, anti-inflammatory, anti-obesity characteristics and currently for its anticancer activity against a number of types of cancer [13]. It was witnessed by unparalleled advances in the field of capsaicin research. These studies and several other reports clearly showed that capsaicin and its analogs possess multiple pharmacological effects and its application in different clinical conditions.

3. Anticancer activity of capsaicin and its analogs

There is a strong epidemiological and experimental evidence that the phytochemical diet found in fruits, vegetables, whole grains, spices, and teas provides various inhibitory effects against the initiation, development, progression, and metastasis of cancer [14]. Capsaicin, a bioactive phytochemical abundant in chili peppers, is in between them. Capsaicin is a derivative of homovanillic acid, which has been shown to modify the function of many genes associated with cancer cell life span, growth arrest, angiogenesis, and metastasis [15, 16].

Tumorigenesis is a multistage process, which usually begins over an extended period. Cancer cells develop special properties not acquired by most healthy cells. Multiple genetic alterations and aberrant signaling pathways initiate and advance cancer. Determining the molecular targets involved in the tumor development process will provide opportunities to develop a successful cancer-fighting strategy. Studies assessing the capsaicin effect to inhibit cell proliferation by mechanisms are not fully understood in many types of cancer cells [17]. The capsaicin's suggested anticancer pathways include increased cell cycle arrest and apoptosis.

3.1 Capsaicin and apoptosis

Apoptosis is a vital mechanism against the growth of cancer, and it is strongly correlated with the loss of apoptotic signals in malignancy. It has been shown that capsaicin induces apoptosis in many different types of cancer cell lines, including pancreas, colon, prostate, liver, esophagus, bladder, skin, leukemia, lung, and endothelial cells, keeping the normal cells unharmed. A recent review noted capsaicin appears to induce apoptosis in more than 40 distinct lines of cancer cells [13, 18].

Two major signaling systems are the intrinsic mitochondrial death pathway and the extrinsic death receptor pathway, which activate executioner/effector caspases and lead to apoptosis. In specific, the mitochondrial pathway is involved in the complete execution of apoptosis; thus, the mitochondrion has been named as apoptotic mechanism's gatekeeper, and the mitochondrial death pathway proteins and pathways had become important targets for new treatments [19]. Many proteins involved in the mitochondrial death pathway have been targeted by capsaicin to induce apoptosis in different cancer cell lines. For instance, capsaicin treatment activated the cluster of differentiation 95 (CD95)-mediated apoptotic intrinsic and extrinsic pathways [20] and suppressed antiapoptotic protein expression, B-cell lymphoma 2, which causes caspase-9 and -3 activation, loss of mitochondrial membrane potential, and subsequent rises in cytochrome c release [21].

Capsaicin's proapoptotic activity has been found to be mediated via transient vanilloid potential receptor (TRPV1) in many types of cancers [21–23]. It is a nonselective cation channel pertaining to the transient receptor potential channel (TRP) family [24]. It does prefer Ca^{2+} to Na^{+}. Thus it contributes to changes in the concentration of cytosolic free Ca^{2+} and it is capsaicin's primary cell target.

3.2 Reactive oxygen species

Earlier research in pancreatic cells showed that the apoptosis effects of capsaicin were correlated with reactive oxygen species (ROS) production, c-Jun N-terminal Kinase (JNK) activation, mitochondrial depolarization, cytochrome c release in the cytosol, and caspase-3 cascade activation [25]. There is also quite a complex relationship that exists between capsaicin exposure and ROS production. ROS is conventionally considered cytotoxic and mutagenic in normal cells and can induce cell death, apoptosis, and senescence at high levels [26].

Capsaicin has been suggested to induce apoptosis in cancerous cells through the production of higher rates of intracellular ROS. This observation reveals capsaicin as the primary signaling molecule [27, 28]. Capsaicin is capable of activating apoptosis through nonreceptor mechanisms [25].

A number of studies in recent years have shown that oxidative stress causes cellular apoptosis through both mitochondria-dependent and mitochondria-independent pathways [29].

Mitochondria account for 50% of the total cytoplasmic volume in most cells and they participate more than any other organelle in metabolic functions, particularly those involved in cellular energy production. It also consumes nearly 90% of cellular oxygen and is the main source of ROS produced during breathing, and it is engaged in maintaining the intracellular redox state. Along with their long-standing role in energetics, mitochondria depict its prime focus in mammalian cells for many cell death signals. Interactions at the mitochondrion eventually determine whether a cell survives or dies in reaction to many physiological or therapeutic stimuli of cell death. A number of proteins involved in the mitochondrial death pathway were shown to be targeted by capsaicin in order to initiate apoptosis in various cancer cell lines.

It was reported that a 12-h exposure to high concentrations of capsaicin was necessary to induce higher apoptosis rates in Cellosaurus Colo 16 (COLO 16) cells [26]. More than half experienced apoptosis, which was correlated with progressive irreversible dispersion of mitochondrial transmembrane potential and elevated superoxide levels, illustrates the destruction of mitochondria and subsequent breakdown of mitochondrial electron transfer.

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a part of the complex I of the mitochondrial electron transport chain. Capsaicin has been shown to specifically inhibit mitochondrial NADPH oxidase activity by competitively binding this enzyme to the ubiquinone/coenzyme Q site. Therefore, if capsaicin blocks the transportation of electrons in mitochondria, irreversible dispersion of mitochondrial transmembrane potential will occur. This will initiate apoptosis and change the mitochondrial permeability to release cytochrome c and eventual activation of proapoptotic pathways.

Capsaicin can block NAPDH oxidase in the plasma membrane by acting as a Q antagonist coenzyme. Capsaicin's vanillyl moiety is structurally similar to coenzyme Q's cyclic part, which could address the fact that vanilloids serve as antagonists to the coenzyme Q. Suppression of plasma membrane NAPDH oxidase was correlated with capsaicin's prooxidant and proapoptotic properties in some transformed cells, and activated T cells [30].

Another mechanism proposed by capsaicin's anticancer activity is interaction with AMP-dependent protein kinase (AMPK), which is the cell's primary metabolic gatekeeper, belonging to the family of protein kinase stimulated during enzyme-depleting metabolic states like hypoxemia, thermal shock, oxidative stress, and physical activity. It acts as a significant metabolic transition for maintaining energy homeostasis and shown to be an intrinsic controller of the mammalian cell cycle [31, 32].

There is an increase in intracellular Adenosine diphosphate (ADP)/Adenosine triphosphate (ATP) and/or Adenosine monophosphate (AMP)/ATP ratios [33] during energetic imbalance and it promotes activation of AMPK. Activation of AMPK increases oxidative stress in many human cancer cells and induces apoptosis. This stimulates catabolic pathways and, at the same time, inhibits the rate of anabolic reactions to regain the correct energy charge for adenylates.

Capsaicin treatment of human colorectal adenocarcinoma (HT29) cell line is shown to cause AMPK activation and inhibition of acetyl-CoA carboxylase (ACC), a well-known AMPK substrate, which indicates capsaicin inhibits lipid biosynthesis. The above concepts were implicated in apoptosis caused by capsaicin.

3.3 Capsaicin and cell cycle

The cycle of cells is a series of stages that cells undergo to allow them to divide and produce new cells. The cell cycle is divided into phases G0/G1, S, and G2/M stages. The cyclins, cyclin-dependent kinases (CDKs), and the CDK inhibitors are essential parts of the cell cycle. There are DNA checkpoints to assure DNA replication integrity. Such checkpoints and repair pathways render cellular responses to damage to DNA easier [14]. When stimulated, the CDKs provide the cells with a driving force to pass from one stage to the next, but if cyclin and/or CDKs are impaired, cell cycle arrest [17, 33, 34] happens. Thus, any alteration in these pathways raises the cancer risk. It was reported that capsaicin inhibits CDK2, CDK4, and CDK6 inhibiting the proliferation of 5637 bladder carcinoma cells via cycle arrest [34].

3.4 Capsaicin and p53

The p53 tumor suppressor prevents cell proliferation by inducing cell cycle arrest and apoptosis in response to cell stress such as damage to DNA, hypoxia, and activation of oncogenes. Phosphorylation is critical for p53-dependent transactivation at the Ser-15 residue [17]. P53 promotes apoptosis by a linear pathway involving Bax transactivation, cytosol-to-membrane Bax translocation, mitochondrial cytochrome c release, and caspase-9 activation, followed by caspase-3, -6, and -7 activation. Research studies indicate p53 is a target of capsaicin's anticancer action. Capsaicin was found to induce p53 phosphorylation of residue Ser-15 and enhanced p53 acetylation by sirtuin 1 downregulation, which is responsible for the initiation of apoptosis [35].

Incubated adenocarcinoma gastric cell line (AGS) of human gastric cancer with different capsaicin concentrations in the presence and absence of p53 siRNA showed that capsaicin induces apoptosis via p53 upregulation in AGS cells and that the apoptotic effect of capsaicin is p53-dependent [36]. It also observed that the tendency of capsaicin to induce the expression of proapoptotic proteins, such as Bax, caspase-3, and caspase-8, was almost entirely diminished by hitting down p53. Effects of capsaicin on the same cell type reported that caspase-3 activity increased with capsaicin exposure, indicating that capsaicin may serve as an anti-tumorigenic agent in human gastric cancer [37].

3.5 Capsaicin and β-catenin

β-catenin is a beneficial 90 kD protein that leads to cell growth under normal physiological conditions. It is a key transcription factor in the signaling of Wingless-Int (Wnt) and plays a vital role in stem cell regeneration and organ regrowth. Abnormal expression of β-catenin causes the malignant conversion of normal cells, and its anomalous activity has been documented in several cancer types. β-catenin is essentially active in many types of cancer cells. In a recent study, it was reported that capsaicin downregulated β-catenin transcription, decreased its protein stability, and caused apoptosis of colorectal cancer cells [38].

3.6 Capsaicin and angiogenesis

Angiogenesis is the development of new blood vessels from preexisting vasculature as well as an important homeostatic process for normal wound healing and embryonic growth. It involves the stimulation of endothelial cells, cell proliferation, invasion, chemotactic migration, and differentiation into new blood vessels [39]. Angiogenesis is a central player in cancer cell growth and tumor metastasis. The development of an angiogenic phenotype is regarded as a vital step in the progression of tumors [40, 41]. The cancer cells grew to 1–2 mm^3 in thickness in absence of circulation and stopped, but in the angiogenic area, they expanded beyond 2 mm^3. Tumors can become necrotic or even apoptotic in the absence of vascular support [42]. Thus, angiogenesis is a significant factor in cancer progression.

Some factors such as growth factors, cytokines, and vascular endothelial growth factor (VGEF) regulate angiogenesis. Nevertheless, VEGF plays a significant role in angiogenesis. In addition, endothelial cells produce growth factors that induce autocrine and paracrine growth of tumors. The initiation of angiogenesis correlates with the increased entry into the circulation of neoplastic cells and thus promotes metastasis [43]. Treatment of endothelial cells with capsaicin blocked the sprouting and development of VEGF-induced vessels in Matrigel mouse assay that was correlated with downregulation of p38 mitogen activated protein kinases (MAPK), protein kinase B (PKB), and focal adhesion kinase (FAK) activation [44, 45].

Furthermore, capsaicin enhanced deterioration of hypoxia-inducible factor1α, which is a crucial transcription factor rising VEGF transcription. Capsaicin thus interferes with typical angiogenic signaling pathways and can have the ability to suppress cancer becoming a malignant one. It was reported that capsaicin's anti-angiogenic activity was associated with reduced cyclin D1 expression, which results in decreased Rb phosphorylation, leading to the GI arrest of human endothelial cells of the umbilical cord (HUVEC) [46]. In addition, capsaicin also inhibited focal adhesion kinase (FAK) and p38 kinase activation caused by VEGF. All of the above results indicate that capsaicin is a dietary anti-angiogenic agent.

3.7 Capsaicin and metastasis

Cancer metastasis is the hallmark of tumor malignancy, which starts with the spread of cancer cells from the principal tumor to nearby tissues and distant organs, and it is the main cause of cancer morbidity and mortality. Metastatic cancer is resistant to treatment and contributes 80% of cancer-related deaths, which remains a great problem for cancer therapy [47]. Invasion and movement of tumor cells include the proteolytic degradation of extracellular matrix components by tumor cell-secreted proteases, involving serine proteases, plasminogen activators, and matrix metalloproteinases (MMPs).

Cancer type	Cell line	Effective doses (μM)	Anticancer mechanism
Human colorectal cancer	HCT 116	100–200	Induced autophagy
	LoVo, SW480	100	Induced anti-tumorigenesis; deregulation of β-catenin/TCF-dependent signaling
	Colo 205	150	Induced cell death, increased ROS, and proapoptotic proteins
Human breast cancer	MCF-7	50–300	Induced autophagy, inhibited growth, and induced apoptosis
	T47D, BT-474, SKBR-3	200	Inhibited growth and increased apoptosis
	MDA-MB231	20–200	Induced apoptosis and dysfunctions in mitochondria. Antiproliferative activity and arrest of cell cycle into G2/M phase. Enhances the apoptotic effects of TRIAL by activating the calcium-CaMKII-Sp1 pathway
Human prostate cancer	LNCaP	40–50	Inhibited proliferation and induced apoptosis
	PC-3	20–50	
	DU-145	500	
	RWPE-1	40	
Human myeloid leukemia	HL-60	>50	Induced G0/G1 phase cell cycle arrest and apoptosis
	U937, THP-1	200	Enhances the apoptotic effects of TRIAL by activating the calcium-CaMKII-Sp1 pathway
Human esophageal epidermoid carcinoma	CE 81 T/VGH	100	Induced apoptosis and G0/G1 phase cell cycle arrest
Human melanoma	A375	100	Inhibited cell growth and promoted apoptosis
Human KB cancer cells	KB cells	150–200	Reduced cell proliferation and viability. Induced cell death and cell cycle arrest in G2/M phase
Mouse melanoma	B16-F10	50	Inhibited cell migration. Induced apoptosis
Human pancreatic cancer	AsPC-1, BxPC-3	150	Inhibited proliferation. Induced apoptosis and generated ROS
	PANC-1	200	Induced G0/G1 phase cell cycle arrest and apoptosis; and inhibited growth
Human multiple myeloma	U266, MM.1S	>5	Inhibited cell proliferation, caused accumulation of cells in G1 phase
Human hepatoma	Hep G2	10–200	Decreased cell viability, generated ROS and activated caspase-3; and induced apoptosis and autophagy
	Hep3B	200	Enhances the apoptotic effects of TRIAL by activating the calcium-CaMKII-Sp1 pathway
Human nasopharyngeal carcinoma	NPC-TW 039	200–400	Induced G0/G1 phase arrest and apoptosis. Increased ROS
Human gastric carcinoma	SMC-1	200	Induced apoptosis
Human bladder cancer	T24	100	Induced ROS production and mitochondrial membrane depolarization

Cancer type	Cell line	Effective doses (μM)	Anticancer mechanism
Human small cell lung cancer	NCI-H69, NCI-H82, DMS53, DMS114	50	Suppressed growth in all four cell lines

TCF: T-cell factor; ROS: reactive oxygen species; TRIAL: TNF-related apoptosis-inducing ligand; CaMKII-Sp1: calcium-calmodulin-dependent kinase II signaling pathway I.

Table 1.
Anticancer potential of capsaicin against various cancer cell lines.

Capsaicin revealed its anti-invasive and anti-migratory activity by modulating signaling pathways including cell invasion and migration and suppressing advanced cancer stages. Treatment with capsaicin significantly reduced the metastatic burden in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice. It has been reported that it significantly inhibited the migration of melanoma cells without leading to apparent cellular cytotoxicity [48]. This effect was associated with the downregulation of the signaling cascade of phosphoinositide 3-kinase (PI3 K) and reduction of the RAS-related c3 botulinum toxin substrate 1 (RAC1), which in itself is the main kinase controlling motility and migration of cells. Capsaicin blocked the invasion and migration of human fibrosarcoma cells triggered by epidermal growth factor (EGF) by downregulation of AKT/FAK, extracellular signal-regulated kinases, and p38 MAPK signaling, and subsequent downregulation of matrix metallopeptidase 9 (MMP9) in invasive fibrosarcoma cells.

Thereby, capsaicin recruits several mechanisms of signaling for controlling migration and invasion. These include epithelial-mesenchymal transition (EMT) activation, AMP-activated protein kinase (AMPK), MMP signaling pathway, intracellular calcium elevation, VEGF, Wnt-Hedgehog control, tumor-associated NADH oxidase (tNOX), protein kinase B (Akt), MMPs, epidermal growth factor receptor (EGFR), extracellular signal-regulated kinase (ERK), p38 MAP kinase, RAC1, nuclear factor “kappa-light-chain-enhancer” of activated B-cells (NF-kB), and AP-1 [49–54]. **Table 1** depicts the anticancer potential of capsaicin against various cancer cell lines.

4. Synergistic anticancer activity of capsaicin with other compounds

Recent development has allowed synergistic drugs to treat a wide array of cancers. Combinations of low doses of chemopreventive agents have progressively been used clinically in the treatment of various cancers in the present days. Compared to the traditional single drug policy, the combination strategy increases therapeutic effects or reduces drug resistance [55]. Computational progress in qualitatively testing and predicting synergistic components has been experienced in the last few years [56–58]. Multiple studies have illustrated that novel combination therapies with different phytochemicals and chemopreventive drugs can elicit increased antitumor activity through additive or synergistic action [59, 60].

The pathways can include parallel action on cancer suppression by modifying carcinogen detoxification and metabolism of hormones, scavenging oxidative stress, and enhancing immunity [61–63]. Hundreds of phytochemicals have various physicochemical and kinetic properties that target different signaling pathways and transcription factors that decide the phenotype of cancer. The use of several bioactive compounds with distinct anticancer pathways could, therefore, be a promising method for successful cancer treatment and prevention.

Capsaicin and other compounds are known to have synergistic anticancer properties. Combined with resveratrol, capsaicin facilitated apoptosis by elevating nitric oxide (NO) via a p53-dependent manner [64]. It has synergistic anticancer activity with pirarubicin, an anthracycline drug, by activating TRPV1 in bladder cancer [65]. Capsaicin shows a synergistic effect with the dietary phytoestrogen, genistein by regulation of AMPK and cyclooxygenase 2 in breast cancer cells [66]. Capsaicin and 3,3′-diindolylmethane, a key in vivo metabolite of indole-3-carbinol, abundantly present in cruciferous vegetables, have recently been reported to work synergistically to induce apoptosis in colorectal cancer, by altering the transcriptional function of the nuclear factor kappa B, p53, and control apoptosis-related genes [67]. Capsaicin, and brassinin, a form of indole derived from cruciferous vegetables, showed synergistic anticancer activity by suppressing MMP2 and -9 expression and enzymatic activities, and invasion, and migration of prostate carcinoma cells [68]. Sometimes, capsaicin also interacts with chemotherapy drugs. For example, in some myeloid leukemia cells [69], capsaicin enhanced the therapeutic efficacy with 12-O-Tetradecanoylphorbol-13-acetate. Capsaicin can, surprisingly, affect the viability of cancer stem cells. Capsaicin has been found to cause cancer stem cell death by inhibiting the NOTCH signaling pathway in stem cells of breast cancer [70].

