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Review Article

Phytochemicals and biopesticides: Development, current challenges and effects on human health and diseases

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Highlights

- Identification of technical problems associated with phytochemical biopesticides with possible solutions.
- Identification and discussion on problems in marketing of biopesticides in Indian and global scale.
- Identification of the barriers for commercializing of phytochemical biopesticides in India.
- Effects of phytochemicals on human health and diseases

Abstract

For the last 50 years, the protection of crops is depended on synthetic pesticides. However, these synthetic pesticides are highly toxic, persistent, have adverse residual effects on crops, groundwater, soil, and their excessive application, leading to increased resistance of pests. On the other hand, biopesticides are less persistent, less toxic to non-target organisms, and eco-friendly. In this review, the complete process of developing biopesticides from plant-based extracts is described along with the marketing aspects that may help the entrepreneur open his venture on botanical pesticides. The complete process of developing biopesticides includes extraction, separation, identification of phytochemicals, the bio-efficacy study of crude extracts, and the formulation of biopesticides.

Though India has a good market, the commercialization of biopesticides is not happening on that large scale. Because the system that is designed for synthetic pesticides generally regulates biopesticides also. It imposes high costs on biopesticides; thus, it makes a barrier to prevent the market entry of biopesticides. Some policy gaps and technological faults have been identified to reduce the utilization of biopesticides. One of the main problems in commercializing biopesticides is the lack of awareness about the effective use of biopesticides and their advantages. Some other problems in promoting biopesticides are limited resources, lack of profile, unhealthy relationships between producers and regulators, and limited capabilities. To increase the use of biopesticides at the country level, it is necessary to understand their mode of action, contribution to sustainable agriculture, and their effects on human health and diseases.

Keywords: Human diseases, Phytochemicals, Extraction, Formulation, Biopesticides, Commercialization

Introduction

Presently, crop loss due to pest attack is a big problem. The losses of crops caused by insect pests are quite high in developed and developing countries as well. Though it is intended that crop protection is aimed at avoiding or preventing crop damage, data on different pests' effect is less available. Almost 10% to 90% of crop damage occurs due to pest infestation. Dhaliwal *et al.* estimated that crops were harmed by around 10,000 insect species and about 1000,000 diseases, which were caused by microorganisms [1]. Oerke *et al.*, estimated losses in cash crops due to pest infestation at 42.1% and in 2006, he updated the data as 32.1 % for the period from 2001-2003 [2]. The estimated crop loss data are 26-29 % (wheat, cotton, and soybean), 31% (maize), 37% (rice) and 40% (potatoes).

During the early 2000s, the losses increased considerably due to the intensification of agriculture.

The maximum losses were indicated in cotton (50%), which was followed by sorghum and millet (30%), oilseeds, maize, and rice, each 25% [1]. The loss has shown a significant increase from 7.2% in the early 1960s to around 23% in the 2000s. An increase in the loss has been maximum in cotton (18-50%) followed by millet and sorghum (3.5 to 30%), maize (5 to 25%), and oilseeds other than groundnut (5 to 25%). The cotton crop continues to suffer the maximum losses (30%) followed by rice (25%), sugarcane, rapeseed-mustard (each 20%), maize (18%), groundnut, pulses (15%), other oilseeds (12%), course serials (8%), and wheat (5%). Indian agriculture suffers annual losses of about US$ 36.0 billion due to the ravages of insects in the field. This is a colossal loss, and all our efforts should be made to bring the losses to the minimum so that access to food is increased for the expanding population. So, to reduce the loss, people started to use chemical pesticides. However, the World Health Organization (WHO) reported that around two hundred thousand people were killed every year due to chemical pesticide poisoning. World Resource Institute reported that more than 500 insects were resistant to insecticides.

However, some plants produce chemical compounds that are used to control different pests. In the past, Chrysanthemum (*Chrysanthemum cinerariaefolium*) was the best-known example that was used as a finely ground form as the botanical insecticide. Some locally available plants like *Derris, Nicotiana*, *Ryania* were used as the first generation botanical pesticides to control phytophagous pests like ectoparasites (lice, fleas). It was an old way to control pests [3].

Cymbopogon spp. were used to protect granaries against damaging insects in Greek (2000-200 B.C.) and in Rome (500 BC-76AD) [4]. In around 400 B.C, during king Xerxes' kingdom, finely powdered dry flowers of pyrethrum were used for delousing children [5]. Pyrethrum is one of the most widely used botanical pesticides globally, which was extracted from Chrysanthemum. In East Africa, this plant was grown as cash crops by farmers [6]. In ancient Rome, some aromatic plants were used to fumigate the storehouses of threshed grains and put at the entry of those storehouses [7,8] like rosemary, juniper, and myrrh. Thus, people came to know the repellent effect of aromatic plant parts and eventually, the concept was developed among the people [9]. In this period, the extracts from the roots of *Helleborus niger* L. were also used as rodenticides.

In Persia, the disease scabies caused by *Sarcoptes scabiei* L, was treated using some essential oils [4]. In the 17th century, nicotine obtained from tobacco leaves was used as a botanical insecticide against palm beetle. New botanical insecticide rotenone was introduced in 1850 from the root of *Derris* sp. [5].

In Europe, after the 2nd world war, botanical insecticides were gradually decreased and replaced by chemical insecticides like organochlorines and organophosphates [10]. People started to use chemical pesticides because they were easy to handle and persisted for a long time. Today, pesticide consumption in some developed countries is almost 3000 g/ha. Since pest management in India mainly depends on synthetic pesticides, excessive use leads to environmental pollution and health hazard.

According to the Environmental Protection Agency (EPA), safer insecticides must-have properties like nontoxic to non-targeted pests, less persistent in the environment. Koul and Dhaliwal in 2001, and Koul and Walia in 2009, reported that botanical pesticides and their active ingredients are the safer formulations of plant extracts [11,12]. Botanical pesticides can be classified into two major groups based on their production procedure. The first group of botanical pesticides is grown and produced by farmers themselves using their conventional traditional knowledge. They are generally called farming products. During the development of these agricultural products, people use some locally available plants with pesticide property [8]. In this process, it is difficult to know how many plant species are used in making botanical pesticides. Many studies have tried to gather information about utilizing locally available medicinal plants in plant protection [13]. Mkenda et al., in 2015, found that extracts obtained from the species of weed like *Tithonia diversifolia*, *Vernonia amygdalina*, *Lippia javanica* can control the major pests of *Phaseolus vulgaris* L [14]. Small companies produce a second group of botanical pesticides. One of the well-known examples of such products is a neem product. These products have the highest share in the global market, obtained from the seeds, bark, and leaves of *Azadirachta indica*. Limonoids are the active ingredients of neem extracts, which have insecticidal, antifeedant, and repellent properties [7,15-17].

Plants with Pesticidal Properties

Sweet flag (*Acorus calamus*)

The common name of *Acorus calamus* is the sweet flag, which belongs to the family *Araceae*. Many ethnobotanical, ethnomedicinal, and insecticidal properties have been found in the rhizome of the plant species [18]. Parekh *et al.,* in 2007, experimented with the analysis of preliminary phytochemicals of screened medicinal plants and the result showed that flavonoid, alkaloid, and cardiac glycoside are present in the plant *Acorus calamus [19].* Raina et al., in 2002, analyzed leaf oils and rhizome oils of *Acorus calamus* by using GC and GC-MS and identified around 30 compounds from leaf oils and 29 compounds from rhizome oils [20]. The major constituents found in rhizome oils were β-asarone (83.2%), α-asarone (9.7%), and β-asarone (85.6%) and linalool (4.7%) in leaf oils. The various constituents of essential oils were obtained from the rhizomes of *Acorus calamus* having insecticidal properties as they have the potentiality to develop natural fumigants to control the book. Structures of α-Asarone, Methyleugenol, β-Asarone, and (E)- methyl isoeugenol were depicted in Figure 1.

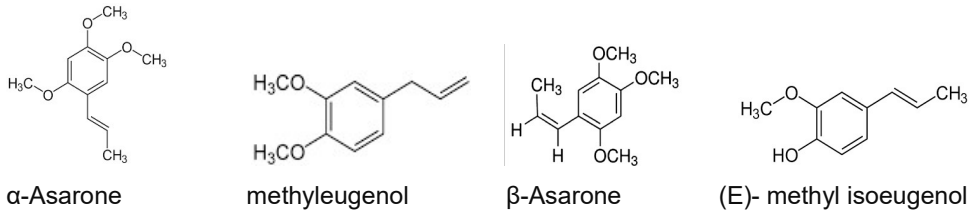
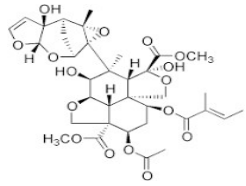


Figure 1: Structures of α-Asarone, Methyleugenol, β-Asarone, and (E)- methyl isoeugenol.

Neem (*Azadirachta indica*)

The common name of *Azadirachta indica* is neem. It is also known as margosa, which belongs to the family *Meliaceae*. Different non-wood products of neem like flowers, leaves, fruits, bark, gum, oil, and neem cake have antifeedant, anti-fungal, insecticidal, larvicidal, nematicidal and other biological activities [21]. The key active ingredient of neem is azadirachtin (Figure 2), which is triterpenoid and is known to cease insect metamorphosis [22]. This component mainly acts on weevils, termites, and aphids. Many neem biopesticides are commercialized in Indian and international scale [23].

