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Importance of Secondary Plant Metabolites in Plant Protection against Agricultural Insect Pests

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Abstract

Pest management is facing the economic and ecological challenge worldwide due to the human and environmental hazards caused by majority of chemical pesticides. Identification of novel effective insecticidal compounds is essential to combat increasing resistance buildup. Secondary plant metabolites have long been as good alternatives to the chemical insecticides for insect pest managements because it reputedly pose little threat to environment or human health. A number of plant have been considered for use as insect antifeedants, repellents, growth regulators (IGRs) and toxicants. In the context of agricultural pest management, secondary plant metabolism are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and postharvest protection of food in developing countries. Improvement in the understanding of plant allele chemical mechanisms of activity offer new prospects for using these substances in crop protection. Essential oils and plant extracts proved to be safe available alternatives to the synthetic pesticides in plant protection in field and during storage. En-capsulation of essential oils inside nano-particle is a new technology of formulation increasing persistence of the active ingredients, achieve high stability of efficacy, which used as delivery systems and considered as a promising strategy to deliver essential oils in agriculture.

Keywords: Plant Secondary Metabolites; Volatile Oils; Plant Extracts; Nano-Formulation

Introduction

Chemical insecticides designed to kill repell or retard the growth of insect pests that damage or interfere with the growth of crops and other vegetation consumed by humans. All chemical pesticides are poisonous and possess long term danger to the environment and human through their persistence in nature or body tissues [1]. Secondary plant metabolites are naturally occurring chemicals derived or extracted from primary plant metabolites [2]. Primary metabolites directly involved in physiological function in the organisms such as normal growth, development and reproduction for example carbohydrates, proteins, lipids, nucleic acid and hormones. Secondary plant metabolites play an important role in plant defensive against insect herbivores and avoid infection by microbial pathogens. Secondary metabolites such Trpenes, Phenolic compound, Coumarin, Lgin, Flavonoids, Tanins sulphur containing, Phytoalexins, Thionin, Lectin, alkaloids, Cyanogenic glucosides and Non-protein amino acids. Indeed, the scientific literature of past 25 years described hundreds of isolated plant secondary metabolites that showed feeding deterrents or toxic effects to insects in laboratory bioassay, and botanical insecticides have been the subject of several recent volumes [3,4]. There are many factors determining using the secondary plant metabolites for pest control as reported by [5], such as the formulation, the active ingredient, the time of application or exposure, to direct or indirect contact, the quantity used the mixture of substances, the climate and season of the year when it is applied [5]. The first secondary metabolites to be used as pesticides came from easily available products; several recent books have reviewed bio pesticides of plant origin [6]. The present review recorded some essential oils and their constituent of Terpenes with plan extracts that proved effects in protection the agricultural crops in field and during storage. Also the importance of the new technology of essential oils formulation improves both their stability and effectiveness [7].

Plant Essential Oils and Terpenes against Agricultural Insect Pests

Steam distillation of aromatic plants yield essential oils, long used as fragrance and flavoring in perfume and food industries, more recently as herbal medicines [8,9]. The oils are generally composed of complex mixture of mono Terpenes, biogenetically related phenols and sesquiterpenes. A number of the source plants have been traditionally used for protection of stored commodities, but interest in the oils was renewed with emerging demonstration of their fumigant and contact insecticidal activities to a wide range of insect pests [10] *Juniper communis* L. (Cupressaceae) is a genus of evergreen shrubs or trees, smoke from burnt juniper branches has been used as fumigant to prevent the spread of pathogenic infections [11]. In addition, there are numerous reports on the biological activity of the essential oil of *J. communis* [12,13]. Sharaby A, et al. [13] reported that *J. communis* essential oil attracted both sex of the RPW. Hashemi SM, et al. [11] who recorded that *J. communis* fruit essential oil had insecticidal activity against two stored product beetles, Insecticidal activity varied with essential oil concentration and exposure time. In some cases, oils also may act as poisons, interacting with the fatty acids of the insect and interfering with normal metabolism. Some researchers have demonstrated that essential oils have neurotoxic, cititoxic and mutagenic actions among others in different organisms, and the essential oils act as multiple levels in the insect, so the possibility of generating resistance is little probable [13]. Dhaliwal GS, et al. [5] mentioned that insects' response varied according to oil type, dose and developmental stage. Oils work primarily by covering and suffocating the pest. In some cases, they can disrupt certain membranes of exoskeleton [14]. Khalaf AA, et al. [15] mentioned that protein decreased in 2nd instars larvae of *Muscina stabulus* treated with oil of *Rosmarium officinalis* and *Cymbopogon citrates*. Breuer M, et al. [16] found that *Melia azedarach* extract inhibited the enzyme activity with 31% in *Leucopphae maderae* larvae than in control. Furthermore, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential oil based pesticides owing to the complex mixture of constituents that characterize many of these oils. Recently Yamane H, et al. [17] determined the biological effects of essential oils isolated from *Cymbopogon nardus, C.flexuosus* and *C. martini* against two Lepidopterous larvae , the all tested oils showed antifeedant activity and dermal contact lethality against *Acharia fusca* and *Euprosterna elaeasa* (Lepidoptera: Emacodidae) at various concentrations. All oils exhibited strong antifeedants and toxicity activity toward the insects. Sharaby A, et al. [18] recorded the toxicity of the tested three different essential oils on the 1st nymphal instar of *H. litoralis littoralis* is shown in Table 1. Latent toxicity was observed, for this LC50 values were recorded after 14 days from the Steam distillation of aromatic plants yield essential oils, long used as fragrance and flavoring in perfume and food industries, more recently as herbal medicines [8,9]. The oils are generally composed of complex mixture of monoterpenes, biogenetically related phenols and sesquiterpenes. A number of the source plants have been traditionally used for protection of stored commodities, but interest in the oils was renewed with emerging demonstration of their fumigant and contact insecticidal activities to a wide range of insect pests [10] *Juniper communis* L. (Cupressaceae) is a genus of evergreen shrubs or trees, smoke from burnt juniper branches has been used as fumigant to prevent the spread of pathogenic infections [11]. In addition, there are numerous reports on the biological activity of the essential oil of *J. communis* [12,13]. Sharaby A, et al. [13] reported that *J. communis* essential oil attracted both sex of the RPW. Hashemi SM, et al. [11] who recorded that *J. communis* fruit essential oil had insecticidal activity