5. Conclusion

An emerging cancer research area is the search for suitable molecular targets and effective anticancer compounds that modify the cancer targets. Capsaicin exhibits significant anticancer activity in various tumor stages by targeting multiple signaling pathways and cancer-associated genes. As a whole, capsaicin's anticancer pathways involve apoptosis initiation, cell growth arrest, and suppression of angiogenesis and metastasis. Capsaicin and its analogs activate the tumor-suppressive signaling pathway and associated transcription factors while inhibiting oncogenic signaling pathways and cancer cell promoters. In addition, capsaicin acts synergistically with other anticancer agents, allowing for the potential use of capsaicin with other chemotherapeutic agents in cancer therapy, and shows double advantage, i.e., capsaicinoids enhances the chemotherapeutic effect and pain relief for cancer patients. More research on capsaicin's and its analogs as anticancer targets holds the potential for future treatments and requires more study to improve our understanding of its efficacy in cancer treatment and prevention.

Conflict of interest

The authors have none to declare.

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Chapter 9

Capsicum Seeds as a Source of Bioactive Compounds: Biological Properties, Extraction Systems, and Industrial Application

Javier Echave, Antía G. Pereira, María Carpena, Miguel Ángel Prieto and Jesús Simal-Gandara

Abstract

Recent research has substantially focused on residual subproducts containing chemical compounds with bioactive properties. Even though there are some culinary or medicinal uses of *Capsicum* seeds, there is still a seed mass waste from pepper processing. Many pepper leading producer countries generally lack the facilities and infrastructure required for such processing technologies and so, pepper seeds are usually either destroyed or employed as landfilling or as animal feed. This involves an inadvertent economic loss for producers as well as a detrimental environmental impact. However, there is a hidden potential within the pepper processing industry related to valorization of pepper seeds to obtain added value by-products and thus reduce generated waste. Pepper seeds are a good source of antioxidants, carotenoids, phenolic acids, flavonoids, and vitamins C, E, and A and are also rich in volatile compounds, among others. The unique alkaloids of this genus are capsaicinoids and capsainoids, which have been linked to many beneficial biochemical and pharmacological effects including anti-oxidative or anti-inflammatory activities. Other prominent bioactive compounds of peppers seeds include saponins, lectins, and polyunsaturated fatty acids. In this context, an overview of the biological properties, extraction systems, and possible industrial application of bioactive compounds of pepper whole fruit and seeds is presented.

Keywords: *Capsicum* spp., seeds, extraction, bioactive, activity

1. Introduction

1.1 Characteristics of the genre

The genus *Capsicum* belongs to the Solanaceae family, which consist of different variants of peppers that can be easily recognized by their size, shape, color and degree of pungency. This latter characteristic allows the classification of chili peppers depending on their Scoville heat units (SHU), a measurement of their pungency, into spicy or hot foods (generally those with small fruits) and sweet or non-pungent (generally those with large fruits). This aspect is very interesting for marketing them and makes them a demanded product [1]. Considering this, we can consider for example the pungency of paprika peppers (10–30 parts per million capsaicinoids), chili peppers (30–600 parts per million) and red peppers (600–13,000 parts per million) [2]. Ripe fruits display a range of colors varying from white to deep red. Likewise, the intensity of red color and the degree of pungency are valued as major quality parameters. That is why the fruit is harvested when its red color has completely developed in order to ensure the highest quality of these features [3].

Capsicum is a very homogeneous genus with 33 species and 10 varieties (**Table 1**), with only five of them being domesticated: *Capsicum baccatum* L., *Capsicum pubescens* Ruiz & Pav., *Capsicum frutescens* L., *Capsicum chinense* Jacq. and *Capsicum. annuum* L. (**Figure 1**) native to Central and South America [5]. Each one of them was farmed independently in Pre-Columbians times in diverse regions of the American tropics [4]. They have been genetically modified to obtain varieties with agronomic interests, as in the case of *C. annuum* L. in which varieties more resistant to adverse factors were created, with a higher content and/or yield of the compounds of interest and fruit quality [6].

Amid the species *C. annuum*, there are different well know varieties like paprika, cayenne, jalapeños or chiltepin. Inside the specie *C. frutescens* are tabasco chilis; among *C. chinense*, the hottest chills (naga, habanero and Scotch bonnet) and among *C. pubescens* and *C. baccatum*, peppers emblematic from South America like rocoto or aji [7].

Although the characteristics of each species differ from each other (**Table 2**), it can be said that the genus *Capsicum* is characterized by being an annual or perennial herb or undershrub with entire or repand leaves that alternate. Its flowers are pedicelled, enclosing five petals; it is axillary and can appear solitary or in groups of two or three. Its sepals connate in a subentire or minutely five-toothed calyx that is much shorter than the fruit. It also has 5 stamens, which are adnate nearly to base of corolla-tube and are characterized for being short filaments. Its carpels connate in a two-celled (in some cases three) ovary, and its anthers are dehiscence longitudinal

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	*Capsicum*
Species	*C. baccatum*, *C. pubescens*, *C. frutescens*, *C. chinense*, *C. annuum*
Varieties	*C. annuum* var. *annuum*, *C. annuum* var. *glabriusculum*, *C. baccatum* var. *pendulum*

Table 1.
Taxonomic classification of the genus Capsicum *[1, 4].*

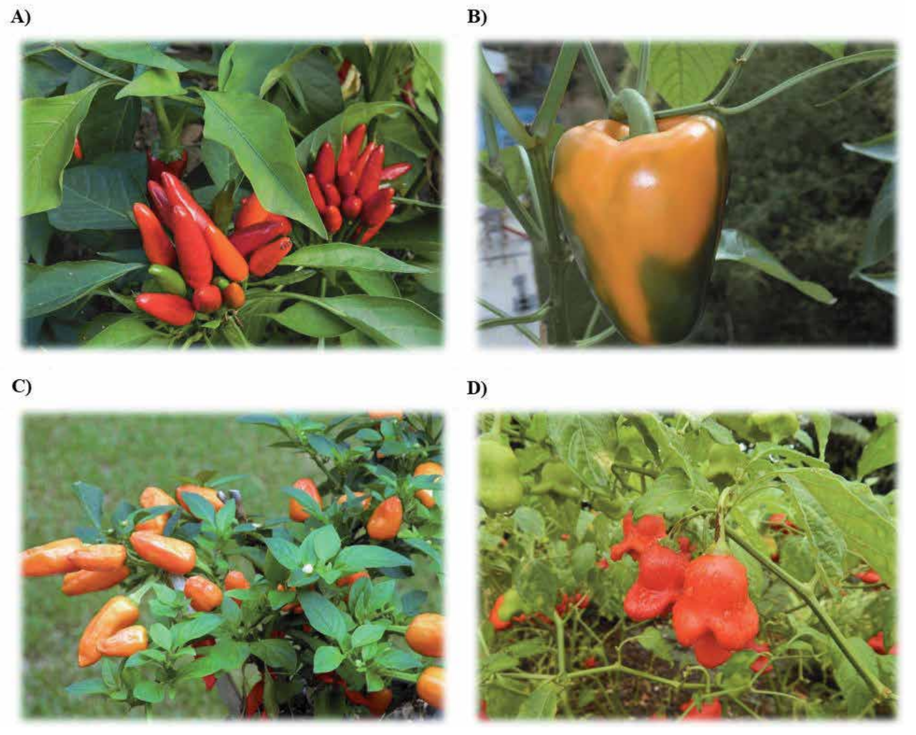

Figure 1.
Some of the major cultivated pepper species. (A) Capsicum frutescens*; (B)* Capsicum annuum*; (C)* Capsicum chinense*, and (D)* Capsicum baccatum*.*

Species	Flower color	N° flower	Seed color	Calyx constriction	Distribution
C. annuum	White	1	Tan	Absent	Colombia, USA
C. frutescens	Green	2–5	Tan	Absent	No studies
C. chinese	White-green	2–5	Tan	Present	South America
C. baccatum	White with yellow spot	1–2	Tan	Absent	South America
C. pubescens	Purple	1–2	Black	Absent	South America

Table 2.
Characteristics that allow to distinguish between the most common species of the Capsicum *genus [8].*

without any exceeding filaments. As for fruit and seeds, the first is irregularly shaped (globose or elongate) with many seeded berries. Seeds are discoid, smooth or subscabrous [8].

Thus, its physical appearance defers from one species to another. For example, *Capsicum annuum* is an annual cultivate that reaches a height of 1 m and has glabrous or pubescent lanceolate leaves, white flowers, and fruit of varying length, color, and pungency depending upon the cultivar and growth conditions. It also the most widely cultivated pepper species around the world. Considering another species, *Capsicum frutescens* is a short-lived perennial with woody stems that reach a height of 2 m, with glabrous or pubescent leaves, with two or more greenish-white flowers per node, and extremely pungent fruit [2].

Being a cold sensitive plant, the best conditions for production are between 7 and 29°C and an annual precipitation of 0.3–4.6 mm. It grows best in well-drained, sandy or silt-loam soil and a soil pH of 4.3–8.7. Hot and dry weather is also desirable for fruit ripening [2]. To carry out its cultivation it is necessary to seed or transplant the peppers, harvesting 3 months after planting [2].

1.2 *Capsicum* seeds sources and production

Chili (a variety of *C. annuum*) is one of the first plants cultivated in Mesoamerica, existing evidence of its use for the last 9000 years. It can be used in multiple ways: fresh, dry, as a spice powder, natural dye, antioxidant, bactericide and fungicide, as a drug in the pharmaceutical industry, in the cosmetology industry or in food industry (sausage, canned meats) [9]. These characteristics made them an essential part of daily cooking in many Latin American and Asian countries, for example, curry blends in India or in many meat sausages both fresh and dehydrated in the Mediterranean region [3].

On the basis of extracts obtained from pre-ceramic in the Coxcatlan caves, it is believed that the domestication of *C. annuum* probably occurred in the northeast or central-east of Mexico, being older than the remains of corn, beans and pumpkin [1]. In fact, peppers presently represent one of the vegetables of greater economic importance just behind the tomato for several American tropical countries [10].

Of the five domesticated species of *Capsicum* spp., the largest cultivated and with higher production per hectare are *C. annuum* and *C. frutescens* [11]. As for its world production, it is estimated that it is nearby 24.9 million tons (*C. annuum*), making it the ninth most produced vegetable in the world. Likewise, it has had an annual average growth of 6.26% in the last 10 years, with Mexico being its main consumer with an annual average of 8 kg/person. Its main producers are China (60.6%), Turkey (8.4%), Mexico (7.8%), Spain (5.0%), USA (4.3%), Indonesia (3.4%), Nigeria (2.8%), Egypt (2.2%), Korea (2.0%) and Italy (1.7%) [7]. In Mexico, one of the countries with the highest production levels, there are about a hundred cultivars of hot *Capsicum* spp. with varying degrees of spiciness, size, shape and colors. Nonetheless, there are also varieties that are cultivated throughout the world. This is the case of *C. annuum* or *C. chinense* [12]. Hence, it is a product that is traded internationally. This means that a series of elements such as aflatoxins, pesticides, residuals, microbial contaminations or infections, capsaicin levels and color values are subjected to inspection. All these aspects are controlled by law in several countries (EU, USA) [8].

Pepper quality depends on their composition, which is determined by factors such as environmental cultivation conditions, variety, ripeness, and pre-harvest and post-harvest handling and preservation [13]. The degree of ripening required may be one of the most important factors in quality, but it will also depend on the destined market, since not the same degree of maturity is desired for all the possible uses. Notably, the moment of harvesting is also important for the maintenance of the quality as metabolic activity persists after harvesting [14].

1.3 Chemical composition

Regardless of the enormous consumption and production of this kind of vegetables, there is little data about the chemical composition of the different varieties (**Table 3**). However, the demand and cultivation of peppers, especially "hot" cultivars, has increased due to its flavoring and medicinal properties. Some of the latter have been described as anticancer, antioxidant and antimicrobial. Its edible and

Common name	State	Carbohydrates	Protein	Fat	Capsaicinoids	Fiber	Ash
Guajillo	D	58.00	12.89	12.43	5.97	Nd	7.52
Ancho	D	60.21	12.05	9.82	8.50	Nd	7.81
Pasado	D	66.18	12.61	5.41	9.74	Nd	7.18
Pasilla	D	60.53	12.28	13.76	11.80	Nd	5.85
Puya	D	63.76	13.25	8.11	12.13	Nd	7.82
M. Tres venas	D	61.05	13.28	9.61	14.40	Nd	7.02
Chiplote Meco	D	57.68	15.22	9.08	29.01	Nd	9.54
Jalapeno	D	63.97	14.36	4.23	58.40	Nd	7.32
Mirasol	D	58.96	14.05	7.49	58.55	Nd	9.61
Morita	D	58.91	14.12	7.60	67.32	Nd	8.59
Serrano	D	67.93	12.78	2.26	102.73	Nd	5.81
Chiplote	D	62.92	12.72	8.66	143.57	Nd	6.92
De Arbol	D	59.41	12.75	13.38	193.51	Nd	8.82
Piquin	D	62.25	13.72	11.02	368.83	Nd	7.28
Habanero	D	61.13	13.52	4.63	1312.10	Nd	7.51
Marako fana	D	35.3	11.8	11.2	Nd	27.3	5.3
Bako local	D	39.5	8.7	9.5	Nd	26.0	7.3
Oda haro	D	37.1	9.2	9.2	Nd	28.6	7.3
Arnoia red	F	6.23	0.15	0.54	Nd	1.62	0.62
Arnoia green	F	3.84	0.14	0.22	Nd	1.63	0.40
Arnoia green B	F	3.51	0.12	0.16	Nd	1.31	0.33
Hot pepper	D	Nd	21.29	23.65	Nd	38.76	4.94
Chunhamuchuk	D	Nd	15.05	29.27	Nd	48.72	3.59
Amhanegosa	D	Nd	14.66	26.70	Nd	52.10	3.28
Hanbando	D	Nd	14.08	27.84	Nd	38.43	3.49
Dachon I	D	Nd	15.99	19.53	Nd	50.61	3.76
Samgang	D	Nd	13.90	23.50	Nd	50.71	3.71
Chunhajeil	D	Nd	14.67	21.87	Nd	52.54	3.46
Daejangbu	D	Nd	14.88	26.50	Nd	53.78	3.47
Hongjangkun	D	Nd	15.17	21.61	Nd	54.66	3.33
Kumbit	D	Nd	15.09	23.28	Nd	46.17	3.18
Dokyachungjung	D	Nd	15.75	25.13	Nd	53.36	3.86
Dangchan	D	Nd	15.36	19.99	Nd	55.63	3.46
Chohyang	D	Nd	13.25	18.05	Nd	59.13	3.77
Taesan	D	Nd	14.71	23.45	Nd	48.80	3.11
Ganggun	D	Nd	15.55	20.63	Nd	52.71	3.43
Chungsan	D	Nd	15.06	20.45	Nd	50.75	3.05
Dachon II	D	Nd	15.89	18.83	Nd	45.73	3.61
Wangdaebak	D	Nd	16.53	23.65	Nd	54.39	3.28
Chunhailpum	D	Nd	15.70	19.79	Nd	53.34	3.38

Common name	State	Carbohydrates	Protein	Fat	Capsaicinoids	Fiber	Ash
Daechan	D	Nd	13.88	20.64	Nd	54.68	3.72
Mixed	D	Nd	14.01	24.09	Nd	50.26	3.26
Sandia	D	Nd	14.95	23.07	Nd	58.34	3.22
R-Naky	D	Nd	14.36	23.57	Nd	60.19	3.57
New Mexico 6	D	Nd	14.79	21.95	Nd	60.61	3.48
LB-25	D	Nd	14.87	25.06	Nd	52.98	3.29

F, fresh; D, dried; Nd, not described.

Table 3.
Different Capsicum *spp. and their proximate chemical composition (g/100 g) [14–18].*

nutritional value is acknowledge as well, since it is rich in vitamins (A, C, B_6, E), carotenoids (β-carotene), flavonoids, oils, oleoresins and alkaloids [19]. Therefore, the compounds that can be found in this genre are carbohydrates (accounting for approximately 85% of dry weight), polyphenols (0.5% of dry weight) and important molecules such as capsaicinoids, carotenoids and vitamins [20]. Given these facts, peppers are considered a good source of most essential nutrients [14].

The capsaicinoids content, which depends on the variety and maturation stage, will determinate the pungency. *C. chinense* and *Capsicum annuum* var. *aviculare* contain larger amounts of capsaicin and dihydrocapsaicin (ratio of 2:1), while some varieties of *Capsicum annuum* var. *annuum* showed an average proportion of 1:1. These variations could be attributed to environment, genetics and extraction methodologies [15]. Even though in some cases peppers are sought to be spicy, this can also be a limitation. This is the case, for example, in obtaining dyes from this raw material in the food industry. In this case, extraction methods need to be improved to prepare non-pungent oleoresins from pungent *Capsicum* fruits. This is achieved by the selective removal of capsaicinoids which allows the exploration of a large number of pungent varieties with good oleoresin yielding [20].

Capsaicinoids are the characteristic pungent compounds of the *Capsicum* genus. They include capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. Capsaicin is the most abundant. As an example, capsaicin constitutes together with dihydrocapsaicin approximately 90% of total capsaicinoids content of chili peppers [15].

The characteristic red color of many peppers is determined by the presence of different carotenoids. Actually, more than 50 different carotenoids can be found in this kind of material. Some of them are capsanthin, capsorubin, and cryptocapsin which give brilliant red color (ripe fruits) or β-carotene, violaxanthin, zeaxanthin and β-cryptoxanthin which give yellow–orange color [3]. Nevertheless, the color will depend on the state of maturity. For example, jalapeño (*C. annuum*) has a green color, and when it is mature it presents an intense red color [7]. Therefore, depending on the degree of maturity, the physicochemical parameters, flavor and mineral composition differs. For example, fat, protein, ascorbic acid (vitamin C), soluble solid content and titratable acidity will increase during ripening [14].

Using jalapeño as an example to study composition, its main component is water, then carbohydrates (5.3%), fiber (2.3%), protein (1.2%), fat (0.1%) and minerals, being the most important potassium (340 mg per 100 g of fresh product). It also has calcium (25 mg per 100 g of fresh product), magnesium (25 mg per 100 g of fresh product), sodium (7 mg per 100 g of fresh product), iron (2 mg per 100 g of fresh product) and zinc (0.3 mg per 100 g of fresh product). As for vitamins, the most important are ascorbic acid, retinol and folic acid (72, 20 and 23 mg per 100 g of fresh product, respectively). Other vitamins that can be found in peppers are thiamine, riboflavin, niacin and pyridoxine. Moreover, jalapeños also contain important amino acids such as lysine, methionine and valine (252, 40 and 23 mg per 100 g of protein, respectively) [7].

2. *Capsicum* seeds as a source of bioactive compounds

Like many fruits and plants, peppers are an excellent nutritional source. As aforementioned, it has an abundance of minerals, vitamins, aminoacids, carotenoids as also phytochemicals like phenolic compounds or polyunsaturated fatty acids (PUFAs). Likewise, capsaicins are unique to the genus *Capsicum*, being responsible of the pungency of many pepper species. Capsaicinoids have demonstrated to induce a vast range of bioactivities, such as anti-inflammatory, anticancer analgesic, antimicrobial, hypotensive and induce lower adipogenesis or lower body temperature by isolated action or in synergy with other compounds [21–25].