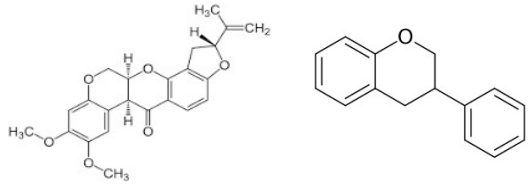


Azadirachtin

Figure 2: Structure of Azadirachtin.

Yam bean (*Pachyrhizus erosus*)

The common name of *Pachyrhizus erosus* is yam bean, tuberous legumes belong to the family *Fabaceae*. It is known to have pesticidal properties and it is considered as potential medicinal plants. The leaves of yam bean have toxic effects on larvae of many insects. The major constituents of yam bean are isoflavonoids. Isoflavonoids and rotenone were also isolated from the seeds of *Pachyrhizus*. Structures of Rotenone, Isoflavonoid were depicted in Figure 3. Seven compounds *i.e.,* 12-a-hydroxydolineone, a-naphthoflavone, 12a-hydroxy rotenone, 12a-dehydropachyrrhizone, 12a-hydropachyrrhizon, Pachyrrhizine, and 12a- hydroxy rotenone were isolated from yam bean showed pesticidal activity against *Aedes albopictus'* larvae. They further reported that six other compounds like quercetin dihydrate, 6-methyl-flavone, 5-methoxy flavone, 4-hydroxy-flavanone, 7-hydroxyflavone, 3-hydroxyflavone and 3-hydroxyflavanone isolated from yam bean showed insecticidal property against *Aphis gossypii* (apterae) [24]. 12a-hydroxydolineone possessed insecticidal activity against *Herse convolvuli* larvae and 12a-hydroxyrotenone exhibited toxicity to the *Plutella xylostella.*



Rotenone Isoflavonoid

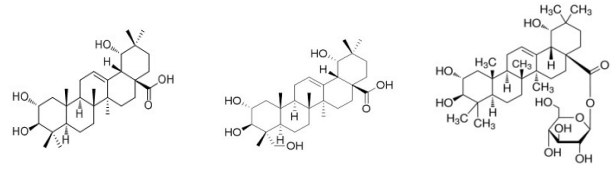
Figure 3: Structures of Rotenone and Isoflavonoid.

Karanja (*Pongamia glabra*)

Pongamia glabra is commonly known as Karanja. Oil extracted from Karanja is prepared as a pesticide by making a nano-emulsion for the control of the *Tribolium castaneum*, a secondary pest of stored grains. The aqueous filtrate obtained from the solid cake causing insecticidal properties [25].

Arjun tree (*Terminalia arjuna*)

The common name of *Terminalia arjuna* is Arjun, which belongs to the family *Combretaceae*. Bark is the major plant part of having pesticidal properties. Arjunic acid, Arjunetin, and Arjugenin are the three major components present in the bark of *Terminalia arjuna* with the antifeedant property *[26]*. Structures of Arjunic acid, Arjungenin, and Arjunjetin were depicted in Figure 4.

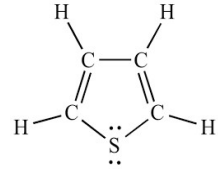


Arjunic acid Arjungenin Arjunjetin

Figure 4: Structures of Arjunic acid, Arjungenin, and Arjunjetin.

Marigold (*Tagetes* sp.)

The common name of *Tagetes* sp. is marigold belongs to the family *Asteraceae*. The whole plant of marigold possesses pesticidal properties. The insecticidal activity of the plants in *Tagetes* L. is mainly originated from Thiophene derivatives. Alfa-Terthienyl is the most effective compound then other thiophene derivatives [27]. Thiophenes possess one, two, and three aromatic sulfur-containing rings linked together by alpha carbons (Figure 5). Marigold contains sulfur-containing compounds with potent insecticidal activities [28].

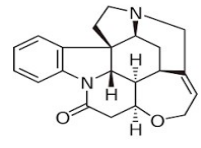


Thiophenes

Figure 5: Structure of Thiophenes.

Kuchla (*Strychnos nuxvomica*)

The common name of *Strychnos nuxvomica* belongs to the family *Annonaceae* [29]. The seed of this plant has the pesticidal properties. The seeds contain approximately 1.5% strychnine and the dried blossoms contain 1.0%. Strychnine, which is used as a pesticide (Figure 6).



Strychnine

Figure 5: Structure of Thiophenes.

Mahua (*Madhuca latifolia*)

The common name of *Madhuca latifolia* is mahua, which belongs

to the family *Sapotaceae*. Seed and bark of this plant have significant pesticidal properties [30]. It contains saponins, triterpenoids, steroids, saponins, flavonoids, and glycosides. Mahua bark contains ethyl cinnamate, sequiterpene alcohol, α-terpeneol, 3β-monocaprilic ester of eythrodiol, and 3β-capryloxyoleanolic acids. α and β amyrin acetates and mahua seeds contain myrisic, palmitic, stearic acid, α-alanine, aspartic acid, cystine, glycine, isoleucine and leucine, lysine, methionine, proline, serine, threonine, saponin, quercetin, linoleic, and oleic acid [31]. Saponin serves as an anti-feedant in plants.

Pre Extraction Methods

For the preservation of the bioactive components of plant samples, the pre-extraction preparation is needed. Phytochemicals occur in various plant parts like leaves, fruits, flowers, and roots. They can be derived from fresh or dried and ground or powdered samples. Azwanida et al., (2015) are reported that dried samples were more efficient for the extracting of bioactive compounds than fresh samples [32]. Extraction from dried samples takes lesser time for experiments than the fresh samples. Experimental work is done after three hours of harvesting to keep the freshness of samples. Fresh samples are soft and get quickly deteriorated than the dried samples. A comparative study between the dried and fresh *Moringa oleifera* leaves showed that phenolic content is not significant but flavonoid content is high in dried samples [33].

On the other hand, particle size is inversely proportional to the surface area. As the powdered samples have smaller particles and are more homogenized than ground samples, it allows the target analytes to more contact surface with the extraction solvents. Particle size is considered as a significant factor in the case of solvent and enzyme-assisted extraction of samples. Those enzymes degrade pectin and polysaccharides of the plant cell wall faster when the particle size is smaller. The size of the biomolecule particles less than 0.5 mm is optimum for extraction. According to the experiment by Suleiman et al. [34], the particle size of samples was 0.4 mm. Extraction from nanoparticles powdered *Centella Asiatica* samples by Planetary Ball Mill produced a higher yield (82%) than the extraction from micro powdered samples using the maceration technique with methanol [35]. Drying of samples is done in many ways like air-drying, freeze-drying, and oven drying.

Different types of plant parts like leaves, flowers, and roots take different durations of time to be air-dried. Heat labile compounds are not dried at high temperatures. Air drying takes a longer time than other methods and there is also a chance for contamination in this method.

The principle of electromagnetic radiation is used in microwave drying. An electric field causes dipolar rotation, so heat is produced. It causes permanent dipole moments. It causes oscillation of molecules, and thus, the collision of molecules occurs, and heat is produced [36]. This method is taken a shorter time than air-drying, but the disadvantage is that it degrades some of the phytochemicals.

The freeze-drying method involves the process of sublimation. In the case of freeze-drying, the quantity of extracted phenolic compounds is more than to air-drying [32]. The freeze-drying method is costlier and difficult compared to air and microwave drying, and this method is not recommended for heat-sensitive materials.

In the oven drying method, heat energy is used to remove the moisture of samples. This method of sample drying is considered the most straightforward method and it preserves bioactive compounds. The extracts of *Cosmos caudatus* yielded the highest antioxidants when the sample is oven-dried at a temperature of 44.5^{0}C for 4 hours using the solvent methanol (80%) [37].

Screening Tests for Different Phytochemicals

Ahmad et al., reported different phytochemical screening tests as per the standard method [38]. As per his report, Egwaikhide et al. carried out the Dragendroff's test [39], Rangarajan et al., carried out Mayer's test for alkaloid [40], and Amin and Sawheny carried out a ferric chloride test for phenolic compounds [41]. Further, he reported, NH_4OH test (Ammonium hydroxide), Alkaline reagent test, Zn (Zinc), and Mg (magnesium) turning tests were carried out for flavonoids and Folin's test and Million's test were carried out for Tyrosine and tryptophan amino acids' test.

In an experiment carried out for terpenoids, steroids, and tannins; for terpenoid and steroid test, 2.5 ml of acetic anhydride and 2.5 ml of chloroform were treated with 20 mg of the extract. Then concentrated sulfuric acid was slowly added and a bluish-green color appeared for steroids and red-violet color for terpenoids. In 2010, he experimented for tannins where ferric chloride (1-2 drops) solution, 1 ml water and 0.5ml of extract were added. The presence of catechol tannins showed green, black color while gallic tannins showed a blue color. He also conducted other tests like the Ninhydrin test and the Xanthoproteic test for amino acids in the same year.

Extraction Method

Extraction is a procedure by which phytochemicals extracted from active parts of the plant are separated by using selective solvent through the standard procedure. Separation of bioactive compounds from the plant samples, which are water-soluble, requires all types of extraction. The crude samples need to process further. The different extraction methods are discussed below-

Maceration is a conventional and most widely adopted extraction method in the research field. In the maceration technique, coarsely ground or powdered plant material is soaked with the selective solvent in a closed container and kept at room temperature for three days at least and agitation is done frequently. In the decoction and infusion method, the same principle is involved like maceration. In both of the methods, samples are soaked in either cold or hot water. The difference between maceration and infusion is that the soaking time in the infusion method is lesser than the maceration method. In the decoction method, volume of crude extract and solvent is specified like 1:4 or 1:16. Heat stable compounds are used in decoction like roots and barks, and mostly the extracted compounds are oil-soluble. In percolation, boiled water is used as a solvent and the maceration period is 2 hours, and the extraction rate is 6 drops/min. Among all the extraction methods, percolation is the simplest method. The limitation of this method is a tremendous amount of solvent is needed. However, the volume of the solvent can be reduced by altering the temperature. Phenolics content and antioxidant activities are high when *Centella asiatica* is boiled at 90°C in the percolation method.

The maximum extract is yielded when *Psidium guajava* L. leaves extracted with ethanol and hydroalcoholic solvent (4:1 v/v) in the presence of carbohydrate, saponin, alkaloid, tannin, and flavonoids

instead of using petroleum ether, water or chloroform [42]. Using petroleum ether as the solvent preserve minimal number of tannins. When water is used as a solvent (1:10 w/v), it showed similar efficiency to ethanol, with no alkaloid [42]. Solvents, which are polar like methanol, are efficient in phytochemical extraction from *Psidium guajava*. Antioxidant activities of phytochemicals are higher when *Garcinia atroviridis* is extracted with methanol (1:10 w/v) than the water (1:10 w/v). But in the case of water, anti-hyperlipidemic activity is high [43]. Phenolic content is more in *Portulaca oleracea* when it is extracted with 70% acetone (1:10 w/v) in the maceration process. Flavonoid content is high in *Cosmos caudatus* when it is extracted with 70% methanol [34]. Phenolics and flavonoid content are highest when *Moringa oleifera* is extracted with 70% ethanol (1:40 w/v) using the maceration technique rather than the percolation and the Soxhlet extraction [33].

In the soxhlet extraction method, a small amount of solvent is needed. However, the limitation is that it creates toxic emission and allows more exposure to flammable organic solvents. This technique is quite expensive because it requires highly pure solvents and also it is not an ecofriendly [44]. Another disadvantage of this method is only dry and finely ground samples can be extracted and many factors also need to be maintained like solvent sample ratio, temperature, and agitation speed [45].

The high volume of phytochemicals can be extracted from the leaf powder of *Azadirachta indica* (neem) using solvents like methanol [46]. The extraction from *Moringa oleifera* using this method showed a very lower amount of phenolics and flavonoids content [47]. However, extracts of *Centella asiatica* are showed the metal chelating activities when the extraction is done by the soxhlet method at the temperature of 25°C, agitation speed 200 rpm, and sample-solvent ratio 1:45 [45].

The microwave-assisted extraction (MAE) method is time-saving and requires fewer solvents than maceration and soxhlet extraction. In MAE, analytes can be recovered and reproduced; however, there is a chance of thermal degradation if proper conditions are not maintained [48]. This method's limitation is that phenolic compounds like gallic acid, quercetin, ellagic acids, isoflavin, and trans-resveratrol cannot be extracted because these molecules are small in size can remain stable up to 100 degree Celsius for 20 minutes in microwave heating conditions. Another limitation is extra cycles in MAE e.g., 4×20s to 5×20s caused a decrease in phenolics and flavonoid yields, mainly because of the oxidation of compounds [49]. Heat-sensitive compounds like anthocyanins and tannins cannot be extracted as it undergoes degradation during process by this method.

When the plant *Centella asiatica* is extracted by this method using absolute ethanol at a temperature of 75°C and 600W power for four cycles resulted in high yield of triterpene [50]. Extraction from *Dioscorea hispida* by MAE was showed a highest yield using 85% ethanol at 100 W power for 20 minutes and 12.5:1 solvent-sample in ratio [51]. In the case of MAE, time duration and irradiation power are two major factors. For example, when the plant *Andrographis paniculata* was extracted at 119.7 W and 39.9 W power for 5 minutes and 17.5 minutes, respectively, yield showed as optimum in both of the cases [52].

The range of ultrasound wave 20 kHz to 2000kHz is used in ultrasound associated extraction (UAE) or sonication extraction method [53]. When the samples are exposed to the ultrasound frequency, the physical and chemical properties of samples change and the plant's cell wall is disrupted, which helps to release bioactive compounds from the cells. The UAE method requires a lesser amount of solvent volume and lesser time for extraction. Mostly for the heat-sensitive or thermos-labile compounds like anthocyanin content of flowers, the UAE method is suitable as it reduces the exposure to high temperature. If the used ultrasound frequency is greater than 20kHz, it forms free radicals and affects the biomolecules.

UAE is an effective extraction method for propolis extraction for 10 to 30 minutes. In 2013, Dhanani et al., found out that the extraction of *Withania somnifera* by the UAE method resulted in the highest yield (11.85%) when water was used as a solvent and an extraction time was 15 minutes as compared to the method where ethanol and water-ethanol were used as solvents and extraction periods were 5 minutes and 15 minutes, respectively [54]. In the case of *Cratoxylum formosum*, it is observed that UAE showed efficacy on phenolic compounds at the temperature of 65^{0}C for 15 minutes, using 50.33% ethanol as solvent [55].

Accelerated solvent extraction (ASE) is more efficient than the soxhlet extraction and maceration method as it requires less solvent. For every individual sample, the temperature and pressure are controlled in this technique and requires less extraction time. The solvent type is the primary factor in the case of ASE. The extraction of *Bixa Orellana* with cyclohexane-acetone (6:4 v/v) at the temperature of 50^{0}C for 5 minutes resulted in the highest yield (68.61%) [56]. From *Rheum palmitin*, 94% of flavonoids were recovered by the ASE method using 80% methanol [57].

In the supercritical fluid extraction (SFE) method, most non-polar analytes and CO_2 are used as a supercritical fluid. As supercritical CO_2 is not soluble in polar compounds, therefore, some methanol and ethanol are added to extracts. By changing temperature and pressure, the strength of the supercritical solvent can be changed. SFE from *Wedelia calendulacea* at 25°C temperature, 25 mPa pressure for 90 minutes with the 10% concentration of modifier showed the highest yield [58]. The high cost of equipment is a disadvantage of this method.

Separation Methods

The phytochemicals having antioxidant properties are mainly separated and purified by thin-layer chromatography. Coumarins, cinnamon acids, and flavonoids are separated from *Taraxacum officinale* extracts by paper chromatography [59]. Due to low cost and more convenience, column and thin-layer chromatography (TLC) are mostly applied for separation and detection of the antioxidant phytochemicals [60]. An efficient separation is done by thin-layer chromatography using amino silica, cellulose, diatomaceous earth, hexane-chloroform as mobile phase, and polyamide as a stationary phase. Carotenoids are separated efficiently mostly by the regular or 2D TLC [61]. The disadvantage of TLC is a huge amount of samples are required, which is not available always, and TLC plates cannot recover the phytochemicals [62]. Nine antioxidant phytochemicals were separated from extracts of the aerial parts of *Hypericum hyssopifolium* and purified by TLC and column chromatography [63]. The flavonoids and phenolic acids are separated from the aromatic plants' extracts, which belong to the family *Lamiaceae* by using TLC [64]. From *Satureja hortensis*, rosmarinic acid was separated by using silica gel and reversed-phase C18 column chromatography.

From medicinal plant polygonum multiflorum, phenolic acids and polyphenols were separated by using spandex LH-20 column chromatography and silica gel.

The conventional technique like LC, TLC, are not highly sensitive and do not have high resolution. However, Gas chromatography and High-Pressure Liquid Chromatography are highly efficient for the separation and detection of the phytochemicals. Gas chromatography is suitable for volatile compounds. As most of the plant-derived antioxidants are non-volatile, its use in phytochemical separation is not as popular as high-performance liquid chromatography. The disadvantage of GC is the difficulty in large-scale applications. It is mostly used in the separation of essential oils. Depending upon the properties of phytochemicals, different columns with different inert materials, polarity, and widths are used in the separation technique. Capillary column and mass spectroscopy detectors are usually used in GC for phytochemical separation. In 2000, it was reported that for the separation of phytochemicals extracted from *Hamamelis virginiana*, *Crataegus oxyacantha,* and *Hydrastis canadensis*, a column DB-5 with 5% biphenyl and 95% dimethylpolysiloxane with medium polarity was found to give the best result [65]. The major active compounds of H. virginiana was identified as 1,2,3-trihydroxybenzene using this method. Sun et al. in 2002, reported that alkylamides in *Echinacea* were separated by GC and GC-MS [66].

HPLC is mostly used for the separation of different non-volatile compounds. The type of HPLC varies. One of such method is the diode array detector (DAD) with a mass spectrometer. HPLC is a preferable separation technique for carotenoids having lipophilic properties. For the separation of carotenoids, a stationary silica phase is used in the adsorption HPLC technique [67]. Saponified carotenoids are separated with silica column of 280 mm × 46 mm and radiant is 95% light petroleum, 2 to 95% acetone [68]. C8 and C18 columns are more favorable for the separation of carotenoids in reverse phase HPLC [66,69]. Separation of free lutein showed promising results using a C18 column [70]. C30 is useful for separation of carotenoid isomers [71,72]. Sander et al., in 1994, reported that the non-polar isomers of carotenoids, zeaxanthin and lutein were not well separated using the C18 column, whereas hydrocarbon carotenoids were separated better using a polymeric C30 column [73]. An experiment showed the mono and diesters of lutein were identified using the C30 column in LC-MS [74]. Another experiment showed that cis isomers of lutein diesters were separated using RP-C18 column and MS and DAD [75].