Different bioactive compounds have been isolated and extracted from *Capsicum* spp. fruits and seeds and its concentrations vary among species. More information on reported compounds studied may be found in (**Table 4**).

2.1 Phenolic compounds

Phenolic compounds, also referred as phenolics, are secondary metabolites that may be found in a wide spectrum of plant species. They are synthesized as a result of adaptation to biotic and abiotic stress through the phenylpropanoid pathway playing an important role in plant development, because they act as a defensive mechanism that eases plant growth against harsh conditions [33, 34]. Based on recorded knowledge, phenolic compounds exhibit numerous potential health benefits that are already well described in scientific literature and are currently a major current focus of nutritional and pharmacological research [35, 36]. Phenolic acids and flavonoids are the main phenolic phytochemicals found in peppers. Likewise, capsaicinoids are synthetized in the same biochemical pathway and exhibit some similar properties such as antioxidative activity [34].

The yield of phenolic compounds recovered from an extraction can be very different, depending largely on the extraction method, the conservation of vegetal material and maturity state [37]. Generally, fresh raw material preserves the highest quantities of phenolic compounds [16]. Several factors contribute to yielding disparity, such as heterogeneous genotypes, growing and harvesting conditions of the samples [38]. Some of the most prominent non-capsaicinoid phenolic compounds because of their valuable health benefits are phenolic acids like gallic acid, caffeic acid, chlorogenic acid or ellagic acid (**Figure 2**). Flavones are another important group of phenolic compounds, being some of the most prominent kaempferol, quercetin, luteolin or rutin (**Figure 3**) [26]. These phenolics have demonstrated great health benefits and many of them are commercially available in purified products extracted from other plant species [39, 40]. Even so, their concentration differs among species but not much among varieties [26]. Other phenolic compounds are reviewed in the next paragraphs as in the case of some carotenoids or vitamins.

Regarding capsaicinois, they are synthesized naturally in the placenta of pepper fruits by enzymatic transformation of vanillylamine, the phenolic portion of the molecule, which confers this alkaloid its antioxidant capacity [41]. The seeds are not the primary source of capsaicinoids but they may absorb them because they are in close proximity to the placenta, which is the richest capsaicin fraction [42]. Their presence in the seed and the high concentrations they achieve, has been observed to

Species and varieties	Bioactive compounds						
	Major phenolic compounds	Vitamins	Pigments	Volatiles	PUFAs	Peptides	Ref.
C. annuum	4-Hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, sinapic acid, ferulic acid, ellagic acid, naringein	Nd	Nd	Nd	Nd	Nd	[26]
C. annuum	Elagic acid, gallic acid, chlorogenic acid, caffeic acid, salicylic acid, rutin	Nd	Nd	Nd	Nd	Nd	[21]
C. annuum	Caffeic acid, gallic acid, ferulic acid, rutin, capsaicin, dihydrocapsaicin	Tocopherols	Capsanthin, zeaxanthin, capsorubin, lutein, β-carotene, cryptocapsin	Nd	Linoleic, palmitic, oleic acids	Nd	[27]
C. annuum	Nd	Nd	Nd	Nd	Nd	Mannose/ glucose Specific lectin	[28]
C. annuum	Nd	Ascorbic acid	Nd	Betulin, β-sitosterol	Oleic, palmitic, linoleic, elaidic acids	Nd	[29]
C. annuum	Gallic acid, caffeic acid, ferulic acid, chlorogenic acid, cathechin, epicathechin capsaicin, dihydrocapsaicin	Ascorbic acid	Nd	Nd	Nd	Nd	[30]
C. annuum var. *annuum*	Caffeic acid, cinnamic acid, coumaric acid, capsaicin, vanillin	Nd	Nd	Nd	Nd	Phenyl-alanine	[31]
C. baccatum	Tannins, flavonoids, coumarins, polyphenols	Nd	Nd	Saponins	Nd	Amino acids	[32]

Nd, not described.

Table 4.
Capsicum *seeds bioactive compounds.*

rise the riper the pepper is [43]. It is also confirmed that fresh seeds yield more capsaicin than dry seeds, which suggests that surface capsaicinoids are sensitive to heat and/or oxidation [29]. "Hot" pepper cultivars attribute their pungency to high levels of capsaicinoids whereas non-pungent or "sweet" peppers (e.g., bell pepper) have very low capsaicinoids quantities [44].

Capsinoids are other non-pungent capsaicin analogues noticeably found in *C. baccatum* var. *praetermissum* and the sweet cultivar CH-19 of *C. annuum* [45, 46].

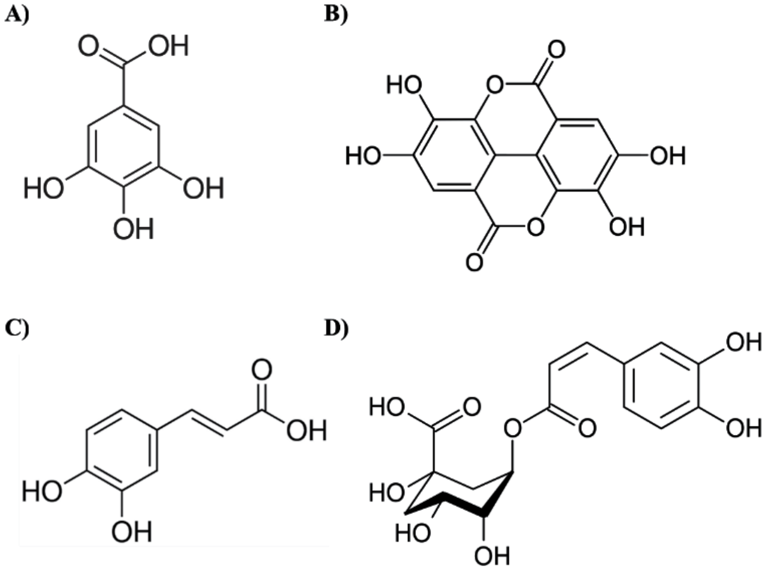

Figure 2.
Chemical structure of some main phenolic acids found in peppers: (A) gallic acid; (B) ellagic acid; (C) caffeic acid and (D) chlorogenic acid.

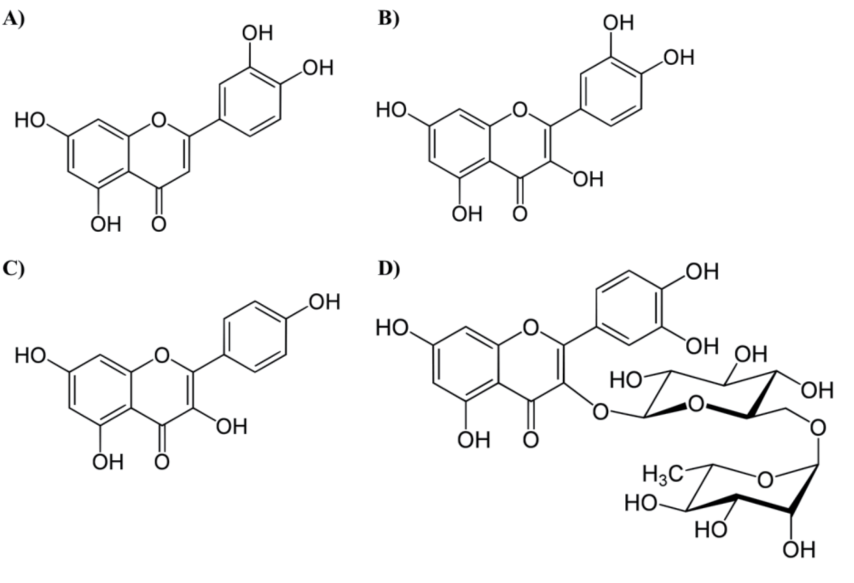

Figure 3.
Chemical structure of the main flavonoids found in pepper: (A) luteolin; (B) quercetin; (C) kaempferol; and (D) rutin.

Capsinoids exhibit biological activities similar to capsaicin due to being capsaicinoids analogs, except that they display much lower pungency in comparison [45]. Capsaicinoids and capsinoids manifest many promising therapeutic properties such as apoptosis induction, antioxidation, anticancer (cytostatic), or immunomodulation, making them attractive targets for pharmaceutical research [24, 47].

Besides, it is worth mentioning that the well-known flavone chrysoeriol (**Figure 4**), present in a multitude of vegetables and at least in *C. annuum* and *C. frutescens* fruit and seeds, has been determined to inhibit the growth of several microorganisms at very low quantities [31].

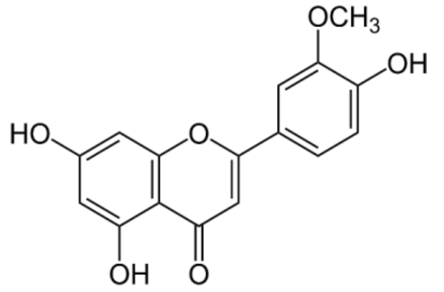

Figure 4.
Chemical structure of chrysoeriol.

2.2 Fatty acids

Peppers are fruits rich in polyunsaturated fatty acids (PUFAs), and their seeds show even greater concentration per gram [42]. The main fatty acids are indeed PUFAs [48]. These PUFAs, which are in the whole fruit and seeds, are mainly linoleic acid, palmitic acid α-linolenic acid and stearic acid [16, 48]. Moreover, peppers appear to have very low levels of saturated fatty acids. Linoleic acid has the highest concentration (≈70%) and the other fatty acids show much lower levels [48, 49]. PUFAs and specifically linoleic acid and α-linolenic acid, are recognized as essential fatty acids and are precursors of other important fatty acids in metabolism like arachidonic acid and eicosapentanoic acid or prostaglandins that in whole contribute to normal physiological performance [50].

2.3 Pigments

Chlorophylls and carotenoids constitute another group of valuable pepper nutrients that affect its color, but they also have important antioxidative, anti-inflammatory effects and promote immune response [22, 51, 52]. Zeaxanthin, β-carotene, violaxanthin, lutein and β-cryptoxanthin are the pigments with highest concentration among *Capsicum* spp. [37, 43]. Capsanthin is another red colored pigment mostly present in red bell pepper (*C. annum*) [53]. They all show strong antioxidative properties and increase their levels the more mature the pepper is [44].

β-carotene and β-cryptoxanthin for instance, possess the added value of being able to be converted to vitamin A [54]. Vitamin A plays a vital role in disease prevention and development [54].

2.4 Vitamins

As it has been mentioned, peppers contain several vitamins like ascorbic acid and tocopherols like α-tocopherol, γ-tocopherol and δ-tocopherol, are all isomers of vitamin E [55, 56]. These essential vitamins contribute to the normal metabolism, promote immune response and also have antioxidative bioactivity [52, 57]. Because of this, ascorbic acid is an essential vitamin that is also used as a natural food preserver [52]. Indeed, peppers contain levels of vitamin C corresponding with those found in many citrus fruits and other vegetables considered good sources of this vitamin [14]. Tocopherols are well-known lipophilic antioxidants and appear to exert a vital part in the normal T lymphocyte maturation, lower age-related increase in tissue inflammation and lower interleukin production [55, 58]. This makes vitamin E an important modulator to an orderly and better inflammatory reaction, among other health benefits [58].

2.5 Volatile compounds

Volatile compounds in *Capsicum* spp. have been studied because of the importance they have in many plants as pollinator attractors and their influence in odor and flavor, which are key features of any edible vegetable [59, 60]. Some of the most valuable are hexyl isopentanoate, hexyl isobutanoate, and β-ionone [61]. Extracts of *C. annuum* have also showed high levels of 4-methoxyphenol, ethylhexadecanoate, hexanal or isopulegol [62].

2.6 Other minor compounds

There are a few other minor and less studied compounds amid the different pepper species, because research has focused almost entirely on finding and studying phytochemicals of metabolic importance. In regard to these bioactive compounds, *C. annuum* or *C. frutescens* saponins have showed antimicrobial activity against several opportunistic and pathogenic fungi genres [63]. Saponins are triterpene glycosides with the ability to disrupt the cell membrane and wall of fungal cells during cell proliferation, which leads to cell lysis [27] As such, saponins do not have effect on non-germinated fungi, but they inhibit its growth. Although there are several present in pepper tissue, CAY-1 has been well-studied (**Figure 5**), presenting a high antifungal activity [60].

Another of these minor chemicals are lectins, a group of proteins with glycoside agglutination properties. Some lectins have been described in *C. frutescens* and *C. annuum* seeds and have been studied as possible antimicrobials and have yielded positive results against some fungi [28, 64].

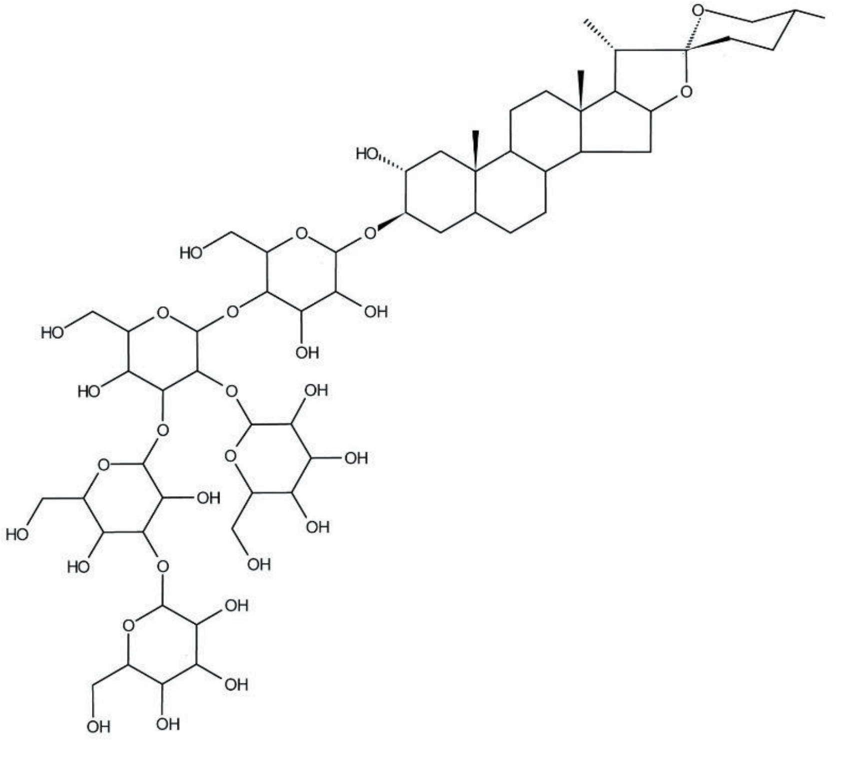

Figure 5.
Chemical structure of CAY-1. Adapted from [60].

3. Bioactivities of the raw material

In recent years, nutraceutical and therapeutic research has focused its view towards both exotic and domestic fruits and vegetables as a source of phytochemicals with the ability to induce beneficial bioactivities [65]. These natural chemicals have been and are already used as a main source of therapeutics in traditional and modern medicine, reaching one third of the total production of therapeutics [66]. Furthermore, processing waste by-products of fruits and vegetables are being researched as a viable source of phytochemicals that would be affordable and reduce economic and ecological impacts of wasted by-products or taking produce out of the food market [67, 68]. Thus, fractions that are not employed in nourishment such as the placenta, seeds or leaves of many species can prove to be a valuable resource instead of end as waste or fertilizer, which is the most common use for vegetable non-edible parts [61].

Taking into account the aforementioned compounds present in the many different species of the genus *Capsicum* and different regional cultivars, as in the case of *C. annuum*, many biological activities are expected to be found in direct or indirect physiological response to the presence of these substances. It is widely known that capasaicinoids, capsinoids, saponins, many phenolic compounds and its valuable nutrients are behind the beneficial effects of this vegetable, which has been traditionally employed as a source of medicinal remedies all across South America, Asia and Africa [63]. The intensity and type of activity may vary depending on the concentration of these compounds and will differ in each species and cultivar of *Capsicum*, the bioavailability of each compound, the ripening state and from which fraction of the vegetable the extracts are obtained [43, 44].

These bioactivities have been described in scientific reports through tissue culture and both animal and human test research as antioxidant, antimicrobial, anti-inflammatory, anticancer, analgesic or even antidiabetic [21, 22, 34]. Considering this with the fact that *Capsicum* spp. is widely grown around the world, it makes it to be considered an excellent source of new therapeutics and dietary supplements that could lead to a healthier well-being. Indeed, many extracts of pepper fruits and seeds with a varying purification degree are marketed and can be purchased [69].

Some of the most recognized bioactivities found by chemicals in the whole pepper and its seeds will be reviewed and a brief compendium of the bioactivity research can be found in **Table 5**.

3.1 Antioxidant

Oxidative stress is caused as a result of the presence of reactive oxygen species (ROS) which may be produced in oxidative metabolism and exposure to the environment [33]. The term ROS englobes the molecules superoxide radical (O_2^{2-}), the hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2). They are produced by the sequential reduction of molecular oxygen in various metabolic reactions [76]. O_2^{2-} is the most unstable form but it may dismutate to H_2O_2 by the action of endogenous superoxide dismutase (SOD) enzyme reaction or non-enzymatically [76]. The effect of oxidative stress mainly translates into changes in the rate of metabolic reduction reactions and an increased rate of DNA mutations and cell mitosis. They are also main signals indicating cell death, which in turn triggers inflammation via the pro-inflammatory factor nitric oxide (NO), which is released by macrophages [33, 76]. Since these are both cause and result of cancer development, it is considered that high levels of ROS are detrimental to health [40, 65, 76]. All living aerobic

Activity	Bioactives	Species	Type of study	Test results	Ref.
Antioxidant	Capsaicin, dihydrocapsaicin	*C. annuum*, *C. baccatum* var. *pendulum*, *C. chinense*, *C. frutescens*	In vitro	DPPH, ABTS	[68]
	Total phenolic compounds	*C. annuum*	In vitro	DPPH, ABTS, ORAC	[26]
	Phenolic extracts	*C. annuum*	In vitro	DPPH, ferrus chelating	[39]
	Seed oil	*C. annuum*	In vitro	DPPH, ABTS	[42]
Antimicrobial	Capsicosides A, G, D	*C. annuum* var. *acunimatum*	In vitro	Gia. various yeasts	[32]
	Capsaicin	*C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*	In vitro	Gia. *Clostridium tetani* & *C. sporogenes*	[31]
	Phenolic extracts	*C. annuum*	In vitro	Gia. *S. aureus*, *L. monocytogenes*, *S. typhimurium*, *B. cereus*	[70]
	CAY-1	*C. frutescens*	In vitro	Gia. *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. niger*, *C. albicans*, *P. carinii*	[60]
	Lectins	*C. annum*	In vitro	Gia. *A. flavus*, *F. graminearum*	[28]
	Total polyphenol content, capsaicins	*C. annuum*	In vitro	Gia. *E. faecalis*, *P. aeruginosa*, *E. coli*, *S. aureus*	[21]
	Jalapeño extracts	*C. annuum* var. *annuum*	In vitro	Gia. *L. monocytogenes*	[71]
	Capsaicinoids, chrysoeriol	*C. frutescens*	In vitro	Gia *E. faecalis*, *B. subtillis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans*	[31]
	Residual aqueous extract	*C. baccatum*	In vitro	Gia *S. epidermidis*, *P. aeruginosa* biofilm	[72]
Anti-inflammatory	Capsaicin	Nd	In vitro	Inhibition of inflammatory transcription factor NF-κB and AP-1	[73]
	Capsaicin	Nd	In vitro	Inhibition of Ikα and Ikβ via NF-κB	[74]
	Capsaicin	Nd	In vitro	Inhibition of adipose tissue inflammation (interleukin 8, c-Jun)	[75]

Nd, not described; Gia., growth inhibition against; DPPH, 2,2-diphenyl-1picrylhydrazyl scavenging method; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt scavenging methods; ORAC, oxygen radical absorbance capacity.