Lu et al., 2004, conducted different types of separation methods for naturally occurring antioxidant phytochemicals [76]. They explained that to separate the polyphenolic antioxidants, a reverse-phase C18 column is used for the HPLC method's chromatographic conditions. In this method, an ultraviolet-vis-diode array detector (DAD), an organic solvent that is polar and an acidified water containing binary solvent are used for flavonoids (A) and phenolic acids (B) separation. Apati et al., 2003, separated antioxidant flavonoids, which included chlorogenic acid and rutin in solidago plants using HPLC-UV [77]. Robards et al., in 2003, used DAD as a multiple wavelength detector and found out that its versatile nature is often neglected [78].

The separation in HSCCC (High-speed counter-current chromatography) was aided by centrifugal force and pressure; the former was produced from the coiled column's planetary and rotational movement. These forces aided in the vigorous mixing of two liquid phases, which were immiscible, leading to stationary phase retention [76]. Lipophilic phytochemicals like carotenoids are separated using the application of HSCCC.

Supercritical fluid chromatography (SFC) is another new technology. Carbon dioxide significantly reduces the solvent waste and its usage eases collecting fractions by removing the solvent. Antioxidant phytochemicals show high diffusion in a supercritical fluid pertaining to low viscosity; thus, there is high homogenous diffusion of the compounds in the packing material, there are higher separation time and high resolution. Kohler et al., in 1997, made the supercritical fluid out of 3% ethanol and carbon dioxide at 500C and 15 MPa [79].

Quantification and Identifications

UV–vis spectrophotometer is required to quantify of organic compounds (these compounds absorb light in the visible and ultraviolet region). An antioxidant photochemical can be identified with high certainty by the matching results of both retention time and UV–vis spectrum. Antioxidant phytochemicals are identified using UV–vis and DAD, while in the case of similar compounds, spectra based approaches cannot be used.

Electroscopy ionization (ESI) was used for the ionization of antioxidant molecules like anthocyanin (ionic and polar in aqueous solution), and atmospheric pressure chemical ionization (APCI) was used for antioxidants that are non-ionic and less polar such as carotenoids [80]. The PI-MS (Peptide identifier-MS) method was used for antioxidants photochemical detection. It was observed that the NI-MS method (Nanostructure-initiator mass spectrometry), which included ESI and APCI, gave the best results for flavonoids analysis [81].

For developing the botanical formulation, knowledge about crude extract's bio efficacy on different pests is also required.

Bio-Efficacy Study of Crude Extracts

Pavela et al., conducted experiments by taking different Lepidopteran species' like *Spodoptera littoralis* (Boisduval), *Tetranychus urticae* and, *Myzus persicae* (Sulzer) to find out the effectiveness of botanical insecticides [3,16]. In the case of the efficacy against aphid, 100% mortality of sample species was caused by the highest concentration on the 12th day after application among all the crude extracts. In the other concentrations, program showed the maximum efficiency ranging from 96%-97% and 76%-82% for 1.0%-0.5% depending on the years. When the concentration of neem extract was 0.5%, it had the minimum efficiency, which was around 57%. While pongam oil's efficiency was increasing with time, the efficiency of pyrethrum and neem were decreasing gradually as new nymphs hatched. In the case of acaricide, the most efficient product was pongam oil and neem against *T. urticae.* The mortality rate was increasing with time for 3% and 1% concentration. In the lowest concentration of the crude extracts, neem showed 60-70% efficiency while pongam showed 49-52% efficiency. With a 0.5% concentration pyrethrum showed a minimum efficiency of 10-20%. When the efficiency was tested against *S. littoralis* larvae, there was not much difference between the efficiency of neem and pyrethrum based products. When the concentration was 0.5%, the pongam oil efficiency did not even reach 50% in two years. The antifeedant index is 92% with a concentration of 0.5% and 100% with 3-0.5%

concentration for the neem-based product. The antifeedant index was estimated as 31% in the case of pyrethrum based product.

Murasing et al., in 2019, conducted an experiment to show the efficacy of fern plant extracts on the species *P. xylostella* under laboratory conditions [82]. They took three fern plants like *Diplazium esculentum, Christelle parasitica,* and *Blechnum orientale* for the study. They observed that larval mortality increased with the increase in concentration and it was maximum at 20% concentration. In the case of *D. esculentum* the maximum larval mortality was 36.6% and 73.33% after 24 hours and 72 hours, respectively, while in untreated control, it was only 0.6 % and 13.3% after 24 hours and 72 hours, respectively. It is also reduced the weight of larva and pupa, altered feeding behavior of *P. xylostella*, malformed pupa, and increased the pupation period. All these indicated that for *P. xylostella* larvae the extracts of *D. esculentum* were most toxic. The result showed that larval mortality was 46.6% for *D. esculentum*, 43.33% for *C. parasitica,* and 40% for *B. Oriental* at 20% concentration. After 72 hours of treatment, maximum mortality was observed 73.3% for *D. esculentum*, 33.33% for *C. parasitica* and 60% for the *B. orientale*.

Ashraf et al., in 2018 carried out the bio-efficacy study of different phytochemicals on brassica aphid [83]. They observed, the highest mortality (48.42%) was exhibited by neem seed extract, followed by 45.54% mortality with dathora extracts and 40.29% mortality with kaner leaf extract after seven days of treatment. It was also observed that aak leaf extracts exhibited a minimum mortality, 26.64%.

Ali et al., in 2011, conducted an experimented to find the biography case study on *Bactrocera cucurbitae* (coquillett) (*Diptera: Tephritidae*) [84]. They found maximum mortality in methomyl and neem seed extracts treatments followed by the parthenium plant extracts and eucalyptus plant extracts.

Yao et al. reported that the ethanol extract of *Acorus calamus* has strong repellency and contact effect to *S. zeamais* and the active constituent of the *Acorus calamus* was characterized as (Z)-asarone by spectroscopic analysis [85].

Basukriad et al. noted that *Pachyrhizus erosus* seed extract has oviposition deterrent activities against *Plutella xylostella* (Lepidoptera: Plutellidae) and the cabbage looper *Trichoplusia ni* (Hubner) [86].

The ethyl acetate leaf extract of *Strychnos nuxvomica* Linn. (Loganiaceae) was found to be the effective larvicide with LC_{50} value of 222.28 and 146.99 ppm after 24 and 48 hours against the Filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae) [87].

Pawar et al. reported that extracts and purified extracts of seeds of *Madhuca latifolia* have insecticidal properties against *H. armigera [88]*. Insect feeding deterrent and growth inhibitor properties of Terminalia Arjuna were noted against lepidopteron insect *Spilarctia oblique [89]*.

Phoofolo et al. reported that aqueous extract of *T. minuta* was as efficient as crude extracts from organic solvent system against cabbage aphids. The aqueous extract of *T. minuta* also had reduced fecundity of cabbage aphids with the magnitude comparable to those obtained from organic solvents [90].

Isman et al. reported that publications numbers are increasing in botanical insecticide research, especially in India, China, and Brazil; but commercialization of phytochemicals and phytoessential oils for insect pest management is significantly less [91]. An alternative way of research is required to fulfill the demand for production of botanical pesticides for promoting organic farming and integrated pest management (IPM) in developing countries. The scientists are working more on isolation and characterization of toxicants from plant and other biological sources, but there is a demand for pesticides. Only a few biopesticides are in commercial use. More research and development should be conducted on the development of pesticide formulations for sustainable pest management strategies.

Different Types of Formulation of Bio-Pesticides

Biopesticides are mainly plants or living organisms' best extracts. So, during the formulation and storage process, these are to be maintained at the level of acceptance. In the process of biopesticide formulation, adjuvants and different carriers are mixed with phytochemicals to improve the storage stability, protect from the environmental conditions, and improve bioactivity. The major factors to be considered in the formulation process are 1. stabilization and storage, 2. protection against adverse environmental conditions, 3. easy application, 4. improvement of bioactivity, 5. maximum interaction with target pest [92]. The formulation of biopesticides can be classified into two groups; 1. dry formulation, 2. liquid formulation.

Dry formulations are produced in several ways like freeze, drying, air-drying, and spray drying. Different wetting agents, dispersant blinder is added to it [93,94]. Liquid formulations are mainly water-based, polymer-based, oil-based, and combinations. Suspension emulsions and concentration and capsule suspension are water-based formulations. Some inert ingredients like stabilizers, surfactants, stickers, and coloring agents are added to this formulation. Some forms of dry formulation are dust, granules, micro granules, powders, water dispersible granules, and wettable powder and soil dispersion, emulsions, suspo-emulsions, suspension concentration, and capsule suspension are the liquid formulations. In the case of dust form, active ingredients are mixed with clay or talc having particle size 50-100 μm for the dust formation. Adhesive materials are added to the formulation to improve adsorption. UV protectants and anti-caking agents are also added. Generally, 10% of active ingredients occur in the dust. This type of formulation was developed before the granules. The disadvantage of this formulation is serious health hazards by inhalation; therefore, it is restricted to use [92].