Table 5.
Properties and mechanisms of bioactive compounds and Capsicum *spp. extracts evaluation.*

organisms have developed defense mechanisms against oxidative stress through the synthesis of reductive biochemicals or enzymes such as SOD. The most important antioxidative biochemicals are phenolic compounds, which are prominently found in plants [52, 66]. These antioxidants present in peppers, mainly identified as the mentioned phenolic compounds, vitamins and pigments, have the potential to reduce biological oxidative stress and thus preventing the incidence of many related diseases, but also to further food preservation by inhibiting the oxidative metabolism of decomposing fungi and bacteria [33, 35, 38]. Phenolic compounds are the principal antioxidants in nature and generally show the greatest antioxidative capacities [65]. However, high concentrations of few phenolic compounds showed pro-oxidant effects due to a concentration imbalance between ROS and the phenols, which is why it is important to maintain intake of different phenolic compounds in order to benefit from their antioxidant properties [52]. Thus, antioxidants have a wide extent of applications, and many natural and synthetic antioxidants have been used by the food industry in order to better preserve raw or precooked products that would otherwise have a much shorter shelf-life [22].

The antioxidative effect of the aforementioned phenolic compounds essentially works by scavenging free superoxide and hydroxyl radicals and thus preventing high levels of ROS, NO and oxidation of sensitive biomolecules like proteins or lipids (**Figure 6**) [36, 40]. This results in a better physiological performance, immunomodulation and DNA mutation protection. Furthermore, the decrease of oxidative stress results in better cardiovascular health. This is due to the fact that low-density lipoprotein (LDL), as the main cholesterol carrier in the circulatory system, is susceptible to oxidation by ROS [58]. Oxidized LDL presence has been found to be cause of atherosclerosis, a vascular ailment caused by the formation of plaques inside the arteries that may weaken blood flow and lead to cardiovascular diseases [77]. On top of that, pepper and its seeds are described to be one of the richest vegetables in phenolic compounds [29]. Then, it should be taken into account the antioxidant properties of the many phenolic acids, capsaicinoids, vitamins and pigments present among the *Capsicum* genus and the fact that these exploitable compounds are available not only in the fruit, but also in the non-edible fractions [39, 57, 78].

3.2 Antimicrobial

Pepper seed extracts and selected pigments, phenols, capsaicins and capsiates have been tested against the most common microorganisms present in foods and/or potential pathogens. The major antimicrobial effect of capsaicinoids has been found to be against common opportunistic and pathogenic fungi like *Fusarium* spp., *Aspergillus* spp. and yeasts like *Saccharomyces cerevisiae* or *Candida albicans*, but also against certain bacteria like *Streptococcus* spp., *Helycobacter pylori*, *Pseudomonas aeruginosa* or

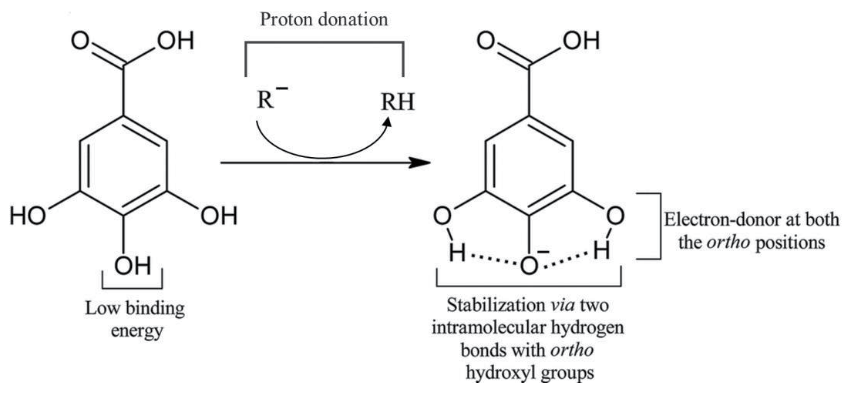

Figure 6.
Scheme of radical scavenging performed by gallic acid as an example of phenolic compounds. Adapted from [36].

Listeria monocytogenes [21, 32, 38, 79]. It has been reported that it can also reduce the virulence of pathogenic infections by *Vibrio cholerae* or *L. monocytogenes*, by inhibiting pro-inflammatory factors or toxin production [79–81]. There are few *in vivo* studies of these effects, though there are some reports of poultry showing less hepatic damage from *Salmonella enteritidis* when given feed supplemented with capsaicinoids, which could be related to its antioxidative activity [24].

Capsaicinoids appear to inhibit, fungi, and both Gram positive and negative bacterial growth in several studies. In all of them, capsaicin and/or dihydrocapsaicin showed to stop or slow colony development in a significant degree [24, 35]. Some interesting results of antimicrobial tests with capsaicinoids reported that it inhibits the growth of *Escherichia coli*, *L. monocytogenes*, *Bacillus subtillis* and many strains of *Staphylococcus aureus* [21].

Even though capsaicinoids possess these interesting antimicrobial properties, the positive correlation between antimicrobial activities, concentration and its pungency and potential irritation effects make them less suitable when it comes to applying this compound as a food preserver [46]. Thus, it is interesting to study the effects on this area of alternative chemicals found in pepper fruit and seeds. CAY-1, chrysoeriol and lectins have been previously mentioned as such.

CAY-1 is found in *C. frutescens* and a study demonstrated to produce cell membrane lysis in fungi like *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Fusarium moniliforme*, *Pneumocystis carinii* or *Cansida albicans* [60]. It is ineffective though against bacteria though, probably because of the different type of cell membrane and wall [60]. It has also been suggested to combine this saponin with certain metallic elements such as silver in order to produce antimicrobial surfactants [82]. Other saponins isolated from *C. annuum* seeds, like capsicosides, which are furostanol saponins, have showed similar effects on growth inhibition against yeasts [27].

Chrysoeriol is another potent antimicrobial compound present in a variety of plants as well as in peppers [83]. Several studies have presented that chrysoeriol is capable of greatly inhibit the growth of *Enterococcus faecalis*, *Klebsiella pneumoniae*, *S. aureus*, *L. monocytogenes*, *B. subtillis* or *C. albicans* [31, 84]. Given the very low concentrations at which it exerts its antimicrobial effect, it may be an optimal food preserver against many opportunistic and nosocomial microbes.

Certain lectins isolated from peppers have showed antifungal properties, inhibiting growth of some common opportunistic fungi like *Aspergillus flavus* and *Fusarium moniliforme* by limiting surface adherence. However, its inhibitory action is not as effective across all species of these genres [28, 85].

Furthermore, a recent study analyzed the antiadhesive capacity of natural peptides from *C. baccatum* var. *pendulum* on biofilms made by *Staphylococcus epidermidis* [64]. Considering that biofilms are a source of significant virulence, new studies on this field present extremely interesting. Altogether, these findings may contribute to develop new uses for natural antimicrobials, which would be an application of vital priority, given the global concern on the appearance of newborn antibiotic resistant microorganisms [31, 84].

3.3 Anti-inflammatory

Although an anti-inflammatory action is carried indirectly by the antioxidant capacity of other compounds to scavenge ROS and inhibit NO production, it is known that capsaicins and capsiates directly induce an anti-inflammatory effect through the activation of the transient receptor potential vanilloid 1 receptor (TRPV1) [24, 86]. TRPV1 is an ion channel receptor located in several glia that gives sensations of heat, but is also relevant in pain perception and induces this sensation, as well as inflammation [87]. By activating this receptor, capsaicins cause the well-known pungency sensation, as well as irritation in high enough concentrations. However, after activation by capsaicinoids, the excited neurons become resistant to further stimuli [87]. Hence, after a brief burning sensation, capsaicin may act as a local analgesic in neuropathic pain [84].

Research on capsaicinoids has showed that these molecules can also decrease inflammation of adipose tissue linked to obesity [73, 75]. It seems that capsaicin is able to inhibit or at least partially decrease the production of pro-inflammatory signals like interleukin 8 (IL-8), nuclear factor kappa-light-chain-enhancer of activated B cells (NF\kappaB) or active protein 1 (AP-1) [74, 80]. This anti-inflammatory effect has the potential to make inflamed tissue less prone to tumor development as well as reduce the infection caused inflammation [25].

Still, the pungency of capsaicins poses a setback for using them without potentially hazardous secondary effects. That is why capsinoids may be a good alternative to induce similar effects to capsaicin, even though they are not found in so many pepper species [41, 45].

Although the importance of researching capsaicinoids bioactivities in pepper is due to its unique presence in this genre, the also present phenolic compounds, vitamins and pigments also bear significant anti-inflammatory properties [25, 88, 89]. Indeed, the antioxidative properties of phenolic compounds could be a key factor in reducing age-related extended tissue inflammation [25, 58]. Nonetheless, further research assessing the suitability of using as anti-inflammatory drug these phytochemicals, and specifically capsaicin, is needed.

4. Extraction systems for bioactive compounds from *Capsicum* spp.

The bioactive compounds obtained from nature are secondary metabolites produced by the organisms. The concentrations of each type of compound are very variable, so in order to have enough quantities, the development of new and advanced technologies is needed.

Although there are several extraction methodologies, there is a demand for more appropriate and standardized extraction strategies. For choosing one method over another, it must be taken into account the quantitative and qualitative characteristics of the compound of interest. In addition, to improve the efficiency of a method it is important that the nature of the source, the different parameters of the method and the possible interaction are taken into account. Experiments carried out with peppers can be seen in **Table 6**.

4.1 Conventional extraction systems

4.1.1 Maceration

Maceration (MA) is a type of solid–liquid extraction, in which the solid would be peppers. This raw material is characterized by having several compounds soluble in the liquid phase. The type of molecules extracted will depend on two factors: the type of starting material and the type of solvent used.

In order to carry out this process, the sample is suspended in the solvent at the desired temperature during the chosen time while stirring. Once the process has been carried out, the sample is centrifuged and the supernatant, which is where the compounds of interest are found, is filtered. Therefore, the process of extraction consists of two stages. On the first (washing) there is a rapid transfer of the compounds from the surface of the solid to the solvent. On the second one (transfer), the matter passes from the interior to the exterior of the solid by diffusion

Compound	**Species**	**Extraction**		**Medium**	**Yield**	**Ref.**
		Type	**Method**			
Pigments	*Capsicum* spp.	Emergent	SFE	—	6%	[90]
	C. annuum	Green	MAE	Acetone-water	—	[91]
		Emergent	SFE	—	17.4%	[92]
				—	93%	[93]
				—	7.2%	[94]
Carotenoids	*C. annuum*	Green	EAE	Viscozyme L	87%	[95]
				Pectinase	80%	[95]
				Cellulase	Low	[95]
				Viscozyme L	78%	[96]
Phenolics	*C. annuum*	Emergent	SFE	—	84%	[93]
				—	100%	[94]
Capsaicinoids	*Capsicum* spp.	Green	MAE	Acetone (30%)	0.48 mg/g	[97]
	C. annuum	Green	MAE	None	230 ppm	[98]
			UAE	None	200 ppm	[97]
				n-hexane (100%)	—	[99]
				Methanol (100%)	100%	[100]
	C. annuum	Green	EAE	Viscozyme L	88.8%	[96]
				Viscozyme L	22%	[101]
				Celluclast	20%	[101]
		Emergent	SFE	—	8.6%	[92]
				—	—	[102]
				—	93%	[93]
				—	710 μg/g	[103]
	C. frutescens	Green	MAE	Acetone (pure)	5.3 mg/g	[104]
				Ethanol (99.5%)	—	[105]
			UAE	Ethanol (95%)	87.4%	[106]
				Methanol (100%)	100%	[100]
				Acetone (100%)	3.92 mg/g	[107]
		Emergent	SFE	—	710 μg/g	[103]
				—	5.2%	[108]
	C. chinense	Green	UAE	Acetone (100%)	0.31 mg/g	[109]
				Methanol (100%)	2.88 mg/g	[110]
		Emergent	SFE	—	0.5%	[111]
	C. baccatum	Green	UAE	Methanol (100%)	50%	[112]
Antioxidants	*C. baccatum*	Green	UAE	Methanol (100%)	27%	[112]
OLEORESIN	*C. baccatum*	Green	UAE	Methanol (100%)	26%	[112]
	C. annuum	Green	EAE	Viscozyme L	6%	[101]
		Emergent	SFE	—	8.2%	[102]
				—	7.4%	[94]
				—	—	[113]
	C. frutescens	Emergent	SFE	—	0.3%	[108]
Other phytochemicals	*Capsicum* spp.	Green	UAE	Methanol (80%)	—	[114]

Table 6.
Bioactive compounds obtained from Capsicum *spp. extracted with different extraction methods.*

being this step the limiting stage. This method is used, for example, for the isolation of phenolic compounds of vegetable origin for their subsequent use in the food, pharmaceutical and cosmetic industries [115].

Water can be used as an extraction solvent, but it comes with the drawback is that it principally extracts the hydrophilic compounds present in vegetable materials. Several studies showed that water is not a very suitable solvent because its high polarity does not allow the extraction of capsaicinoids that are non-polar. However, it has the advantages or being safe, cheap and the simplest form to obtain essential oils. This technique can also be done with other solvents, being the ratio of solvent another parameter to take into account. Polarity is the reason why the most common solvents used are methanol, ethanol, water or a mixture of them [3].

Another parameter to optimize will be temperature. This makes it possible to differentiate two types of MA: cold maceration and heat maceration. Maceration with or without stirring, mild heating or heating under reflux are also possible variations to the method.

It is a quite old simple method with the inconvenience of long extraction times and large amounts of sample and solvents. Additionally, high temperatures can destroy thermolabile compounds such as phenolic compounds. In a study in which the effect of MA and ultrasound was related, it was detected that they have similar extraction yield of oleoresin; nevertheless, MA needs longer extraction times with the consequent loss of quality due to their higher times of exposure to high temperatures. It also was observed that n-hexane was a better solvent than ethanol [116].

4.1.2 Heat assisted extraction

This procedure is also a type of solid-liquid extraction in which the extraction is usually carried out in thermostatic and sealed water baths so that the solvent does not evaporate. The determining parameters are extraction time, temperature and the proportion of solvent that is used as well as the solid-liquid ratio. Due to the application of high temperatures (as far as 100°C), it is not the best extraction process for this type of matrix because certain compounds present in peppers (vitamins, phenolic compounds, etc.) are degraded by heat.

4.1.3 Cold pressure extraction

Cold pressure extraction is one of the oldest techniques of extraction for obtaining oils. It consists of mechanical pressing with the absence of heating. By using this method, little to no heat is generated, however it gives low yields. This technique was applied to Capia pepper seed, resulting in a lower extraction yield compared to traditional Soxhlet extraction with hexane. Moreover, the final oil was unpopular among consumers [117].

4.1.4 Organic solvent extraction

Organic solvent extraction (OSE) allows the extraction of many compounds (oils, fats and proteins). Normally, after the extraction process another step is done. It consists of concentrating the extract by removing the solvent under atmospheric or reduced pressure. OSE is the most extensively used technique to obtain oleoresins from peppers. These oleoresins are. Usually used as color additives [118]. In this method, the most determining limitation will be the solvent properties (polarity). When choosing the solvent, it must also be taken into account if the legislation allows its use. In the EU is regulated in Commission Regulation N° 231/2012.

In order to improve these conventional extraction techniques, other methods have been developed (ultrasound assisted extraction, pressurized hot water extraction, negative pressure cavitation-assisted extraction and pulsed electric) to partner with them and solve some of the inopportuneness of traditional techniques. In this way the extraction efficiency is substantially improved.

4.2 Green extraction techniques

This group includes several techniques with the aim of not only preventing pollution but also reducing sample preparation costs by, for example, lowering solvents consumption.

4.2.1 Microwave-assisted extraction

Microwave-assisted extraction (MAE) is based on the heating of solvents that are in contact with solid samples with the use of microwaves. This allows the partition of compounds of interest from the sample into the solvent. Microwave energy accelerates a great variety of chemical reactions as well as the extraction of organic compounds from different matrices [98]. During the process, electromagnetic energy is transformed into calorific energy by two mechanisms: ionic conduction and dipole rotation [119]. It is a method that could be an alternative for avoiding thermal degradation and oxidation, with no influence on cell integrity and shape. However, due to thermal stress and localized high pressures, cell rupture is more rapid than with another techniques, which is an inconvenience, for example, in the extraction of volatile oils [91].

Some of the advantages of this technique are simplicity, effectiveness, low processing time, low solvent consumption and energy, no generation of secondary waste and can be used for larger volumes [120]. Another advantage of this method is that it produces a uniform heating, so extraction is simultaneous regardless of the area where the compound is [121]. By using this method, lipids, pigments, carbohydrates, vitamins and proteins can be extracted [122]. Moreover, it is characterized by having a superior extraction rate of volatile compounds in *Capsicum* spp.

The selectivity and efficiency of MAE largely depends on the dielectric constant of the extraction solvent mixture, which defines its chemical polarity and thus what compounds will be extracted [91]. It can be expected that by using water as a single solvent, the quantity of capsaicinoids extracted decrease due to its negligible polarity [109]. Therefore, the parameters to take into account in this type of extraction are time, power, temperature and type of solvent and its ratios.

Several experiments can be seen in a summarized form in **Table 7**, which shows the variation between the values of the parameters among different experiments with solvents of different polarity. It is worth highlighting the values obtained for capsaicinoids using ethanol [105] as solvents since it is the one with the highest yield.

4.2.2 Ultrasound assisted extraction

In every extraction process there is a critical step which is the degradation of cell walls and membranes. Ultrasound assisted extraction (UAE) makes this possible by applying pressure waves that are transmitted across a medium as compression and expansion (rarefaction) cycles, finding an area with maximum pressure in the compression phase and one with minimum pressure in the rarefaction phase. This pressure difference makes the cavitation phenomenon possible, which breaks the

Target	Species	Extracting conditions				Yield	Ref.
		Temp.	Time	Power	Solvent ratios		
		°C	min	W			
Volatiles	*Capsicum annuum*	55	10	250	n-hexane (pure)	42 compounds	[59]
Pigments	*Capsicum annuum*	60	2	50	Acetone-water (50%)	—	[91]
Capsaicinoids	*Capsicum* spp.	21	15	300	Acetone (30%)	0.48 mg/g	[98]
	Capsicum chinense	50	5	800	Methanol (100%)	0.32 mg/g	[109]
	Capsicum frutescens	—	20	320	Ethanol (99.5%)	5.3 mg/g	[105]
	Capsicum frutescens	120	15	150	Acetone (pure)	0.673%	[104]
	Capsicum annuum	76	1	500	Methanol (60%)	230 ppm	[97]

—, not described.