In the case of powdered form, active ingredients, inert material, and powder carrier are added to this formulation to enhance the seed coat's adherence. This form of biopesticides is generally applied to seed directly. This is an old method for coating seeds and the seed pigment on the seed indicates that they are dressed seeds [95].

In the case of a granular form, the size of granule particles is generally 100-1000 microns and for micro granules, it is 100-600 microns. Silica, kaolin, polymer, starch, and plant residues are generally used to formulate granule biopesticides. 5 to 20% of active ingredients occur in granule form. This type of formulation is generally applied to soil to kill the target insect living in soil, to control soil nematodes, and weeds. Granules release active ingredients in the presence of soil moisture after application [96].

Wettable powders (WP) are a powder water suspension. In this formulation process, surfactants and dispersing agents are blended with wettable powder. Then they are granted to 5-micron particle

size. This formulation causes serious health issues for manufacturers during inhalation and also causes eye and skin irritation. Another problem, the wettable powder is suppressed by water-dispersible granules due to its dustiness [96]. This type of formulation is more preferable for its easy application by spraying long-duration storage stability and good solubility to water [93].

Water Dispersible Granules (WG) is developed by suspension in water like a wettable powder but does not create dustiness. It is free from dust and its storage stability is good. Both dispersing agents and wetting agents are present in this formulation. However, the concentration of the dispersing agent is high. This product may be formulated in different ways like granulation, spray drying, and fluid bed granulation. This formulation is expensive; but it is environment-friendly and easy to apply, so preferred by many users [94].

In emulsions, the size of the droplet is 0.1 to 10 microns, which is dispersed to the liquid (immiscible). There are two types of emulsion; 1. normal emulsion oil in water, 2. invert emulsion water in oil. Emulsifiers should be appropriately selected to overcome the problem of instability. The evaporation loss is minimal in the case of invert emulsion [93]. Sometimes it produces toxicity to plants, and it has low stability. However, in current days, many different emulsifying agents are used to improve invert emulsions.

In suspension concentrates, active ingredients are dispersed in water. This mixture is agitated frequently so that solid particles dissolve correctly. To enhance the stability, wetting agents, and antifoaming agents are added. Its particle size is 1-10 micron. Inert materials adsorbed on the particles and do not let them re-aggregate. Solid small particles have a large surface area, so it allows maximum interaction between plant tissue and active ingredients. This kind of formulations is environment-friendly, easy to handle, and safe to users [95,96].

In oil dispersions (OD), solid active ingredients are diluted in oil. It improves spreading, retention, and penetration of the product. Water sensitive active ingredients are delivered by this formulation, which increases the pest control ability. To overcome the problem of instability and inert ingredients should be appropriately selected.

In suspo-emulsions (SE), suspension concentrates are mixed with emulsions. It is preferred to use due to its stability as it forms a homogeneous emulsion [94].

In capsule suspension (CS), encapsulated active ingredients are diluted in water. Active ingredients are coated or encapsulated with cellulose, starch, and gelatin. Thus, the bioagents are protected against UV radiation and environmental conditions. Encapsulation of microcapsules increases the efficiency of fungal biopesticides [97]. Surfactants are added to stabilize the formulation. The formulation is expensive, and its commercialization is slow in progress [98,99].

Toxicity and Persistence of Botanical Pesticides

Toxicity and persistence of biopesticides depend upon the type of solvent [100]. Biopesticides are eco-friendly, harmless to non-target organisms, so they are an alternative to chemical pesticides. However, the chemical compounds extracted from biopesticides have a toxic effect on humans also [101]. Mpumi et. al. reported that the oral LD_{50} values of pyrethrins, sabadilla, rotenone, nicotine, linalool and neem are 1200-1500 mg/kg , 4000 mg/kg, 60 to 1500 mg/kg, 50-60 mg/kg, 2440 to 3180 mg/kg, 13000 mg/kg, respectively and the dermal LD_{50} values of pyrethrins, rotenone, nicotine, and linalool are more than 1800 mg/kg, 940 to 3000 mg/kg, 50 mg/kg and 3578 to 8374 mg/kg, respectively [102]. Their persistence in the environment is still not well known [101]. Some plants like *Derris, Tephrosia* and *Lonchocarpus* contain a large amount of rotenone [103]. Rotenone has low toxic insecticidal property used in the garden and high toxic property on fish [103]. For rats, rotenone has the LD_{50} value of 132-1500mg/kg [100,101]. Rotenone has moderately toxicity effect on human beings and its LD_{50} value is 300 to 1500 mg/kg [14,101]. As it is lipophilic, it has a high toxicity effect on insects and fish. It is advantages for the environment that in sunlight, rotenone breaks down [103]. As it easily breaks down, it cannot persist for a longer time in the environment; thus, it is less toxic to non-targeted organisms [100]. several factors like sunlight, pH, temperature, and turbidity of water cause the decomposition of rotenone when applied in water. In general, the half-life of rotenone is 4 days [6,100,101,103]; however, Henn et al. reported that the half-life of rotenone is half a day and 3.5 days at 24°C and 0°C, respectively [100].

Azadiractin has toxicity with an LD_{50} value of 15ug/g in *S. littoralis* and 3540 mg/kg in the rat, which indicates that it is less harmful to mammals [104]. Some environmental factors like heat, moisture, and sunlight impact the rapid degradation of azadirachtin. Under UV radiation and sunlight, the half-life of azadirachtin is 48 minutes to 3.98 days; however, it could be 2.47 days [6,100].

Pyrethrins possess an axonic toxic effect [100]. They have a high toxicity effect on fish and less on birds and mammals. Oral LD_{50} value of pyrethrins is 1200 to 1500 mg/kg on rat [6]. Under sunlight, air, and moisture pyrethrin start to degrade [6]. The half-life of pyrethrins is 2 hours or could be less [100].

Vernodalin, epivernodalol, and vernodalol are the primary active ingredients of *V. amygdalina* with a low toxicity effect [7]. The LD_{50} value of vernodalol was 1265 mg/kg on mice [105]. However, these sesquiterpene compounds have antibacterial and antifungal properties.

Challenges Associated with Market and Commercialization of Biopesticides

Market share of biopesticides in global and Indian perspectives

As per UNDP report on India biodiversity awards in 2018, India possesses the largest diversity of flora having 47000 plant species and accounts for 7-8% of the world's recorded species. In the Indian perspective, there is a needful demand to increase the productivity of biopesticides for pest management, green, and sustainable agriculture. Botanical pesticides are potential alternative sources as biopesticides in India. Many experts forecast a huge potential of botanicals over the next decade. Biopesticides could grow from 4-5 % of the global pesticide market to 20% by 2025. Growth in botanicals may perhaps be even higher, going from 1-2 % of the market share to somewhere, possibly around 7% of the total market share [91]. The report of credence research showed the growth of the global insecticides market and its prospects from 2018 to 2026. The global insecticides market was valued at 18.47 bn in 2017, and it is likely to be reached to 25.9 percent from 2018 to 2026. In the global biopesticides market, the value of biopesticides was USD 3147 during 2018 and it is expected to reach a CAGR

of 14.1% from 2019 to 2024. The highest growth is expected in South America during that period and there is a possibility to reach a CAGR of 16.4%. It is also expected that the United States will be the largest market for biopesticides during that period.

As crop protection, human, animal, and environmental health are a major concern on a global scale; many countries are giving more emphasis on using biopesticides rather than chemical pesticides. It is expected that Asia Pacific, North America, and Latin America will lead the biopesticide market globally scale. North America holds around 41.5% of total global biopesticides. The major factor which drives the use of biopesticides is the concern of sustainable agriculture. Various factors help in the growth of global biopesticide markets like increasing adoption of organic products, concerned about the harmful effects of using synthetic pesticides, and the management of integrated pest management. In the global biopesticides market, various companies are competing to hold the largest share in the market and focus on the quality of product and promotion. They are focusing on strategic moves and launching new products. The major companies like Koppert BV, Bayer crop science AG, Valent crop sciences, and corporations are investing in developing the market of biopesticides. It indicates that the demand for biopesticides is gradually increasing.

The development of various organic products is spreading awareness of using biopesticides over the chemical pesticides in the developed and developing countries and leading to the adoption of biopesticides in the global pesticide market. The government of many countries across the world is promoting the advantages of using biopesticides, which helps in the biopesticides market's growth. In the Indian pesticide industry, biopesticide is a small segment. It can take large-scale growth in the coming years, gaining support from the government and spreading awareness about the advantages of biopesticide use like safe for environment and non-toxicity to non-target organisms. In the pesticide market, biopesticide contributed almost 7 to 8% in 2013. In 2009, the revenue by biopesticide was 2294.8 INR million in India. In the Indian pesticide market biopesticides have a comparatively low contribution than chemical pesticides, but due to excessive use of chemical pesticides, a large number of injuries and deaths of farmers were reported by the Maharashtra government. Indian Agriculture Ministry showed that biopesticides have a positive trend in India and their use is increasing more than chemical pesticides. The use of biopesticide has improved about 23%, while synthetic pesticides increased 2% only. The directorate of plan protection, Quarantine and Storage, Union Ministry of Agriculture and Farmers Welfare estimated the data of biopesticide consumption in 2010-11 in India was 5151 tons, which had reached 6340 tons in 2016-17. Though the data is provisional, which indicate a positive trend in India.

Barriers for commercialization of biopesticides

Despite the demand and large scale of this research enterprise, only a few bioinsecticides are in commercial use. Because the system, which is designed for chemical pesticides, is generally regulated biopesticides also. It imposes high costs on biopesticides; thus, it makes a barrier to prevent the market entry of biopesticides. Some policy gaps and technological faults have been identified to make efficient utilization of biopesticides. These gaps need to be known at the country level. To lessen the excessive application of chemical pesticides and increase biopesticides, some policy measures should be taken. One of the main problems in commercializing biopesticides is the lack of awareness about the effective use of biopesticides and their advantages. They are still not well addressed in India. It indicates the weakness of policy networks. Some other problems in promoting biopesticides are limited resources, lack of profile, unhealthy relationships between producers and regulators, and limited capabilities. To increase the profile of biopesticides at country level, it's important to understand the mode of action, their contribution to sustainable agriculture, the issues in their adoption, and their effectivity. The safety of the environment is a worldwide concern; so common people, policymakers, manufacturers, government agencies, and farmers need to be aware of the use of biopesticides [106,107].

The science behind biopesticides is still not well known completely. Numerous researches have been conducted on extraction, separation, and quantification of biopesticides but not on the formulation part. Some research should be conducted regarding the formulation and commercialization of biopesticides.

Effects of Phytochemicals on Human Health and Diseases

Phytochemicals are using as biopesticides and also adequate resources of therapeutic compounds and drugs [108]. Dietary guidelines worldwide prescribed increased consumption of phytochemicals or foods to fight chronic diseases like diabetes, cancer, osteoporosis, cardiovascular diseases, and pathogens' infections [109-116]. Plants are an excellent source of phytochemicals in fruits, legumes, vegetables, and cereals, which contain several health beneficial phytochemicals such as carotenoids, terpenoids, phytosterols, flavonoids, isoflavones, isothiocyanates, and fibers.

As a critical etiological agent in the causation of chronic diseases, oxidative stress has received a great deal of interest in recent years. The high reactive oxygen species (ROS) causes oxidative stress that induces oxidative damage to critical cellular biomolecules such as the DNA, lipids, and proteins, which can accumulate in cells and drive to increased risk of chronic pathological conditions [117]. The ROS are generated endogenously as by-products of normal metabolic processes and lifestyle factors like smoking, diet, and exercise. Under their ability to interact with ROS, antioxidants can lessen their damaging effects and play a vital role in controlling pathological conditions [118]. The principal sources of antioxidants are plants like vitamins (A, C, and E), minerals, and phytochemicals (terpenoids, carotenoids, and polyphenols) [119-122]. Besides, several phytochemicals have been shown anti-viral, anti-bacterial, and anti-fungal properties [121,123-125]. Furthermore, more research should be conducted for finding novels phytochemicals or repurposed the existing phytochemicals as a drug against several metabolic and pathogens related diseases like coronaviruses disease-2019 (COVID19) [126-128].

Conclusion

For developing biopesticides, a tremendous amount of plant materials is required. Research on biopesticides should be given more focus on more production of plant materials through biotechnology intervention. The barrier to standardization of chemically complex extracts could be manifested individually or in combination depending on the chemical enrichment of plant species with potential pesticides and interactions between them. The scientists are working more on isolation and characterization of toxicants from

plant and other biological sources, but there is a demand for the use of biopesticides. Only a few biopesticides are in commercial use; therefore, more research and development should be conducted on pesticide formulations for sustainable pest management strategies.

Biopesticides should have entrepreneurial opportunities in agricultural sectors, particularly that of integrated pest management in high-value fruit and vegetable crops in addition to ectoparasite control in animals. Ever-growing urbanization should produce an expanding market with opportunities for botanical insecticides as human safety should also push the demand concerning professional pest control, consumer products, and vector management. Even, there may be some small-scale entrepreneurial opportunities to supply plant materials to the biopesticide industries. In the context of agricultural pest management, botanical insecticides are best suited for organic food production in industrialized countries but can play a more significant role in the production and post-harvest protection of the food in developing countries.

Finally, phytochemicals are excellent sources for finding good biopesticides and also therapeutic drugs to fight against human metabolic diseases and pathogens' infections. Furthermore, research should be focused on identifying novel biopesticides and therapeutic drugs from bioresources.

Conflict of Research Interest

Authors declare no conflict of research interests.

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References

1. Dhaliwal GS, Arora R, Dhawan A. Crop losses due to insect pests in Indian agriculture: An update. Indian Journal of Ecology. 2004;31:1-7.
2. Oerke E-C. CHAPTER 3 - Estimated crop losses due to pathogens, animal pests and weeds. In: Oerke EC, Dehne HW, SchÖNbeck F, Weber A, editors. Crop Production and Crop Protection. Amsterdam: Elsevier; 1999. p. 72-741.
3. Pavela R, Žabka M, Bednář J, Tříska J, Vrchotová N. New knowledge for yield, composition and insecticidal activity of essential oils obtained from the aerial parts or seeds of fennel (Foeniculum vulgare Mill.). Industrial Crops and Products. 2016;83:275-82.
4. Anjarwalla P, Belmain S, Ofori DA, Jamnadass R, Stevenson PC, Sola P. Handbook on Pesticidal Plants2016.
5. A.M AEG. Plant Extracts as Effective Compounds in Pest Control: Natural Products. LAP Lambert Academic Publishing AG & Co KG. 2012:132.
6. Khater HF. Prospects of botanical biopesticides in insect pest management. Pharmacologia. 2012;3(12):641-56.
7. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology. 2006;51:45-66.
8. Grzywacz D, Stevenson PC, Mushobozi WL, Belmain S, Wilson K. The use of indigenous ecological resources for pest control in Africa. Food Security. 2014;6(1):71-86.
9. Dubey NK. Natural Products in Plant Pest Management: CABI; 2011.
10. Ware GW, Whitacre DM. The pesticide book. Willoughby, OH: MeisterPro Information Resources; 2004.
11. Koul O, Dhaliwal GS. Microbial biopesticides: CRC Press; 2001.
12. Koul O, Walia S. Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. Cab Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2009;4.
13. Asase A, Oteng-Yeboah AA, Odamtten GT, Simmonds MS. Ethnobotanical study of some Ghanaian anti-malarial plants. Journal of Ethnopharmacology. 2005;99(2):273-9.
14. Mkenda P, Mwanauta R, Stevenson PC, Ndakidemi P, Mtei K, Belmain SR. Extracts from Field Margin Weeds Provide Economically Viable and Environmentally Benign Pest Control Compared to Synthetic Pesticides. PLoS One. 2015;10(11):e0143530.
15. Benelli G. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. Parasitology Research. 2015;114(9):3201-12.
16. Pavela R, Kazda J, Herda G. Effectiveness of Neem (Azadirachta indica) Insecticides against Brassica Pod Midge (Dasineura Brassicae Winn.). Journal of Pest Science. 2009;82:235-40.
17. Sidhu OP, Kumar V, Behl HM. Variability in Neem (Azadirachta indica) with respect to azadirachtin content. J Agric Food Chem. 2003;51(4):910-5.
18. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. Phytotherapy research : PTR. 2007;21(12):1142-5.
19. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research. 2007;10(2).
20. Raina VK, Srivastava SK, Jain N, Ahmad A, Syamasundar KV, Aggarwal KK. Essential oil composition of Curcuma longa L. cv. Roma from the plains of northern India. Flavour and Fragrance Journal. 2002;17(2):99-102.
21. K G, S S. Neem – A Green Treasure. Electronic Journal of Biology. 2008;4:102-11.
22. Tomlin C, British Crop Protection C. The pesticide manual : a world compendium. Farnham, Surrey, UK: British Crop Protection Council; 1997.
23. Brahmachari G. Neem--an omnipotent plant: a retrospection. Chembiochem : a European journal of chemical biology. 2004;5(4):408-21.
24. Mustika A, Hadi UK, Wardhana A, Rahminiwati M, Wientarsih I, editors. The Efficacy Of Larvicides Of Leaves Of Yam Bean (Pachyrhizuserosus)As Botanical Insecticides Against Fly Larvae Myiasis Chrysomyabezziana2016.
25. Vismaya, Belagihally SM, Rajashekhar S, Jayaram VB, Dharmesh SM, Thirumakudalu SKC. Gastroprotective Properties of Karanjin from Karanja (Pongamia pinnata) Seeds; Role as Antioxidant and H, K-ATPase Inhibitor. Evid Based Complement Alternat Med. 2011;2011:747246-.
26. Dwivedi S, Chopra D. Revisiting Terminalia arjuna - An Ancient Cardiovascular Drug. Journal of traditional and complementary medicine. 2014;4(4):224-31.
27. Ibrahim SRM, Abdallah HM, El-Halawany AM, Mohamed GA.

Naturally occurring thiophenes: isolation, purification, structural elucidation, and evaluation of bioactivities. Phytochemistry Reviews. 2016;15(2):197-220.

28. Mir RA, Ahanger MA, Agarwal RM. Marigold: From Mandap to Medicine and from Ornamentation to Remediation. American Journal of Plant Sciences. 2019;Vol.10No.02:30.

29. Bhati R, Singh A, Saharan VA, Ram V, Bhandari A. Strychnos nux-vomica seeds: Pharmacognostical standardization, extraction, and antidiabetic activity. J Ayurveda Integr Med. 2012;3(2):80-4.

30. Gupta A, Chaudhary R, Sharma S. Potential Applications of Mahua (Madhuca indica) Biomass. Waste and Biomass Valorization. 2012;3(2):175-89.