Table 7.
Different experimental conditions carried out in peppers by microwave-assisted extraction.

structure to make the compounds accessible and therefore available to extract them [123].

This technique has been applied to extract different bioactive compounds from herbs or algae due to its ease to disrupt their cell walls despite its resistance which differentiates this method from the previous ones, since strong disruption of the cell wall is achieved and as a result the extraction of intracellular materials is increased with the increment of energy input [106, 120, 124].

The ultrasonic technique, as well as the other green techniques, has been proven to have several advantages such as reduction of solvents consumption, temperature and time, very important parameters in the extraction of thermolabile and unstable compounds as it has no effect on the chemical structure and biological properties. Furthermore, UAE has low equipment investment and easy implementation, so it can be basically industrially employed in local companies [106]. Moreover, there are studies that showed that this method also achieves a greater supercritical extraction of pungent compounds from ginger owing to physical effects on the surface of particles [106].

There are several parameters to take into account to optimize the method which include ultrasound power intensities, frequency, wavelength and time. There are several studies about it (**Table 8**). One of them studied the influence of several of these parameters on the extraction of capsaicinoids, observing that their release was very fast in the first 5 min of the process and then decreased. Temperature influence was also observed since an increase of 15°C (up to 45°C) improved the extraction using 95% ethanol (v/v) as a solvent. Higher temperatures did not lead to significant improvements [106]. The effect of the solvent was also studied, being the most common solvents used for extracting capsaicinoids methanol, ethanol, acetone, acetonitrile and water. It was proved that the yield of extraction is worse with the addition of water. Methanol and ethanol have similar recoveries but ethanol was better than acetone, the best concentration of ethanol being 95% [100, 106].

Target	Species	Conditions					Yield	Ref.
		Freq.	Intensity	Time	Temp.	Solvent		
		kHz	W/cm^3	min	°C	(%)		
Capsaicinoids	*Capsicum chinense*	20	—	10	40	Acetone (100)	0.31 mg/g	[109]
	Capsicum chinense	40	—	10	24	Methanol (100)	2.88 mg/g	[110]
	Capsicum annuum	31.5	—	20	76	Methanol (60)	200 ppm	[97]
	Capsicum annuum	—	—	20	50	n-hexane (100)	—	[99]
	Capsicum annuum	20	360	10	50	Methanol (100)	100%	[100]
	Capsicum frutescens	35	600	180	45	Ethanol (95)	87.4%	[106]
	Capsicum frutescens	20	360	10	50	Methanol (100)	100%	[100]
	Capsicum frutescens	—	—	40	25	Acetone (100)	3.92 mg/g	[107]
	Capsicum baccatum	20	150	20	40	Methanol (100)	50%	[112]
Antioxidants	*Capsicum baccatum*	20	150	20	40	Methanol (100)	27%	[112]
Oleoresin	*Capsicum baccatum*	20	450	20	60	Methanol (100)	26%	[112]
Phytochemicals	*Capsicum*	35	—	20	50	Methanol (80)	—	[114]

—, not described.

Table 8.
Different experimental conditions carried out in peppers by ultrasound assisted extraction.

4.2.3 Enzyme-assisted extraction

Enzyme-assisted extraction (EAE) is an extraction system that allows the avoidance of processing conditions like temperature or drastic pH changes and so, maintain the quality and yield of multiple biomolecules [125].

This type of extraction supports isolation for recovering bioingredients from different plant materials. An enzymatic pre-treatment before applying traditional methods will help to isolate high yields of bioingredients due to enzyme assisted extraction facilities. This is due to the fact that degradation of cell walls and membranes is the critical step of extraction which [101]. Among its advantages over traditional methods are high selectivity, overall efficacy, eco-friendly procedures, low-energy consumption, minimal usage of harsh chemicals, maximum yield, low to no wasteful protection or deprotection steps, easy recovery, and process recyclability [126]. However, it also presents some drawbacks such as the cost of enzymes, requirement of holding tanks that may require long term incubation, lack of knowledge about optimal or compatible enzyme formulations for cell disruption and inability to completely hydrolyse the bonds in plant cell wall [127].

The factors to take into account in this technique are fundamentally temperature, pH, and type of enzyme. A range of enzymes (lipases, carbohydrase, celluloses, proteases, pectinases) have been widely used as specific catalysts. Each enzyme has different substrates. For example, cellulase and hemi-cellulase have their greater hydrolysing activity on the cellulose found in plant cell walls, hence their name. This enzymatic processing increases the permeability of the cell wall, resulting in a better recovery of some compounds like volatile oil and resin, which are prone to degradation when extracted with more disruptive methods [101].

Several studies (**Table 9**) show that viscozyme L is an enzyme with a superior recovery rate of bioactive compounds fractions like total carotenoid content, total phenolic content, total flavonoids and total antioxidant activity with high total suspended solids (TSS). It also has at this moment the better extract yield [95, 96]. Among its applications is the extraction of pigments or capsaicinoids. In almost all cases the pH of the medium is 4.5 as it is the pH of the sample and it is in the range of optimal activity of the different enzymes [95].

4.3 Emerging technologies for extraction

Due to the disadvantages of traditional techniques, there is an interest in the development of new extraction techniques. Some of the most sought-after features include shortened extraction time, automation or reduced organic solvent consumption.

4.3.1 Hydrostatic high-pressure extraction

Hydrostatic high pressure (HHP) is considered an emerging technology that have been applied in the preservation of food since the end of eighties [128]. The first food products treated with this method began commercialization in Japan in 1990 [129]. This method is established on the application of high pressures (100–900 MPa) to the product of interest.

Among the advantages of this type of extraction there is the possibility to conduct it at room temperature, meaning no thermal degradation and derived bioactivity loss from extracted components. It also does not modify chemical structures of the different compounds of interest's independent form of molecular weight [130]. Moreover, in comparison with conventional techniques it is faster,

Target	Enzyme	Conditions			Yield	Ref.
		Temp	pH	Time		
		°C		h	%	
Carotenoids	Viscozyme L	60	4.5	1	87	[95]
	Pectinase	60	4.5	1	80	[95]
	Cellulase	60	4.5	1	low	[95]
	Viscozyme L	50	4.5	5	78	[96]
Capsaicinoids	Viscozyme L	50	4.5	5	88.8	[96]
	Viscozyme L	45	4.5	1	22	[101]
	Celluclast	45	4.5	1	20	[101]
Oleoresin	Viscozyme L	45	4.5	1	6	[101]

Table 9.
Different experimental conditions carried out in C. annuum *by enzyme-assisted extraction.*

gives higher extraction yields and fewer impurities [131] giving, unlike other preservation technologies as thermal treatment, uniform and nearly instantaneous effects throughout the foodstuff and thus independent of foodstuff geometry and equipment size which makes an easy scale-up from laboratory findings to full-scale production possible [132].

This method can be applied to multiple matrices for the extraction of natural compounds [133] like fruit and vegetables for different targets as for example carotenoids [134], antioxidants [131] and pigments [135].

The main parameters to be taken into account are pressure, time, temperature and type and quantity of solvent, studying each parameter for each variety in particular.

HHP can change physiological and biochemical properties of pepper based on a study carried out with *C. annuum* [136]. In this research, different pressures (50, 100, 200 and 300 MPa) where applied for 5 min at 25°C observing that it produces remarkable changes in seedlings, but no or very little physiological and biochemical variations, creating an antioxidant system that is positive for the plant itself in the process. Therefore, HHP (500 MPa) can be used as a preservation treatment similar to pasteurization which was the ttraditional thermal treatment for sweet pepper preservation [137].

4.3.2 Supercritical-fluid extraction

In supercritical-fluid extraction (SFE), the fluid must reach temperature and pressure above the critical point, so as the fluid behaves like liquid and gas simultaneously which makes extraction easier. This technology has been used in a wide diversity of fields (food, pharmaceutical, chemical and fuel industries) due to its advantages, there is an absence of toxic residue in the final product which allows not only the extraction valuable active compounds (fatty acids, pigments, polyphenols and vitamins) free of solvents, but also to remove undesirable compounds (pollutants, toxins and pesticides) [138]. Additional advantages are great extraction selectivity, short processing times, requirement of minimal solvents, low degradability of the extracted product and the fact that the remaining biomass can be treated with other techniques in order to continue extraction.

The most important conditions are temperature, pressure and co-solvent. The selection of each parameter will depend on the specific compound searched [138]. The most used solvent is CO_2 due to its thermodynamics and heat transfer properties. Moreover, it has a low critical point (31°C, 73 bar). Furthermore, the polarity of CO_2 can be modified by the use of co-solvents such as ethanol, and in this way also extract polar components.

Studies demonstrate that the extracts obtained with this technique are better than natural spice for flavoring purposes as SFE could reduce aflatoxin in the final products [139]. In addition, numerous bibliographic references show that carotenoid extraction is better at higher pressures [113].

Furthermore, when analyzing the extraction of β-carotene and capsaicin at the same time, capsaicin shows lower solubility, yet the solubility of β-carotene did not change in the presence of capsaicin, a factor to bear in mind when designing the separation process of coloring and hot components from paprika [140]. Several pepper species have been used to obtain natural compounds of interest using supercritical fluid extraction including capsaicinoids, oleoresins, pigments, tocopherols and even aflatoxins (**Table 10**) observing large variations between the pressures applied in each of the methods referred to in the bibliography. The greatest amount of studies is related to the specie *C. annuum* since it is the best known, widespread and cultivated specie of the genus *Capsicum*.

Target	Species	Pressure	Temp.	Flow rate	Time	Pepper	Yield	Ref.
		MPa	°C	cm s^{-1}	min	g	%	
Capsaicinoids	*C. frutescens*	20.5	40	0.064	10	50	5.2	[108]
	C. frutescens	7.84×10^{-3}	55	1	—	70	7.1×10^{-4}	[103]
	C. annuum	55	40	—	—	—	8.6	[92]
	C. annuum	12	40	—	390	—	—	[102]
	C. annuum	40	55	0.9–1.2[a]	—		93	[93]
	C. annuum	7.84×10^{-3}	55	1	—	250	7.1×10^{-4}	[103]
	C. chinense	15	60	2.15×10^{-3}	90	2.5	0.5	[111]
Oleoresins	*C. frutescens*	21.5	40	0.071	10	50	0.3	[108]
	C. annuum	30	40	—	360	—	8.2	[102]
	C. annuum	40	35	1–1.5	—		7.4	[94]
	C. annuum	43–54	40	1	—	25–30	—	[113]
Pigments	*Capsicum* spp.	47.5	80	sm	—	—	6	[90]
	C. annuum	36	45	—	—	—	17.4	[92]
	C. annuum	20	35	0.9–1.2	—	—	93	[93]
	C. annuum	40	35	1–1.5	—	—	7.2	[94]
Aflatoxins	*C. annuum*	30	50	—	2	7.8	16.2	[139]
Tocopherols	*C. annuum*	5	25	0.9–1.2[a]	—	—	84	[93]
	C. annuum	40	35	1–1.5	—	—	100	[94]

sm, semi-continuous; —, not described.
[a]Propane.

Table 10.
Different experimental conditions carried out in peppers by supercritical-fluid extraction.

4.3.3 Pulse electric field extraction

Pulsed electric field (PEF) consists of a non-thermal method that is extensively used in food processing applications due to, among other things, its ability to kill microorganisms in liquid foods. This method involves the application of short duration electric pulses, making several pores on the cell membrane in what is called electropermeabilization or electroporation. This makes it possible for the selective recovery of intracellular components with low energy consumption as the dielectric breakdown theory explains. According to this theory, the membrane of the cell has a low-dielectric constant that when exposed to a strong electric field provokes ion migration, forming free charges of the opposite sign which accumulate at both membrane sides, generating a potential difference across the membrane which depends on the size and shape of the cells and the concentration of cells in suspension. This difference in charges makes cell walls undergo a compression, reducing the membrane thickness that results in the formation of micropores and increasing permeability (electroporation) [119, 141]. It can be used directly or as a pre-treatment prior to solvent extraction [142]. The parameters to take into account with this method are number of pulses, length of the pulses, energy input and biomass concentration.

Among the advantages of PEF is the short extraction time (usually under 1 s), efficiency at low temperatures, decreased energy losses and a successful cell wall breakdown [120]. The disadvantages are that membrane changes can be reversible, air bubbles make the process less effective and the efficiency of the method depends on electric field strength and electrode gap [142].

Further research is necessary since there is no data on the extraction of compounds with this technique. However, there are studies of the application of PEF for the production of dehydrated products or juices. As for drying, a study [143] done with red bell pepper at 320 J/kg, 2.0 kV/cm, 1 Hz and 30 pulses of 400 μs reported good results. Another study [144] with the same aim used different extraction conditions, which were 2.5 kV/cm, 100 Hz and 30 μs as the pulse time. As for juice production, *C. annuum* was studied with extracting conditions of 500 J/kg, 1.7 kV/cm, 1 Hz and 30 pulses of 300 μs demonstrating that juice from PEF treated paprika compared well in quality with enzymatic treatment [145].

5. Seed valorisation and industrial application

As it is being already done by some factories and companies in pepper growing countries, seeds, stalks or peels are extracted through whole fruit processing with or without semi-automated equipment. This is mainly done by the food industry in order to take away the non-edible parts of the vegetable to elaborate commodities as pepper powders (e.g., paprika), sauces, jams or pickled peppers. This way, stalks, seeds and placenta are usually removed and discarded.

But in many cases, placenta and seeds are subjected to extraction techniques in order to obtain essential oils to treat chronic ailments via topical administration in traditional remedies [146]. Even so, it is clear that pepper seeds are a multivalent source of many important compounds that could be used in different applications.

The elevated phenolic concentrations found in seeds make them an excellent target for extraction and use of these phenolic compounds, whether it is as antioxidant supplements, as food coating in order to increase shelf-life or as a potential source of functional animal feed. Moreover, the implementation of an extraction process in the food industry is dependent on the type of compounds of interest, as the yields of phytochemicals obtained may differ greatly. It should be noted that highly efficient and sustainable extraction methods continue to be a focus of research both in industry and academia [65].

Also, antimicrobial potential of phytochemicals present in pepper should be considered. Even though this would involve highly specialized equipment and maintain strict aseptic conditions, there is promise in their use as food preservers from common food-borne pathogenic microorganisms besides their antioxidative capability. Furthermore, their use as alternative antibiotics has hardly been researched and the safety of their administration or clinical use has not yet been assessed.

Regarding their nutrition attributes, the high linoleic acid and palmitic acid present in the seeds could lead to use them as a primary source of cooking oil, or as a main component in foods such as margarine, or preserver in certain pickled products [48]. Also, as they are a rich natural source of vitamins E and C, extracts of these vitamins could also be incorporated as constituents of many juices and other foods. Pepper-processing industry could find very rewarding to investigate on the yet unknown beneficial properties of its by-products and obtain additional profit by integrating existing extraction and purification technologies into their process chain.

6. Conclusions

It is clear that the wide spectrum of historical applications of peppers is supported by the latest findings on the properties of peppers. The diversity among

Capsicum species properties have been studied over the years, although many of the possible bioactivities have only been characterized in the whole fruit and there are few *in vivo* experiments reported testing phytochemicals of this genus or assessing its safety. Moreover, research on produce by-products has acquired weight within the scientific community and industry as of lately. The exploitation of food processing by-products means not only an affordable raw material for the extraction of valuable phytochemicals, but also a reduction of ecological and economic impact of agricultural and industrial activities. Further studies are necessary to explore in depth more efficient bio-active compounds from the underutilized and non-edible biomass of *Capsicum* spp. Furthermore, the great genotypic diversity of the many pepper varieties grown in different regions, have a high variety of bioactive compounds, some still to be discovered. Currently available knowledge about the medicinal phytochemicals found in the genus *Capsicum* should be considered to develop novel drugs to treat many diseases and ailments.

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Chapter 10

Use of Capsaicin for Nonlethal Technology

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Abstract

Hot peppers have been used as a seasoning for food, because of both the characteristic flavor and the peculiar feature of pungent sensation. In the same way, it has been used as cosmetics and in other forms such as topical stimulants and body creams. Nonetheless, this chapter shows a not-so-common application of pepper's properties, like the so-called pepper spray for defense technology. According to the premise that the action of the police officer must be adapted to each situation, it is necessary to prepare him for the gradual and proportional use of force, avoiding excesses and even lethal resources. In this way, pepper spray is a resource that can place police action within this premise. The main aspects of pepper spraying technology are discussed and the main concepts related to use, hazards, solution formulations and capsaicinoids quantification tests are pointed out. The advance showed here is around the use of capsaicinoids to extract raw material instead of OC to produce pepper spray solutions. The absence of oils and resin is the main point, as nonflammability is achieved much easier and avoids the use of hazardous organic solvents. The chemical analyses by GC-MS/FID applied to quantify the capsaicinoids, with capsaicin and dihydrocapsaicin together, are demonstrated and can be reproducible for the quality control of this product.

Keywords: pepper spray, nonlethal technology, capsaicinoids, capsaicin

1. Introduction

The Article XII in the Declaration of the Rights of Man and of the Citizen—France—1789, declares that "*The guarantee of the rights of man and of the citizen necessitates a public force; this force is thus instituted for the advantage of all and not for the particular utility of those in whom it is trusted*".[1] The exercise of police work in civic security is very complex and thorough; it requires, at the same time, the application of the law to those who do not want to comply with it and requires the correct, just and necessary application of force to maintain law and order. If, on the one hand, the violation of the law is not correct, the same law provides that this citizen must be treated by the State according to the citizen protective premises of that law [1].

The State, through the Police, has to act with legality and attention to the protection of the human person; in other words, "police solutions" to violations of the law

[1] Declaration of the Rights of Man and of the Citizen—France—1791.

must not incur excesses by law enforcement officers. In order to adjust the conduct of law enforcement officers, there is a need to prepare to use force gradually, according to the premise that police action should suit each situation—considering that most police cases do not involve the use of lethal weapons—so, the police response must include nonlethal solutions [2].

The gradual use of force is an internationally recognized concept, which aims to point out the best way, in terms of containment, during police assistance [3]. Resisting the use of nonlethal technologies means exposing public security professionals to irreparable excesses and errors, due to their effects.

Among the nonlethal solutions for the use of force (in response to police incidents) are disabling agents that do not require physical contact between the police and the lawbreaker (for example, pepper spray), with the advantage of maintaining the offender at a safe distance and unable to harm others, including himself and the police officer.

We must be aware of the growing issue of police officers who are injured during police duties. There is great public attention, fully justified and correct, regarding the excessive actions that can result from the confrontation between citizens and police officers, with a primary focus on avoiding lethality in these situations. However, it is important to understand that the State, through its agents, vulnerability is also not desirable, that is, the impediment to the use of lethal actions could be compensated by the access to nonlethal solutions, more immediate equipment, as previously said, the pepper spray is an example.

Still with respect to the vulnerability of the police, there is the attack of animals (usually dogs), against police officers or third parties; it also strongly indicates the use of pepper spray as a nonlethal solution. There are reports of criminals who train dogs to attack police officers, or even police dogs; such a condition would be mitigated with the use of pepper spray.