31. Verma N, Jha K, Kumar U, Deepak K, Singh NK, Singh A, et al. Biological Properties, Phytochemistry and Traditional Uses of Mahua (Madhuca longifolia): A Review. 2014.

32. Nn A. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Medicinal and Aromatic plants. 2015;4:1-6.

33. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera Leaf extract by the appropriate extraction method. Industrial Crops and Products. 2013;44:566– 71.

34. Sulaiman SF, Sajak AAB, Ooi KL, Supriatno, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. Journal of Food Composition and Analysis. 2011;24(4):506-15.

35. Borhan MZ, Ahmad R, Rusop M, Abdullah S. Impact of Nanopowders on Exraction Yield of Centella asiatica. Advanced Materials Research. 2013;667:246-50.

36. Kaufmann B, Christen P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. Phytochemical Analysis : PCA. 2002;13(2):105-13.

37. Mediani A, Abas F, Khatib A, Tan C. Cosmos Caudatus as a Potential Source of Polyphenolic Compounds: Optimisation of Oven Drying Conditions and Characterisation of Its Functional Properties. Molecules (Basel, Switzerland). 2013;18:10452-64.

38. Ahmad T, Singh S, Pandey S, editors. Phytochemical Screening and Physicochemical Parameters of Crude Drugs : A Brief Review2014.

39. Egwaikhide P, Gimba C. Analysis of the Phytochemical Content and Anti-microbial Activity of Plectranthus glandulosis Whole Plant. Middle-East Journal of Scientific Research. 2007;2.

40. Narasimhan R, Mohan A. Phytochemical screening of sesamum indicum seed extract. World Journal of Pharmacy and Pharmaceutical Sciences. 2012;1:1298-308.

41. Mir Ma, Sawhney SS, Jassal MMS. Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Journal of Pharmacy and Pharmocology. 2013;2:001-5.

42. Arya V, Thakur N, Kashyap C. Preliminary Phytochemical Analysis of the Extracts of Psidium Leaves. Middle - East Journal of Scientific Research. 2014;19.

43. Al-Mansoub MA, Asmawi MZ, Murugaiyah V. Effect of extraction solvents and plant parts used on the antihyperlipidemic and antioxidant effects of Garcinia atroviridis: a comparative study. Journal of the Science of Food and Agriculture. 2014;94(8):1552-8.

44. Naudé Y, Beer WHJ, Jooste S, Merwe L, Rensburg SJ. Comparison of Supercritical Fluid Extraction and Soxhlet Extraction for the Determination of DDT, DDD and DDE in Sediment. Water SA. 1998;24:205-14.

45. Amid A, Salim R, Adenan M. The factors affecting the extraction condition for neuroprotective activity of Centella asiatica evaluated by metal chelating activity assay. Journal of Applied Sciences. 2010;10(10):837-42.

46. Hossain MA, Al-Toubi WA, Weli AM, Al-Riyami QA, Al-Sabahi JN. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. Journal of Taibah University for Science. 2013;7(4):181-8.

47. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Industrial Crops and Products. 2013;44:566-71.

48. Kaufmann B, Christen P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques. 2002;13(2):105-13.

49. Trusheva B, Trunkova D, Bankova V. Different extraction methods of biologically active components from propolis: a preliminary study. Chemistry Central Journal. 2007;1(1):13.

50. Puttarak P, Panichayupakaranant P. A new method for preparing pentacyclic triterpene rich Centella asiatica extracts. Natural Product Research. 2013;27(7):684-6.

51. Kumoro AC, Hartati I. Microwave assisted extraction of dioscorin from Gadung (Dioscorea hispida Dennst) tuber flour. Procedia Chemistry. 2015;14:47-55.

52. Vasu S, Palaniyappan V, Badami S. A novel microwave-assisted extraction for the isolation of andrographolide from Andrographis paniculata and its in vitro antioxidant activity. Natural Product Research. 2010;24(16):1560-7.

53. United Nations Industrial Development O, Handa SS, Khanuja SPS, Longo G, Rakesh DD, United Nations Industrial Development O, et al. Extraction technologies for medicinal and aromatic plants. Trieste (Italy): Earth, Environmental and Marine Sciences and Technologies; 2008.

54. Dhanani T, Tapadia S, Gajbhiye N, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian Journal of Chemistry. 2013.

55. Yingngam B, Monschein M, Brantner A. Ultrasound-assisted extraction of phenolic compounds from Cratoxylum formosum ssp. formosum leaves using central composite design and evaluation of its protective ability against H2O2-induced cell death. Asian Pacific Journal of Tropical Medicine. 2014;7:S497-S505.

56. Rahmalia W, Fabre J-F, Mouloungui Z. Effects of Cyclohexane/Acetone Ratio on Bixin Extraction Yield by Accelerated Solvent Extraction Method. Procedia Chemistry. 2015;14:455-64.

57. Tan L, Ji T, Jiang G, Hu F. Simultaneous identification and quantification of five flavonoids in the seeds of Rheum palmatum L. by using accelerated solvent extraction and HPLC–PDA–ESI/MSn. Arabian Journal of Chemistry. 2019;12(7):1345-52.

58. Patil AA, Sachin BS, Wakte PS, Shinde DB. Optimization of supercritical fluid extraction and HPLC identification of wedelolactone from Wedelia calendulacea by orthogonal array design. Journal of Advanced Research. 2014;5(6):629-35.

59. Williams CA, Goldstone F, Greenham J. Flavonoids, cinnamic acids and coumarins from the different tissues and medicinal preparations of Taraxacum officinale. Phytochemistry. 1996;42(1):121-7.

60. Gwatidzo L, Dzomba P, Mangena M. TLC separation and antioxidant activity of flavonoids from Carissa bispinosa, Ficus sycomorus, and Grewia bicolar fruits. Nutrire. 2018;43(1):3.

61. Butnariu M. Methods of analysis (extraction, separation, identification and quantification) of carotenoids from natural products. J Ecosyst Ecography. 2016;6(2):1-19.

62. Mercadante AZ, Steck A, Pfander H. Carotenoids from Guava (Psidium guajava L.): Isolation and Structure Elucidation. Journal of Agricultural and Food Chemistry. 1999;47(1):145-51.

63. Cakir A, Mavi A, Yildirim A, Duru ME, Harmandar M, Kazaz C. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of Hypericum hyssopifolium L. by activity-guided fractionation. Journal of Ethnopharmacology. 2003;87(1):73-83.

64. Triantaphyllou GB, Dimitrios Boskou, Kalliopi. Antioxidative properties of water extracts obtained from herbs of the species Lamiaceae. International Journal of Food Sciences and Nutrition. 2001;52(4):313-7.

65. Silva A, Rocha R, Oliveira C, Mira L, Duarte M, Florêncio M. Antioxidants in medicinal plant extracts. A research study of the antioxidant capacity of Crataegus, Hamamelis and Hydrastis. Phytotherapy Research : PTR. 2001;14:612-6.

66. Sun L, Rezaei KA, Temelli F, Ooraikul B. Supercritical fluid extraction of alkylamides from Echinacea angustifolia. Journal of Agricultural and Food Chemistry. 2002;50(14):3947-53.

67. Petry FC, Mercadante AZ. New Method for Carotenoid Extraction and Analysis by HPLC-DAD-MS/MS in Freeze-Dried Citrus and Mango Pulps. Journal of the Brazilian Chemical Society. 2018;29:205-15.

68. Almela L, López-Roca J-M, Candela ME, Alcázar MD. Separation and determination of individual carotenoids in a Capsicumcultivar by normal-phase high-performance liquid chromatography. Journal of Chromatography A. 1990;502:95-106.

69. Dachtler M, Glaser T, Kohler K, Albert K. Combined HPLC-MS and HPLC-NMR on-line coupling for the separation and determination of lutein and zeaxanthin stereoisomers in spinach and in retina. Analytical Chemistry. 2001;73(3):667-74.

70. Ligor M, Kováčová J, Gadzała-Kopciuch RM, Studzińska S, Bocian S, Lehotay J, et al. Study of RP HPLC Retention Behaviours in Analysis of Carotenoids. Chromatographia. 2014;77(15-16):1047-57.

71. Emenhiser C, Simunovic N, Sander L, Schwartz S. Separation of Geometrical Carotenoid Isomers in Biological Extracts Using a Polymeric C30 Column in Reversed-Phase Liquid Chromatography. Journal of Agricultural and Food Chemistry. 1996;44:3887-93.

72. Humphries JM, Khachik F. Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. Journal of Agricultural and Food Chemistry. 2003;51(5):1322-7.

73. Sander LC, Sharpless KE, Craft NE, Wise SA. Development of engineered stationary phases for the separation of carotenoid isomers. Analytical Chemistry. 1994;66(10):1667-74.

74. Breithaupt DE, Wirt U, Bamedi A. Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (Tagetes erecta L.) and several fruits by liquid chromatography-mass spectrometry. J Agric Food Chem. 2002;50(1):66-70.

75. Tsao R, Deng Z. Separation procedures for naturally occurring antioxidant phytochemicals. Journal of Chromatography B. 2004;812(1):85-99.

76. Lu H-T, Jiang Y, Chen F. Application of preparative high-speed counter-current chromatography for separation of chlorogenic acid from Flos Lonicerae. Journal of Chromatography A. 2004;1026(1-2):185-90.