2. Pepper spray

Usually, hot peppers are used as a seasoning for food, because of both the characteristic flavor and the peculiar feature of pungent sensation. In the same traditional way, they have also been used as cosmetics and in other forms such as topical stimulants and body creams. Nonetheless, this chapter shows a not-so-common application of hot pepper's properties, like the so-called pepper spray for defense technology. Since the 1970s, pepper spray is used as a nonlethal defense solution by law enforcement forces and civilians around the world [4].

The irritating effect—when the solution contained in the spray is sprayed on the face—allows for disabling the opponent by providing some advantage in self-defense (or third party), crowd controlling, and dispersing civilian disturbances. The immediate and involuntary closure of the eyelids and lacrimation were the main expected effects. Burning eyes, cough, nasal discharge, difficulty breathing, burning in the mouth, and other reactions occur within seconds of exposure. Those pain effects, together with the psychological (or moral) reflexes, cause temporary disability, thereby allowing police officers to avoid the opponent's resistance or even counteracts. The temporary incapacitation lasts for about 10–15 min and, after a few hours, the individual self-regains full capacity without any permanent effect, in other words, without irreversible harm. These properties take the pepper spray for defense as a nonlethal defense technology, an alternative to lethal force.

There are cases of serious injuries from the use of pepper spray, like the situations reported were the mechanical damage to the eye by the high-pressure aerosol jet applied directly to the eye [5], but this can be avoided through training and respecting the safe distance for application. Use in closed spaces, or "in door" uses, is dangerous too, because the spray can asphyxiate in closed spaces, for no other reason other than the aerosol taking the place of breathable air. Several authors have studied the permanent injury cases with the use of pepper sprays (when used improperly), and the most important permanent injury is cornea damage, followed by pulmonary injury cases [6–10]. Nevertheless, in general, the reported cases of death always involve adverse circumstances, due to either the environment or the individual, or even both, which leads to death for reasons other than the single effect of the irritant. The inverse problem is dangerous too, because the inefficiency of the incapacitating properties of the spray solution leads the law agents at risk [11]. Both injuries and inefficiency should be considered when developing pepper spray solutions. The challenge is to find the best formulation that is efficient without causing damage.

Although there are several types of hot pepper extracts, the most widely used irritant agent in the production of pepper sprays is the oleoresin capsicum (OC) [12]. OC is a viscous liquid extracted from the hot peppers fruit (*Capsicum*), which contains more than a hundred different types of substances, consisting of complex mixtures, mainly of lipophilic molecules, and does not have a simple chemical formula [13].

Oleoresin can be just a colorant red type (paprika), colorant and pungency type (red pepper), or high pungent type (capsicum). With regard to industrial production, the capsicum OC pepper spray type has been the most supplied to police forces.

The nonivamide (pelargonic acid vanillylamide or PAVA) is a surrogate substance and some manufacturers use it instead of OC or capsicum extracts. This irritant can be found in some pepper extracts at lower concentrations; however, PAVA can be synthetically made, at low costs, and used alone or together with OC in some formulations. PAVA is much less irritating and its effect is felt much later than capsaicin.

Notwithstanding, there are formulation improvements by the application of another irritant capsaicin content that presents more homogeneous composition and can be more effortlessly controlled than OC. This irritant is an alternative type of pepper extract named natural capsaicin (N.Cap) [14] that engenders high-quality sprays. In practice, the irritant effect is not so different from OC but have a better-defined chemical composition and other advantages. The first advantage point is the absence of red dyes plus the characteristic seasoning smell of the OC, and the second point is the absence of oils and resins making possible the totally nonflammable solution formulation. Therefore, the N.Cap spray solutions cause just pungent effects and nothing else.

The N.Cap can be purchased from manufacturers that supply common pepper extracts to the pharmaceutical market. The product presents a white crystalline coarse powder with the presence of flat-shaped flakes. The irritant content requirement meets a minimum of 95% capsaicin plus dihydrocapsaicin. The remaining 5% consists of the extraction residues at pharmaceutical levels and by other capsaicinoids. Furthermore, it meets maximum drying loss of 1%, maximum ignition residue of 1%, and residual solvent (methanol) max 3000 ppm (USP-467). It has a melting range of 57–66°C. Metal content is <10.0 ppm and arsenic <2.0 ppm.

3. The chemical composition of OC

The chemical composition of OC depends on several factors—the species of plant, the extraction method, the season of the year in which the crop is harvested, and the region of the plant where it is cultivated—as well as processing way and production. All of these and a lot of other factors contribute to make it difficult to standardize the concentration of irritating agent in pepper spray solutions, thereby leading to a wide range of capsaicin amounts contained in different commercial products and also in separate lots from the same supplier.

Since they produce the desired irritant effect, the capsaicinoids are the most important substances present in the OC used in pepper spray solutions. Other substances such as phenols, acids, alcohols, aldehydes, carotenes, esters, oils, and resins are also present in the OC, but cause a little or no irritant effect compared to capsaicinoids. It should be noted that oils and resin compounds are potentially flammable, and they become undesirable by increasing the flammability of the spray solution. If the spray solution has flammable properties, a jet of flame can be produced if there is an ignition source in the path of the spray jet.

Furthermore, the OC normally has a strong spice smell, a striking reddish brown color, and has hydrophobic characteristics, thereby requiring organic solvents (or even emulsions) for its complete dissolution. All these features mentioned are undesirable in pepper spray technology.

The OC extraction has wide types of processes, and the most simple is the extraction through ground powder fruits. Organic solvents like hexane, ether, alcohols, acetates, and ethylene dichloride are common extraction liquid phase media. In general, the extractions pass by two or three times, removing and replacing with pure solvent at each time. Other simple extractions consist basically to macerate the fruit with olive oil or other seed oils, but the impurity is high and the standardization is far from ideal to use as a raw material for pepper sprays. Modern processes of extractions are advanced Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction, ultrasound-assisted extraction, and pressurized liquid extraction. All they are more suitable for better-controlled extractions. However, these methods are very expensive and greatly increase the production costs [13].

The concentration of capsaicinoids in OC, in general, shows variations from 1 to 10%, depending on the type of fruit, solvent, process, and so on. Some types of OC capsicum have an approximate concentration range of 3–6% of capsaicinoids, but some Indian suppliers may have OC with 20% of capsaicinoids [15]. Of course, this large amount of concentrations and side substances present in the OC leads to a difficult standardization of raw material for the pepper spray proposal [12].

4. Capsaicinoids

The substances so-called capsaicinoids can be represented, in a simple way, by the binding of a vanillylamide group with fatty acids as a principal characteristic molecular structure, where the fatty chain would have 9–11 carbon atoms. The capsicum fruits synthetizes the capsaicinoids in the placenta-fruit region, close to the tissue adjacent to the seeds. This type of metabolite is unique to *Capsicum* genus. Although little data exist on this compound's biosynthesis, there are reports that the principal two pathways are phenylpropanoid and fatty acid metabolism [16]. The amount of capsaicinoids in the pepper fruit reaches the maximum concentration in about 2 or 3 months of natural fruit development, and after the deceleration initiated by the action of the peroxidase enzymes, the concentration decreases generating secondary compounds. Certainly, the concentration in the fruit varies with the plant species, cultivation conditions, soil, place, seasonality, and so on.

From the point of irritant effect, the capsaicin is the main substance in the midst of capsaicinoids compounds, followed by dihydrocapsaicin. The total capsaicinoids content in the fruits has a typical concentration range of 0.1–1.0% on a dry basis [17], but it is not a rule and some species have lowest concentration like 0.003% and others have a far high concentration like 1.86% [18]. In general, the capsaicin and dihydrocapsaicin together correspond to ~90% of the total capsaicinoids present in the ready fruit. The typical distribution is 60–70% capsaicin, 20–30% dihydrocapsaicin, 3–7% nordihydrocapsaicin, ~1% homocapsaicin, and ~ 1% homodihydrocapsaicin. However, this is not true in all cases; once, for example, there is evidence that the degree of fruit maturation and incidence of sunlight in the cultivation area, temperatures, and water availability are important factors in the accumulation and proportion of capsaicinoids in the fruit [19]. **Table 1** shows the name, chemical structure, and molecular formula of the major capsaicinoids.

Unfortunately, there are associations between OC and the molecular capsaicin formula in some pepper spray technicians media, thereby leading to erroneous perception that OC and capsaicin are synonymous with the same substance. Nevertheless, the molecular formula $C_{18}H_{27}NO_3$ is not the OC formula but only the capsaicin molecular formula, which is contained in OC.

4.1 The biological action of capsaicinoids

The biological action of capsaicinoids is complex and multi-targeted mechanisms. The action involves, above all, the activation of peripheral nerve receptors

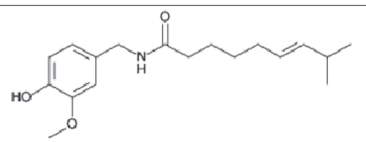
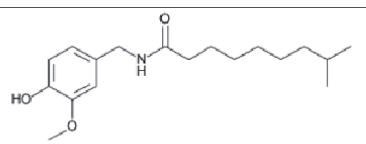
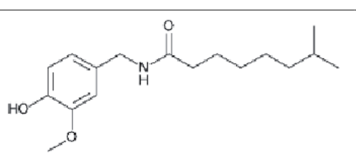
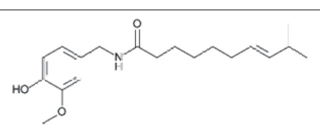
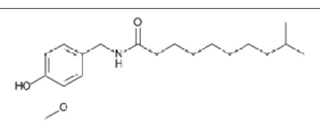
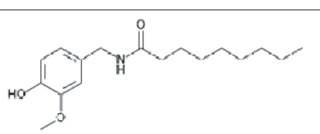
Homolog name	Chemical structure	Molecular formula	CAS
Capsaicin		$C_{18}H_{27}NO_3$	404-86-4
Dihydrocapsaicin		$C_{18}H_{29}NO_3$	19408-84-5
Nordihydrocapsaicin		$C_{17}H_{27}NO_3$	28789–35-7
Homocapsaicin		$C_{19}H_{29}NO_3$	58493-48-4
Homodihydrocapsaicin		$C_{19}H_{31}NO_3$	20279-06-5
Nonivamide (PAVA)		$C_{17}H_{27}NO_3$	2444-46-4

Table 1.
Name, chemical structure, molecular formula, and CAS number of the major capsaicinoids.

in the mucous membranes, by interactions of the capsaicinoids with the vanilloid transient receptor potential type-1 cation channels (TRPV1). These receptors are normally activated by temperatures between 37 and 45°C when opening the calcium channels and induce reflexes of burn sensation. However, when TRPV1 binds with the capsaicinoids, it causes these channels to open below 37°C and the burn sensation occurs at normal body temperature. This is why capsaicinoids are linked to the sensation of heat [20].

Furthermore, the interactions of the TRPV1 with capsaicin are strictly related to pain by nociceptor activation and the release of substance-P. Nociceptor is a nervous sensory receptor responsible for pain mechanisms in the human body [21]. Prolonged contact may cause nerve endings to be desensitized; however, it does not lead to a permanent desensitization state and can be reversed by discontinuing contact.

The capsaicin and dihydrocapsaicin content in the pepper spray solution is determinant for the irritant properties and the disabling effect of the spray. Thus, to evaluate the effectiveness of the disabling properties of the spray, the capsaicinoids concentration in the solution can be measured, particularly the capsaicin together with dihydrocapsaicin [14]. Obviously, the limits to this concentration must take into account the toxicity and security against injury. So, some connections among the concentration and the irritant effect must be evaluated.

The Scoville test, whose unit of measure is SHU, is a known form of evaluating the effect of pungency or blazing of the peppers. This method was developed in 1912 by Wilbor Scoville [22] giving a five-level scale for pungency: nonpungent (0–700 SHU), low pungency (700–3000 SHU), moderate pungency (3000–25,000 SHU), high pungency (25,000–70,000 SHU), and very high pungency (>80,000 SHU). However, the Scoville method is a taste organoleptic method made by a dilution series of the pepper extracts. It makes subjective responses and unreproducible results. Qualitative and quantitative information can be precisely obtained by modern instrumental chemical methods with many advantages [12, 23].

5. Toxicology of capsaicin

Since the peppers are largely used as a food, through several forms and quantities around the word for centuries, it is not expected that it is a poison. The individual consumption of capsaicin in India may be around 7–120 mg/day. In Northeast Thailand, the individual consumption may be 26 g of (jaew) pepper per meal [24]. The main concern with the use of pepper spray is with the possible toxic properties of the solution. Another important aspect is the observation of the tactical way of use, which involves the amount of solution applied, environment conditions, closed spaces and psychological state of the aggressor; all of these characteristics must be well studied in action using pepper spray.

Previous study shows that the toxic level of 60-kg human consumption of capsaicin corresponds to consumption of 1.94 kg of dry weight of capsicum fruit. Obviously, there is no person who can consume this amount of dry pepper at once; due to the pain and pungent sensation, it prevents over consumption [25].

There is no consensus in the literature for the acceptable dose of capsaicin for oral, skin, and eyes human contact. The studies are commonly connected with lethal doses values (LD_{50}), which means a quantity limit that kills 50%, at least, of the population studied. This kind of test programs normally uses rats or mouse to access results. Rabbits, dogs, and guinea pig are common too. It is sustained by a presumption that these animals have a close correlation with human responses to capsaicin [26].

An oral LD_{50} value was reported at range of 60–75 mg/kg (Swiss male albino mice). On the other hand, another experiment with the same mice type and procedure, just changing the solution vehicle, showed LD_{50} at 190 mg/kg [25]. Another work reports male mice with LD_{50} values of 118.8 mg/kg and for female mice of 97.4 mg/kg [27]. The U.S. National Library of Medicine—National Center for Biotechnology Information—reports 47.2 mg/kg for mouse. OSHA or NIOSH has reported no occupational exposure limits for capsaicin or OC. As can be seen in these short examples above, there is not a single value to use to computation and derive secure concentrations of spray solutions to manufactories.

Some inhalation response studies showed no evidences that inhalation of capsicum oleoresin spray causes respiratory compromise [28]. An investigation made with the concern on the respiratory effects of OC concludes that the exposure did not result in abnormal hypoxemia or hypoventilation. This experiment was made by aerosol delivery exposure box with a hood for the subject attached to one end of the exposure box. The aerosol was 5.5% OC (with 0.92% of capsaicinoids) solution with isopropyl alcohol as carrier agent and isobutane/propane as propellant [29]. These types of studs are more conclusive and can take better access for more suitable results to manufactories.

The ocular contact with capsaicin is the primary incapacitation response. Furthermore, the hydrophobic properties of capsaicin (and capsaicinoids) allow them to penetrate the eye tissue, accessing the terminal nervous and, consequently, causing pain and great lacrimation response. The use of contact lenses can lead to an increase in the duration of the effect, due to the accumulation under the lens. In these cases, care should be taken to remove the lens as soon as possible.

6. Experimental

The quantification of capsaicin in pepper spray can be made by GC-MS/FID [14]. This experimental result presented here aims to exemplify the quantitative and qualitative analysis of the samples of the commercial Brazilian pepper spray. About 500 μl of solution sample was weighed on an analytical balance and the contents were solubilized with HPLC grade acetone and transferred quantitatively to a 1 ml volumetric flask. The solution was swollen and an aliquot was conducted for analysis by gas chromatography.

A model GC-2010 equipped with a mass spectrometer GCMS-QP2010 Ultra and an automatic sampler AOC-5000 (Shimadzu) was used. The chromatographic separation was performed using RTx-5MS capillary column (Restek) with a stationary phase containing 5% phenylmethyl and 95% polydimethylsiloxane (30 m × 0.25 mm × 0.25 μm of film). The temperatures of the injector and the transfer line of the mass spectrometer were 300°C. The samples were injected with a split ratio of 1:50. The oven temperature program followed the following conditions: 50°C (2 min), heating rate from 10°C/min to 300°C (2 min). Helium was used as the carrier gas in a flow rate of 1.2 ml/min. The mass spectrometer was operated in electron impact ionization mode, with acquisition in the scan mode with m/z between 50 and 500.

To identify the compounds, a comparison of the spectra with the NIST library was carried out. Only substances whose similarity was greater than 80% were considered, compared with data from the library. **Figure 1** shows the chromatogram result with the solvent around 7.2 min and capsaicin and dihydrocapsaicin peak around 24 min.

With the purpose of quantifying, the capsaicin and dihydrocapsaicin are integrated together and the calibration curve was made by this result against

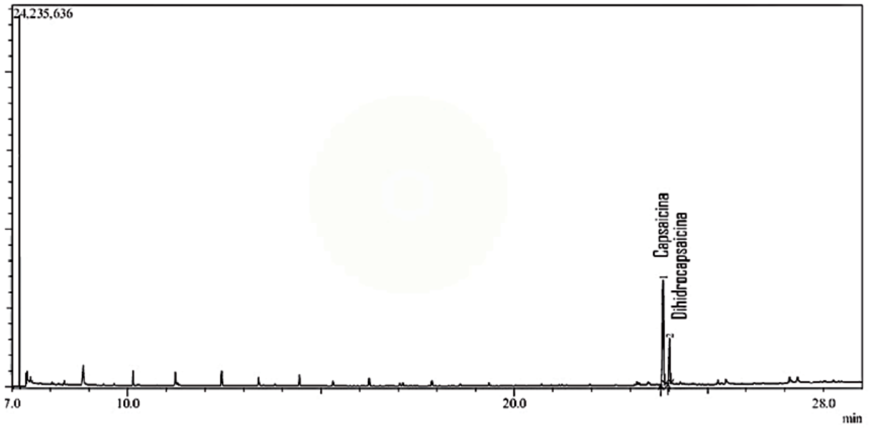


Figure 1.
GC-FID chromatogram of capsaicin and dihydrocapsaicin peaks with retention time around 24 min.

standard solutions made with Sigma-Aldrich Capsaicin (purity capsaicin: 61.1%, dihydrocapsaicin: 31.2% LOT#: LRAA9221 09 September 2015). The regression coefficient R^2 was 0.999 and uncertainty was 0.9%. The subsequent dilution was satisfactory by the statistical linear fit results. In this way, it is possible to quantify the capsaicinoids, that is, capsaicin and dihydrocapsaicin together, on the pepper spray solution.

7. NMR analyses

Nuclear magnetic resonance (NMR) is a powerful analytical tool for identification and quantification of capsaicinoids in raw materials for pepper spray solutions. Its ability to analyze samples with minimum pre-treatment allows a safe analysis that preserves the original characteristics. In the specific case of organic molecules, their sensitivity is increased by the detection of the isotope ^1H. Thus, the ability of NMR to evaluate the quality control of the nonlethal arms industry becomes clear.

In order to verify the N.Cap content, a sample of 26.4 mg was weighed and solubilized in 600 µl of CDCl3 in a 5-mm NMR tube. The analysis was performed at 25°C using glass capillary for the quantification of the maleic acid present in the solution with 100 mmol of D_2O. The broad band inverse (BBI) probe inserted into Bruker Avance III 11.75 T equipment was used. The simple pulse sequence used was zg. The spectra were processed with 0.3 Hz line broadening and zero filling.

Figure 2 shows the graphical results, and the relative chemical shift integration of hydrogens of capsaicin and dihydrocapsaicin are presented. All the signals of the main chemical groups present in the sample were identified through the NMR analyses. Groups 1 from capsaicin and 2 from dihydrocapsaicin (amplified in **Figure 2**) can be used for relative integration of the signals. In addition to being unobstructed, they had a higher signal-to-noise ratio due to having six hydrogens contributing to the integration of each signal. Thus, it was possible to verify the proportion of these compounds among themselves present in the mixture. This led to the proportion of 33% dihydrocapsaicin (minority) and 66% capsaicin (majority), the latter being twice the concentration of the former.

The ^1H NMR technique proved efficient in the identification and relative quantification of the components present in the mixture of pepper derivatives. The spectra were acquired quickly and with a simple sample preparation step.

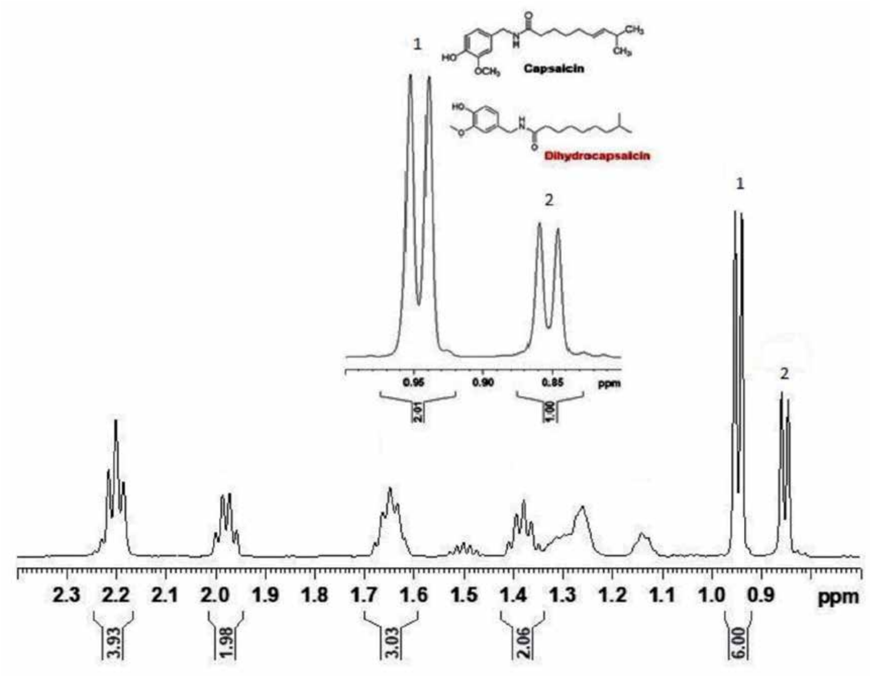


Figure 2.
^{1}H NMR identification and relative quantification of the components present in the N.Cap.

The analyses allowed a broad view of the absence of other compounds, even if minority, thereby proving the purity of the material and absence of degradation products. On the other hand, the presence of other components could be identified concomitantly in a mixture of higher complexity. Thus, this work suggests that ^{1}H NMR can be used as a quality control tool in the nonlethal industry.

8. Conclusion

The main aspects of pepper spraying technology were discussed and the main concepts related to use, hazardous, solution formulations and quantification tests were pointed out. This technology is relatively recent, and a wide field of research is not addressed yet. The advance showed here is around the use of capsaicinoids to extract raw material N.Cap instead of OC to produce pepper spray. The absence of oils and resin is the main point, as nonflammability is achieved much more easily and without the use of hazardous organic solvents. Chemical analyses by GC-MS/FID applied to quantify capsaicinoids (capsaicin and dihydrocapsaicin together) have been demonstrated and can be reproducible for the quality control of this product. The ^{1}H NMR provides information about raw material and how to access quality information and impurity with our relative concentration.

The concentration range between 0.2 and 0.3% (mass of solution) of capsaicinoids guarantees satisfactory spray efficiency, allowing the aggressor to be incapacitated without serious injury. Toxicological study preferred with human voluntaries is the way for a better evaluation of limits.

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Section 4

Capsicum and Neurobehavioral Parameters

Chapter 11

The Effects of Consumption of *Capsicum* on Some Neurobehavioural Parameters

Eme Efiom Osim, Anyauba Nmaju, Azubuike Nwankwo, Udemeobong Okon and Joshua Iwasam

Abstract

Capsicum annum, an extensively cultivated vegetable, is commonly used to spice many dishes prepared in several parts of the world. It contains capsaicinoids, which give it a characteristic pungency. The most active and well known amongst these capsaicinoids is capsaicin (8-methyl-N-vanillyl-6-nonenamide), which is neurogenic and may affect neuronal functions. Therefore, our study investigated the effects of consumption of chilli pepper and capsaicin diets on neurobehaviour of CD-1 mice. The neurobehavioural parameters assessed were anxiety, motor coordination, pain, social behaviour, learning and memory. The animals were randomly assigned into three groups of 10 mice each, namely, control, pepper-diet (20% w/w), and capsaicin-diet (10% w/w) groups. Their learning and memory abilities were assessed through their ability to locate the hidden platform model of Morris water maze apparatus. The elevated plus maze and light-dark transition box were used to assess anxiety-related behaviour, while the beam walking test and nesting behaviour were used to determine motor coordination and social behaviour, respectively. Tail immersion, hot plate, and formalin tests were conducted to assess pain perception. Consumption of the chilli pepper and capsaicin diets decreased pain perception, increased anxiety, and impaired learning and memory but enhanced social behaviour and motor coordination in mice.

Keywords: *Capsicum annum*, capsaicin, pain, social behaviour, anxiety, motor coordination, learning and memory

1. Introduction

Spices are used all over the world not only for their flavours but also for their medicinal properties [1]. One of such spices is chilli pepper. Chilli pepper or chillies are vegetables used world over for culinary purposes and belong to the family Solanaceae [2].

These peppers are widely used as spices in food industry and in a broad variety of medicinal applications worldwide [3]. These spices are remarkable sources of antioxidant compounds including phenolic compounds and flavonoids, of which their consumption has potential health benefits due to their activity as free radical scavengers and may also help prevent inflammatory diseases and pathologies associated with oxidative damage such as atherosclerosis and Alzheimer's disease [3].

Chilli peppers are called *Ntokon* in Efik, *Osé* in Ibo and *Brukunu* in Hausa languages in Nigeria. They are usually red or green in colour. It is the most commonly consumed pepper in Nigeria.

The substances that give chillies their hot sensation and intensity when ingested or contacted are pungent chemical compounds collectively known as Capsaicinoids with Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) as the most abundant [4]. Exposure to the skin causes intense burning sensation while exposure to the eyes leads to intense tearing, conjunctivitis and blepharospasm [5].

The burning sensations associated with Capsaicin ingestion result from the activation of transient receptor potential, vanilloid 1 (TRPV1) located in the gut and other organs [6]. The stimulation of these TRPV1 receptors brings about the influx of sodium and calcium ions which results in the depolarization of nociceptive neurons, leading to the firing of action potentials and finally the sensation of spiciness [7]. Capsaicin is an alkaloid which is highly volatile, odourless, colourless and hydrophobic [8]. It structurally, belongs to a group of chemicals known as vanilloids, having a vanillyl (methylcatechol) head group (A-region) and an aliphatic tail (hydrophobic—C-region) linked by a central amide bond (B-region). This combination of these regions is accountable for the pharmacological activities of capsaicin [8].

Numerous health benefits are believed to emanate from Chilli pepper consumption [7]. Kempaiah et al. [9] reported that Capsaicin demonstrated protective effects against obesity and cholesterol by speeding up metabolism through stress hormone release. It is used as a topical agent in formulations against arthritis [10] and also in preparation of defensive sprays because of its irritant properties and ability to cause neurogenic inflammation (stinging sensation of hands, eyes and mouth) [11, 12].

Capsicum annum contains capsaicin which is neurogenic [11] and therefore, can affect neuronal activities in the body [13]. It is therefore conceivable that it may affect some nervous function parameters such as pain, social behaviour, anxiety, motor coordination, learning and memory. Hence, we present the report of our study that investigated the comparative effects of long-term consumption of capsaicin and chillies on the aforementioned neurobehavioural parameters using mice as experimental models to ascertain whether the effects obtained with pepper-diet consumption can be attributed to capsaicin.

2. Materials and methods

2.1 Preparation and storage of experimental diets

Half-washed basin of fresh red chilli pepper (*Capsicum annum*) was procured from a local market in Nigeria. It was washed and sun-dried for 4 days. The dried samples were then pulverised using electric blender to obtain a fine powder. The pepper powder was then stored in air-tight rubber container from which pepper diets were prepared.

Capsaicin (95% pure) was obtained from Wuxi Gorunjie natural-Pharma Co. Ltd., Jiangsu China.

2.2 Animal treatment

Thirty (30) male mice of CD-1 strain weighing between 22 and 34 g were used for the study. They were kept in a well-ventilated room under room temperature (25 ± 2°C), humidity of 8 ± 5% and 12/12 h light/dark cycle and allowed 1 week for acclimatisation to the research environment before the experiments. They were randomly assigned into three groups, namely; control group that received normal rodent chow, pepper group that were fed 20% chilli pepper diet and capsaicin group that were given 10% capsaicin diet. Each group comprised 10 mice. Each mouse was allowed drinking water *ad libitum*. This treatment was done for 28 days and within this period, their beddings, feed and water were hygienically handled and changed daily.

2.3 Approval for animal use

Approval for this study was obtained from the Faculty Animal Ethics Committee of Faculty of Basic Medical Sciences, University of Calabar with Protocol number 014PY20314.

2.4 Behavioural protocol

2.4.1 Assessment of anxiety levels

2.4.1.1 The elevated plus maze

The elevated plus maze apparatus designed according to the description of Lister [14], and the test protocol adapted by Okon et al. [15] were used in this present study. This apparatus is used to assess the anxiety and fear levels of the mice. The test is based on the inborn aversion of rodents to open or bright illuminated spaces. The maze has two open arms (45×5 cm^2) with 0.25 cm high edges and two closed arms (40×5 cm^2) with 15 cm high walls radiating from a central square (5×5 cm). The open arms contain a slight edge (4 mm high) to prevent the mice from slipping and falling off the edge [16].

Prior to the test, the plus maze arms, surfaces and closed sides were cleaned with methylated spirit to eliminate olfactory clues and to remove faecal boll and urine. The mice were placed in the central square of the plus maze such that the mice faced an open arm away from the experimenter upon placement. Immediately after placement, a stop watch was started and the mice were allowed to explore the apparatus for 5 min. The test sessions were recorded and videotaped. Behaviours scored included open arm entry, open arm entry duration; head dip, rearing and stretch attend posture frequencies.

2.4.2 Assessment of motor coordination

2.4.2.1 Beam walking

The beam walking assesses fine motor coordination and balance [17]. This test examines the ability of the mice to remain upright and to walk on an elevated and relatively narrow beam [17]. The beam has a length of 120 cm, a width of 0.6 cm and is suspended about 60 above some foam pads. The beam is marked at 5 cm and 1 cm intervals. It is composed of wood and is coated with black paint. The mouse was placed on one end of the beam. The trial was started after the mouse has secured its grip on the beam and lasted for approximately 5 min. The tests were videotaped for scoring. The parameters scored included the number of foot slips and falls.

2.4.3 Assessment of pain perception

2.4.3.1 Tail immersion test

This test used by Ramabadran et al. [18] assesses the basic pain response in mice to thermal stimuli. This test measures spinally driven aspects of pain. Here, the animals were immobilised using tube restrainer (which also allowed free movement of the tail). The distal half of each mouse tail was immersed in water bath (at 50°C) contained in a beaker with 20 s time-out. The duration of time taken for the mice to flick its tail away from the heat of the hot water was measured in seconds. This is known as latency of flick [19].

2.4.3.2 Hot plate test

This is a test of thermal nociception, model of short duration stimuli [20]. Each mouse was exposed to a hot surface within a confined glass cage (whose temperature was maintained at 55 ± 0.5°C) for maximum duration of 30 s [21]. The time it took for each mouse to start licking its foot pad was recorded. Higher frequency and duration of paw lick indicate higher pain perception. The time taken for it to jump (latency of jump) was also recorded. The longer the latency of jump, the less pain it felt. These behaviours are the most common measures of pain threshold and are considered supra-spinally integrated [20].

2.4.4 Assessment of social behaviour

Nesting behaviour test used by Bender et al. [22] and Deacon [23] as an assay for social behaviour was used in this present study. The test was conducted in individual home cages. In total, 1 h before giving the mice nestling materials, all enrichment objects were removed from the home cages of the mice. About 3.0 g of nestling material was supplied to each mouse in its home cage and allowed for 24 h after which the nests were assessed using the rating scale supplied by Deacon [23] (**Table 1**). This assessment was based on what was seen in the home cages of the mice. Extreme care was taken while observation was carried out, as causing panic to a mouse could result in the destruction of the nest that was built.

Rating	Requirements
1	Nestlet not noticeably touched (90% or more intact)
2	Nestlet partially torn (50–90% intact)
3	Nestlet mostly shredded, often no identifiable nest site, 50–90% shredded, also, less than 50% remains intact, but less than 90% is within a quarter of the cage floor (i.e., not gathered into a nest site but spread throughout cage)
4	An identifiable, but flat nest, more than 90% of the nestlet is torn, the nest is uneven, material is gathered into a nest within a quarter of the cage floor, but the nest is flat with walls higher than mouse body height for less than 50% of its circumference
5	A (near) perfect nest, more than 90% of the nestlet is torn, nest is fairly even, the nest is a crater, with walls higher than the mouse body for more than 50% of its circumference

Source: Deacon [23].

Table 1.
Nesting behaviour rating scale.

2.4.5 Assessment of learning and memory

The Morris water maze developed by Morris [24] for assessing visuospatial learning and memory was used in this study. It was made of a circular polypropylene pool which was divided into four quadrants: Northwest, Northeast, Southwest and Southeast. It measured about 85 cm and 20 cm in diameter and depth respectively. The pool was filled to depth of 14 cm with water. The water was left to sit overnight in order to achieve room temperature (about 26 ± 2°C) and made opaque with the addition of milk to ensure camouflage of the escape platform. The platform was submerged to about 1 cm below the water surface. The pool was located in the laboratory with posters of diagrams hung on the walls to act as visual cues. During testing, the room was dimly lit with diffuse white light. The performance of the animals in the maze was recorded using a camcorder.

Testing in the Morris water maze lasted for 8 days. The first 3 days were for acquisition training with an invisible platform. The next 3 days were for reversal training with the hidden platform in an opposite quadrant. On the seventh day, a probe trial was conducted with no escape platform. On day eight, 4 trials were conducted with a visible platform. Sixty (60) seconds were allocated for each mouse to locate the platform during each trial. Mice which were unable to locate the platform were guided to the position of the platform. The timer was stopped when the mice located the platform within the 60 s. The time it took the mice to locate the platform was recorded as swim latency. After each trial, mice were placed in cages with shredded paper towel beddings to make them dry easily and a heating lamp was also provided to prevent animals from developing hypothermia.

2.5 Statistical analysis

The data derived from the tests were analysed by one-way analysis of variance (ANOVA) followed by post hoc student's Neuma using the computer software SPSS 2007 and Microsoft Excel 2007 for windows vista (Brain Series, China). Data were presented as mean ± SEM (Standard error of the mean) and p value less than 0.05 was considered statistically significant.

3. Results

3.1 Effects of consumption pepper (*Capsicum annum*) and capsaicin on fear and anxiety in the elevated plus maze

The open arm entry frequency of the pepper group was not significantly different compared to the control, whereas that of the capsaicin group was significantly lower ($p < 0.001$) compared to the control (**Figure 1a**). Both the pepper and capsaicin groups had significantly shorter ($p < 0.001$ and $p < 0.01$ respectively) open arm durations compared to the control (**Figure 1b**).

There was no significant difference in the head dips frequency of the pepper-diet fed mice compared to the control (**Figure 1c**). However, that of the capsaicin-diet fed mice was significantly higher compared to the control ($p < 0.01$). While the stretch attends posture of the pepper group was not significantly different compared to control, that of the capsaicin group was significantly higher compared to bot and pepper groups ($p < 0.001$) (**Figure 1d**).

3.2 Effects of consumption of chilli pepper (*Capsicum annum*) and capsaicin on motor coordination in the beam walking test

The foot slips of both the capsaicin and pepper groups were significantly lower compared to the control ($p < 0.001$) (**Figure 2A**). Also, the number of falls of both the capsaicin and pepper groups were significantly lower ($p < 0.001$ and $p < 0.01$ respectively) compared to control (**Figure 2B**).

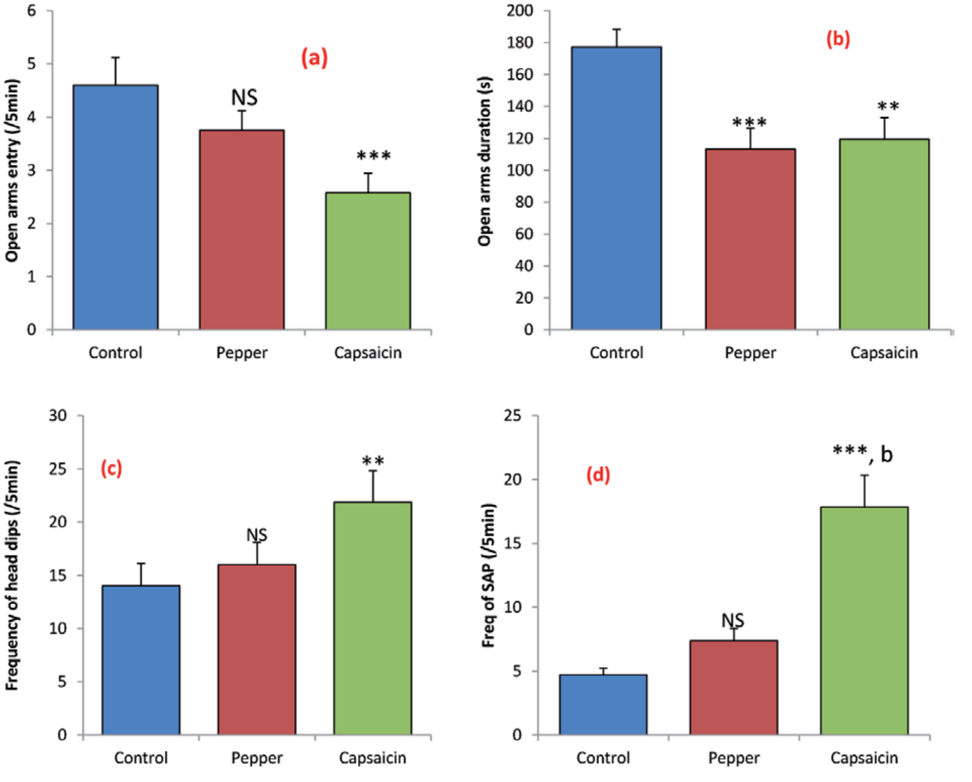


Figure 1.
*Comparison of (a) open arm entry frequency, (b) open arm duration, (c) head dip frequency and (d) stretch attend posture in the elevated plus maze test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. NS = not significant, ** = p < 0.01, *** = p < 0.001 vs control; b = p < 0.001 vs pepper group.*

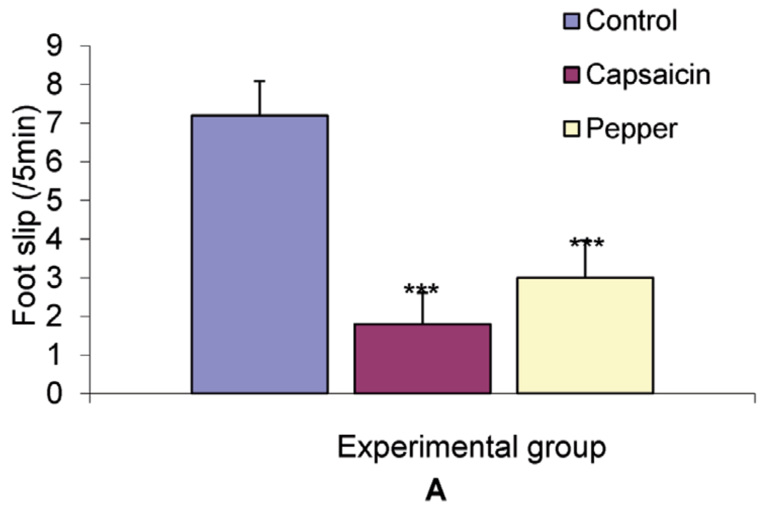
3.3 Effects of consumption of chilli pepper (*Capsicum annum*) and capsaicin on pain

3.3.1 Tail flick test

During the first test, the latency of tail flick of both the pepper and capsaicin groups were significantly longer ($p < 0.01$ and $p < 0.001$, respectively) compared to control. The value for the capsaicin group was significantly longer ($p < 0.01$) compared to that of pepper group (**Figure 3**).