77. Apáti P, Szentmihályi K, Kristó ST, Papp I, Vinkler P, Szoke E, et al. Herbal remedies of Solidago--correlation of phytochemical characteristics and antioxidative properties. Journal of Pharmaceutical and Biomedical Analysis. 2003;32(4-5):1045-53.

78. Robards K. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. J Chromatogr A. 2003;1000(1-2):657-91.

79. Kohler M, Haerdi W, Christen P, Veuthey JL. Supercritical fluid extraction and chromatography of artemisinin and artemisinic acid. An improved method for the analysis of Artemisia annua samples. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques. 1997;8(5):223-7.

80. Careri M, Bianchi F, Corradini C. Recent advances in the application of mass spectrometry in food-related analysis. Journal of Chromatography A. 2002;970(1-2):3-64.

81. Greving MP, Patti GJ, Siuzdak G. Nanostructure-initiator mass spectrometry metabolite analysis and imaging. ACS Publications; 2011.

82. Mk G, mohan kumar V. Bio-efficacy study on alcoholic extracts of fern plants against diamondback moth, Plutella xylostella L. in cabbage. Innovation Journal. 2019;8:5-07.

83. Ashraf HJ, Abbasi A, Khan M, Abbas A. Efficacy of different plant extracts against Brevicoryne brassicae and their effects on pollinators. 2018.

84. Ali H, Ahmad S, Hassan G, Amin A, Hussain Z, Naeem M. Bioefficacy of different plant extracts against melon fruit fly in bitter gourd. Pakistan Journal of Weed Science Research. 2011;17:143-9.

85. Yao Y, Cai W, Yang C, Xue D, Huang Y. Isolation and characterization of insecticidal activity of (Z)-asarone from Acorus calamus L. Insect Science. 2008;15(3):229-36.

86. Basukriadi A, Wilkins RM. Oviposition deterrent activities of Pachyrhizus erosus seed extract and other natural products on Plutella xylostella (Lepidoptera: Plutellidae). Journal of Insect Science. 2014;14(1):244.

87. Arivoli S, Tennyson S. Larvicidal efficacy of Strychnos nuxvomica Linn.(Loganiaceae) leaf extracts against the filarial vector Culex quinquefasciatus Say (Diptera: Culicidae). World Journal of Zoology. 2012;7(1):06-11.

88. Pawar P, Joseph M, Tungikar V, Joshi S. Bioactivity of non-edible oil seed extracts and purified extracts against Helicoverpa armigera (Hubner). Indian Journal of Experimental Biology. 2004;42(1):91-5.

89. Singh DV, Gupta MM, Tripathi AK, Prajapati V, Kumar S. Arjunetin from Terminalia arjuna as an insect feeding-deterrent and growth inhibitor. Phytotherapy Research : PTR. 2004;18(2):131-4.

90. Phoofolo M, Mabaleha S, Mekbib SB. Laboratory assessment of insecticidal properties of Tagetes minuta crude extracts against Brevicoryne brassicae on cabbage. Journal of Entomology and Nematology. 2013;5(6):70-6.

91. Isman MB, Grieneisen ML. Botanical insecticide research: many publications, limited useful data. Trends in Plant Science. 2014;19(3):140-5.

92. Seaman D. Trends in the formulation of pesticides—an overview. Pesticide Science. 1990;29(4):437-49.

93. Brar SK, Verma M, Tyagi R, Valéro J. Recent advances in downstream processing and formulations of Bacillus thuringiensis based biopesticides. Process Biochemistry. 2006;41(2):323-42.

94. Knowles A. Recent developments of safer formulations of agrochemicals. The Environmentalist. 2008;28(1):35-44.

95. Pesticide Formulations. Encyclopedia of Agrochemicals.

96. Knowles A. New developments in crop protection product formulation. T&F Informa UK Ltd. 2005.

97. Winder R, Wheeler J, Conder N, Otvos I, Nevill R, Duan L. Microencapsulation: a strategy for formulation of inoculum. Biocontrol Science and Technology. 2003;13(2):155-69.

98. El-Sayed W. Biological control of weeds with pathogens: Current status and future trends / Biologische Schadpflanzenbekämpfung mit Pathogenen: Aktueller Status und Trends von morgen. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz / Journal of Plant Diseases and Protection. 2005;112(3):209-21.

99. Chen K-N, Chen C-Y, Lin Y-C, Chen M-J. Formulation of a novel antagonistic bacterium based biopesticide using microencapsulated techniques in fungal disease control. Journal of Agricultural Science. 2013;5.

100. Henn T, Weinzierl R, Division INHS, Service UoIaU-CCE. Botanical Insecticides and Insecticidal Soaps: University of Illinois at Urbana-Champaign, College of Agriculture, Cooperative Extension Service; 1989.

101. El-Wakeil N. Botanical Pesticides and Their Mode of Action. Gesunde Pflanzen. 2013;65.

102. Mpumi N, Mtei K, Machunda R, Ndakidemi P. The Toxicity, Persistence and Mode of Actions of Selected Botanical Pesticides in Africa against Insect Pests in Common Beans, P. vulgaris : A Review. American Journal of Plant Sciences. 2016;07:138-51.

103. Hinson D. Rotenone characterization and toxicity in aquatic systems. Principles of Environmental Toxicity University of Idaho, Moscow. 2000.

104. Khalil MS. Abamectin and azadirachtin as eco-friendly promising biorational tools in integrated nematodes management programs. Journal of Plant Pathology and Microbiology. 2013;4(4):2-3.

105. Abdullahi M, Uba A, Yaro A, Maxwell O, Yusuf A, Kabir S, et al., editors. Phytochemical Screening, Acute Toxicity study and Evaluation of Antidiabetic properties of the methanolic leaf extract of Vernonia glaberrima (Asteraceae)2015.

106. Keswani C, Dilnashin H, Birla H, Singh SP. Regulatory barriers to Agricultural Research commercialization: A case study of biopesticides in India. Rhizosphere. 2019;11:100155.

107. Koul O, Dhaliwal G. Phytochemical biopesticides: CRC Press; 2000.

108. Dillard CJ, German JB. Phytochemicals: nutraceuticals and human health. Journal of the Science of Food and AGRICULTURE. 2000;80(12):1744-56.

109. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. The American Journal of Clinical Nutrition. 2002;76(3):560-8.

110. Khalesi S, Irwin C, Schubert M. Flaxseed consumption may reduce blood pressure: a systematic review and meta-analysis of controlled trials. The Journal of Nutrition. 2015;145(4):758-65.

111. Medina-Remón A, Tresserra-Rimbau A, Pons A, Tur JA, Martorell M, Ros E, et al. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. Nutrition, Metabolism and Cardiovascular Diseases. 2015;25(1):60-7.

112. Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, et al. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition. 2008;88(1):38-50.

113. Park E-J, M Pezzuto J. Flavonoids in cancer prevention. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2012;12(8):836-51.

114. Wang Y, Cui R, Xiao Y, Fang J, Xu Q. Effect of carotene and lycopene on the risk of prostate cancer: a systematic review and dose-response meta-analysis of observational studies. PLoS One. 2015;10(9):e0137427.

115. Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues J-F. Intake of flavonoids and risk of dementia. European Journal of Epidemiology. 2000;16(4):357-63.

116. Sharma V, Makhdoomi M, Singh L, Kumar P, Khan N, Singh S, et al. Trehalose limits opportunistic mycobacterial survival during HIV co-infection by reversing HIV-mediated autophagy block. Autophagy. 2020:1-20.

117. Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD. ROS and the DNA damage response in cancer. Redox Biology. 2019;25:101084.

118. Forni C, Facchiano F, Bartoli M, Pieretti S, Facchiano A, D'Arcangelo D, et al. Beneficial Role of Phytochemicals on Oxidative Stress and Age-Related Diseases. BioMed Research International. 2019;2019:8748253-.

119. Sarker U, Oba S, Daramy MA. Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem amaranth. Sci Rep. 2020;10(1):3892-.

120. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The American Journal of Clinical Nutrition. 2003;78(3):517S-20S.

121. Martinez KB, Mackert JD, McIntosh MK. Chapter 18 - Polyphenols and Intestinal Health. In: Watson RR, editor. Nutrition and Functional Foods for Healthy Aging: Academic Press; 2017. p. 191-210.

122. Duma M, Alsina I, Zeipina S, Lepse L, Dubova L. Leaf vegetables as source of phytochemicals2014.

123. Zhang Y-J, Gan R-Y, Li S, Zhou Y, Li A-N, Xu D-P, et al. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Molecules (Basel, Switzerland). 2015;20(12):21138-56.

124. Ghildiyal R, Prakash V, Chaudhary VK, Gupta V, Gabrani R. Phytochemicals as Antiviral Agents: Recent Updates. Plant-derived Bioactives. 2020:279-95.

125. Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, et al. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiological Research. 2017;196:44-68.

126. Khan N. Possible protective role of 17β-estradiol against COVID-19. Journal of Allergy and Infectious Diseases. 2020;1(2):38-48.

127. Chojnacka K, Witek-Krowiak A, Skrzypczak D, Mikula K, Młynarz P. Phytochemicals containing biologically active polyphenols as an effective agent against Covid-19-inducing coronavirus. Journal of Functional Foods. 2020;73:104146.

128. Mani JS, Johnson JB, Steel JC, Broszczak DA, Neilsen PM, Walsh KB, et al. Natural product-derived phytochemicals as potential agents against coronaviruses: A review. Virus Research. 2020;284:197989.