3.3.2 Hot plate test

The latency of jump of both pepper and capsaicin groups were significantly longer compared to control ($p < 0.01$ and $p < 0.001$, respectively). Meanwhile, the latency of jump of the capsaicin group was significantly longer compared to the pepper group ($p < 0.001$) (**Figure 4a**). While the frequency of hind paw lick of the pepper group was not significantly different compared to the control, the value for capsaicin group was significantly lower compared to both control and pepper groups ($p < 0.001$ and $p < 0.05$, respectively) (**Figure 4b**). Pepper group had a paw lick duration that was not significantly different compared to control. However, the value for capsaicin group was significantly shorter ($p < 0.01$) compared to control (**Figure 4c**).



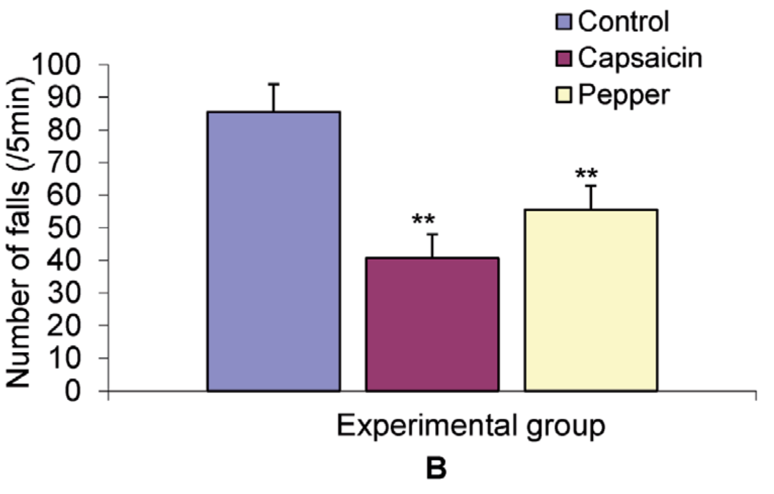


Figure 2.
*Comparison of (A) foot slips and (B) number of falls in the beam walking test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. ** = p < 0.01, *** = p < 0.001 vs control.*

3.4 Effects of consumption of chilli pepper (*Capsicum annum*) and capsaicin on social behaviour in the nesting behaviour test

The nesting score of the capsaicin group was significantly higher ($p < 0.01$) compared to control. The value for the pepper group appeared higher than control but was not significant. However, it was significantly lower ($p < 0.05$) compared to that of the capsaicin group (**Figure 5**).

3.5 Comparison of swim latency in the Morris water maze test for learning

During the acquisition training, the capsaicin group had a significant longer ($p < 0.001$) swim latency on days 2 and 3 compared to control (**Figure 6A**). The swim latency during the reversal training was significantly longer in both capsaicin and pepper groups on day 1 compared to control ($p < 0.001$) but not different on days 2 and 3 (**Figure 6B**).

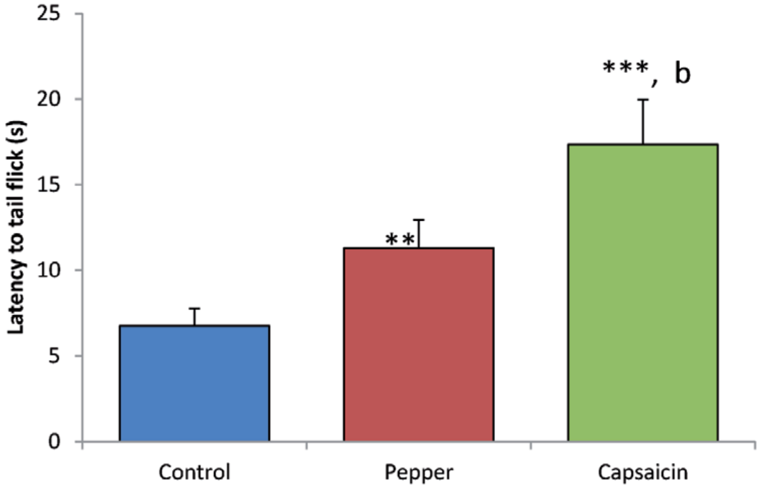


Figure 3.
*Comparison of latency of tail flick in the tail immersion test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. ** = p < 0.01, *** = p < 0.001 vs control; b = p < 0.01 vs pepper group.*

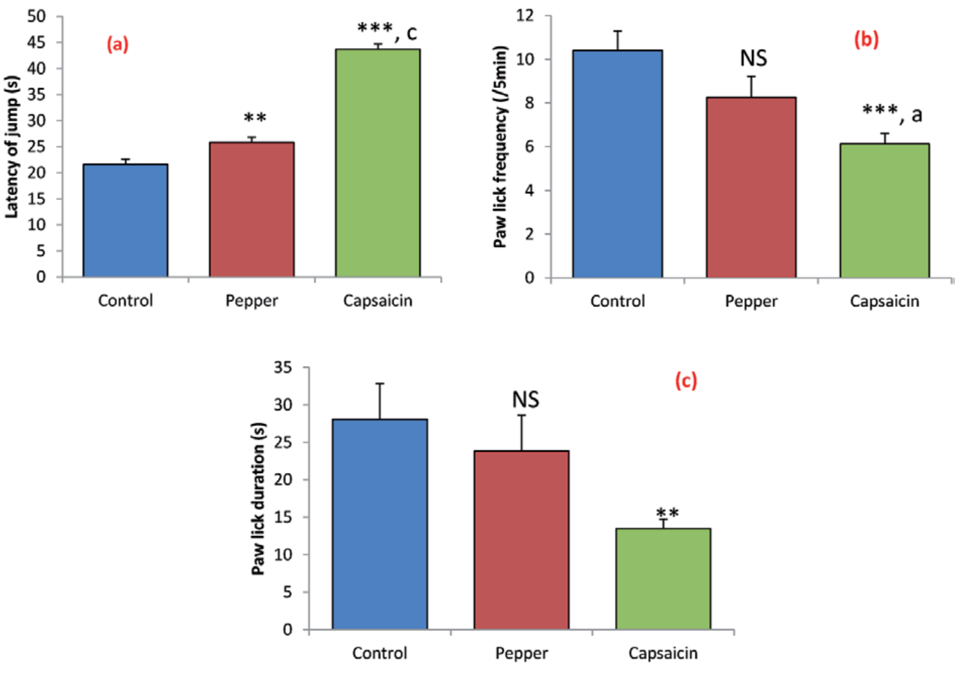


Figure 4.
*Comparison of (a) latency of jump, (b) paw lick frequency and (c) paw lick duration in the hot plate test of the different experimental groups. Values are expressed as mean ± SEM, n = 10. N = not significant, ** = p < 0.01, *** = p < 0.001 vs control; a = p < 0.05, c = p < 0.001 vs pepper group.*

3.6 Comparison of quadrant duration in the Morris water maze test for memory in the different experimental groups

In the probe trial, the pepper and capsaicin groups showed a significantly shorter quadrant duration compared to control ($p < 0.001$ and $p < 0.01$ respectively) (**Figure 7**).

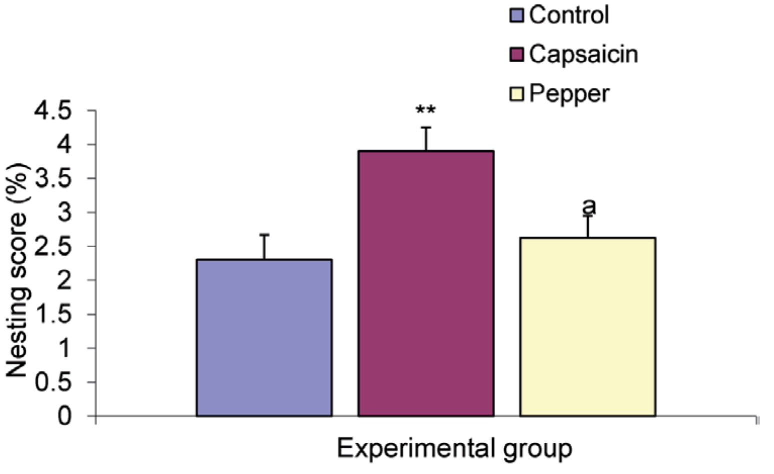


Figure 5.
*Comparison of the nesting score of the different experimental groups. Values are expressed as mean ± SEM, n =10. ** = p < 0.01 vs control; a = p < 0.05 vs capsaicin group.*

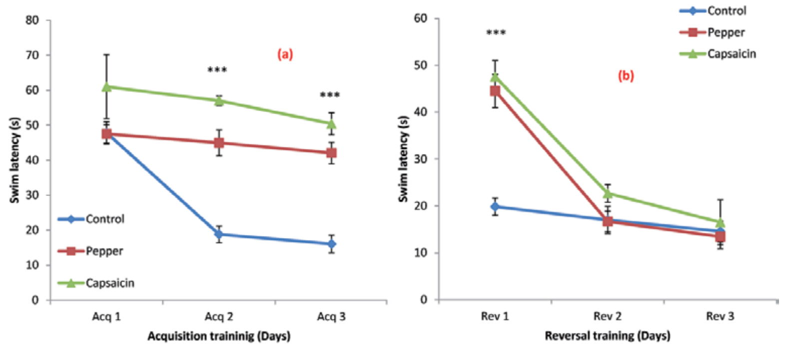


Figure 6.
*Comparison of the swim latencies of the different experimental groups in (a) acquisition and (b) reversal trainings. Values are expressed as mean ± SEM, n = 10. *** = p < 0.001 vs control.*

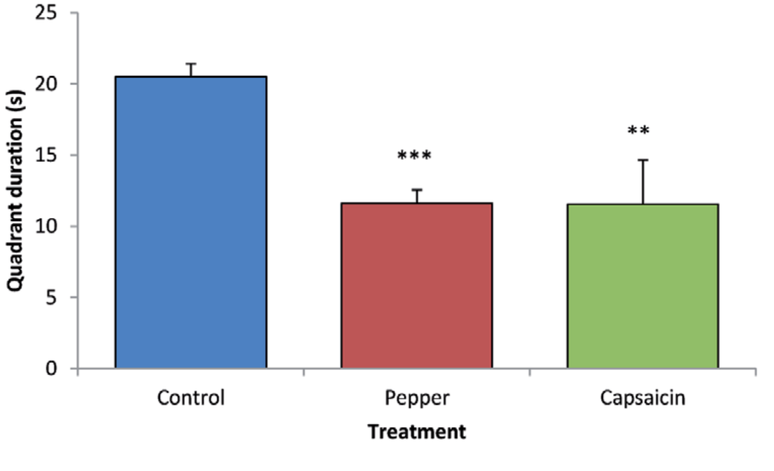


Figure 7.
*Comparison of quadrant duration in Morris water maze test of the different experimental groups Values are expressed mean ± SEM, n = 10. ** = p < 0.01, *** = p < 0.001 vs control.*

4. Discussion

4.1 Anxiety

Following the consumption of pepper and capsaicin diets, the pepper and capsaicin groups had lower open arm entry frequency and duration in the elevated plus maze test. Since fearful mice tend to avoid open areas (especially when they are brightly lit), favouring darker and more enclosed spaces [25], these results imply that chilli pepper and capsaicin caused increase in anxiety in the mice [26].

Increase in risk assessment behaviours such as head dipping and stretch attend posture indicate increased anxiety levels [27]. The results showed that though not significant, the head dips and stretch attend postures of the pepper group were slightly higher than control. On the other hand, the head dips and stretch attend postures of the capsaicin group was significantly higher than the control. These results showed that both pepper and capsaicin increased anxiety in the mice, but the anxiogenic effects of capsaicin were greater [26].

These behaviours confirm the anxiogenic tendencies of long term administration of pepper and capsaicin. However, its mechanism of action has not been ascertained. This is in agreement with the study of Choi et al. [28]. Hakimizadeh et al. [29] also reported that direct injection of capsaicin in the hippocampus induces anxiety-like behaviours, but the report of Santos et al. [30] was on the contrary.

4.2 Motor coordination

Capsaicin and pepper-diet fed mice had significantly reduced foot slips and falls compared to control and these typify improvement in their motor coordination because the lower the frequency of foot slips and number of falls, the better coordinated the animal was [26]. Capsaicin, as the most abundant and commonly occurring capsaicinoid might have achieved this feat (improvement of motor coordination) by aiding in the integration of proprioceptive information with neural processes (TRPV1) in the spinal cord and in the brain (specifically cerebellum) [31].

4.3 Pain

Tail-flick response of a mouse to thermal stimuli is believed to be a spinally mediated reflex behaviour [32]. From the results of our study, the latencies of tail flick of both the pepper and capsaicin-diet fed mice were significantly longer than those of control in the immersion test. Also, the tail-flick latency of the capsaicin group was longer than that of the pepper group. These results imply that pepper or capsaicin diet increased the pain threshold of the mice leading to decreased pain sensitivity. These results point to a more effective analgesic potential in capsaicin than pepper [33].

In the hot plate test, when an animal perceives pain, it attempts to jump away from the object that elicited the painful stimuli. This supra-spinally controlled behaviour (latency of jump) models an escape behaviour. The latencies of jump of both pepper and capsaicin groups were significantly longer than the control. Also, the latency of jump of the capsaicin group was significantly longer than that of pepper group. These results depicted that both capsaicin and pepper exhibited anti-nociceptive tendencies because it took a long time for mice fed such diets to experience pain compared to control. These results further confirm the analgesic potentials of pepper and capsaicin [33].

Capsaicin may have acted by initially activating heat sensitive TRPV1 receptors which induce pain. Repeated and prolonged exposure to capsaicin might have resulted in the reduction of responsiveness of the receptors and ion channels, thus leading to the "defunctionalization" of nociceptor fibres as reported by Anand and Bley [34]. Therapeutic uses of capsaicin for pain treatment were reported; Evangelista [35] and Chung and Campbell [36] reported that capsaicin can be used in treatment of neuropathic pain. Another study reported the analgesic effects of topically applied capsaicin [37]. These reports are in keeping with the results of the present study.

4.4 Social behaviour

The nesting score is an assessment of social behaviour. This nesting behaviour is a reflection of the social behaviour in mice. Nest building in mice correlates to organised behaviour in humans and is very distinct from the findings reported in a research by Alleva et al. [38], where they reported aggressive behaviour in mice treated with capsaicin. A poor performance in the nesting task may indicate impairment in social relationship in mice and likelihood of the presence of autistic behaviour. The nesting score of the capsaicin group was significantly higher compared to that of control. The value for pepper group appeared higher but was not significantly higher than the control. This result indicates enhanced organised social behaviour in mice fed with capsaicin diet. However, its mechanism of action has not been ascertained [33].

4.5 Learning and memory

The hidden-platform task of the Morris water maze is a test of visuospatial learning and memory in the mice and is also hippocampus dependent [39]. The use of extra-maze cues was employed in this task. On the other hand, visible-platform (cued) task of the Morris water maze is a non-hippocampal task and dependent on the dorsal striatum (caudate nucleus and putamen) of the basal ganglia [39]. The visible (cued) platform used a unique intramaze visual cue placed at the location of the escape platform.

The shorter the swim latency, the better the training process. Mice with learning disabilities or impairments were not able to quickly figure out the spatial location/position of the hidden platform, i.e., it took them a long time. Also, the steeper the gradient of swim latencies within the 3 day acquisition or reversal trainings, the better the learning curve, hence faster learning. Following the consumption of pepper and capsaicin diets, the swim latencies of the pepper and capsaicin groups were significantly longer than control in the first 3 days (acquisition training). This shows that pepper and capsaicin delayed learning process during the acquisition training [40].

During reversal training, the swim latencies of the test groups were also significantly longer on day 1 of the 3 day reversal training task, while on days 2 and 3, the values did not differ from control. This means that on days 2 and 3, the three groups learned equally while the control learned better on day 1. Visuospatial memory was also assessed during the probe trial (exploration without hidden platform). During this trial, it was expected that mice with good memory of the spatial location/position of the hidden platform would spend more time exploring the quadrant which had the platform during reversal training (North-East quadrant), but this was not observed in mice treated with pepper and capsaicin diets. They spent less time in the North-East quadrant. This means that they had memory impairment [40]. This is in contrast to the work by Kong et al. [41] which reported that capsaicin did not significantly alter the learning and memory performance in young adult mice but reduced the number of newly generated cells in the hippocampus. However, this is in line with the work by Kooshki et al. [42]. It is possible that the nociceptive effects of Capsaicin might have also affected learning and memory in the mice.

Learning and memory which are complex cognitive functions of the higher nervous centres encompass a variety of subcomponents with many interactions and overlaps [43]. Memories are stored in the brain by changing the basic sensitivity of synaptic transmission between neurons as a result of previous neural activity. The effects observed might have been due to the presence of the alkaloid called capsaicin. Since capsaicin is neurotoxic [9], it is likely that it impaired synaptic transmission between neurons by interfering with the basic sensitivity of the transmission in the hippocampus leading to impairment of learning and memory of the mice.

5. Conclusion

Consumption of both chilli pepper and capsaicin diets decreased pain perception, increased anxiety related behaviours, impaired learning and memory but enhanced social behaviour and motor coordination in mice. Therefore, it is likely that capsaicin, which is a powerful and stable alkaloid in chillies, may be one of the principles responsible for the observed neurobehavioural parameters in the experimental animals.

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Capsicum, also known as chili or bell pepper, is one of the most economically important vegetable crops worldwide due to its antioxidant, anti-inflammatory, and anticancer properties. This book provides information on many aspects of this plant, such as its botanical information, nutritional values, bioactive compounds, pharmacology, cultivation, its use in treating diseases, and its applications in the food and pharmaceutical industries.

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