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Plant Essential Oil	**LC values mg./100ml. diet**			**Fiducial limit**		**Slope**
	LC_{25}	**LC_{50}**	**LC_{90}**	**Upper**	**Lower**	
Garlic Allium sativum	0.009	0.067	2.98	0.1038	0.031	0.7843
Eucalyptus Eucalyptus globulus	0.008	0.075	5.23	0.1187	0.0315	0.6951
Mint Mintha pipreta	0.001	0.84	215.6	0.1723	0.0052	0.376

Table 1: Susceptibility of the grasshopper *H. littoralis* 1St instars nymph to the natural plant essential oils mixed with the artificial diet.

Chaubey MK, et al. [26] found that α-pinene and β-caryophyllene have been evaluated for their repellent, acute toxicity and development inhibitory activities alone and in binary combination against red flour beetle *T. castanium*. In repellency assay, α-pinine and β-caryophyllene repelled *T.castanium* adults significantly even at 0.025% concentration. Sharaby A, et al. [27] recorded the insecticidal activity of some terpenes against the cotton leafworm *S. littoralis*, also Sharaby A, et al. [28] mentioned that terpene Farnesol caused inhibitory effect on growth and development of *S. littoralos* larvae. Terpenes and terpenoids are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures with diverse functions [29]. Sharaby A, et al. [30] mentioned that the effect of a lot of plants which applied in pests control, are growth regulators because they disturb the growth of pests Figure 1.

Plant Extracts

Several researches have recorded the entomotoxic properties of the plant extracts from different plant species [31-33]. Sharaby A, et al. [34] who found that *Dodonea viscosa* extract at 5% concentration induced 75% death to *Spodoptera littoralis* 2nd instar larva fed for one day. The toxicity of *D. viscosa* because of the containment from sterols, viscosol, tannins, and high percentage of flavonoids, alkaloids saponins, coumarins and phenols [35]. Jewers K, et al. [36] recorded *R. stricta* extract had toxic effect on *Culex pipienes*, LC50 reached 190 ppm. After 10 day post treatment, also alcoholic extract gave larval mortality within two day and LC50 was 251 ppm. *R. stricta* contained high percentage of alkaloids. Dawidar AM, et al. [37] recorded that *C. schoenanthus* contained 11 terpines. Sharaby A, et al. [38] found that 2% *C. citratus* oil leading to high toxicity of 3rd instar larvae of *Spodoptera. exigua* within 2 h. from treatment LC50 reached 0.215% after 24 hours.

Sharaby A, et al. [34] reported that the extract of some plants as *Allium cepa*, *Curcuma longa, Dodonea viscose* and *Thuja orientals* have adverse effect on the potato tuber moth eggs, Their effects are similar to that caused by JH on the eggs of other insects. Plant extract of *Myrtillocactus geometrizans* showed insecticidal and insect growth regulatory activity on *Spodoptera frugiperda* [39]. Melia extracts have antifeedants activity through their effect on JH that disrupt the growth and development of insects [40]. 30 All plant extracts of *Eucalyptus rostrata, Dodonea viscose, Rhyza stricta* and *Cymbopogon schoenanthus* induced different effects as toxicant and growth regulators on tomato fruit worm *Helioverpa armigera* (Figure 1).

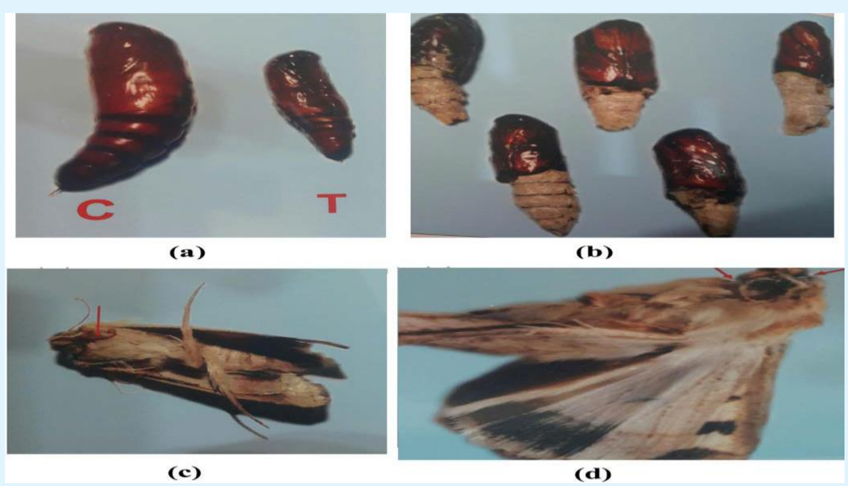
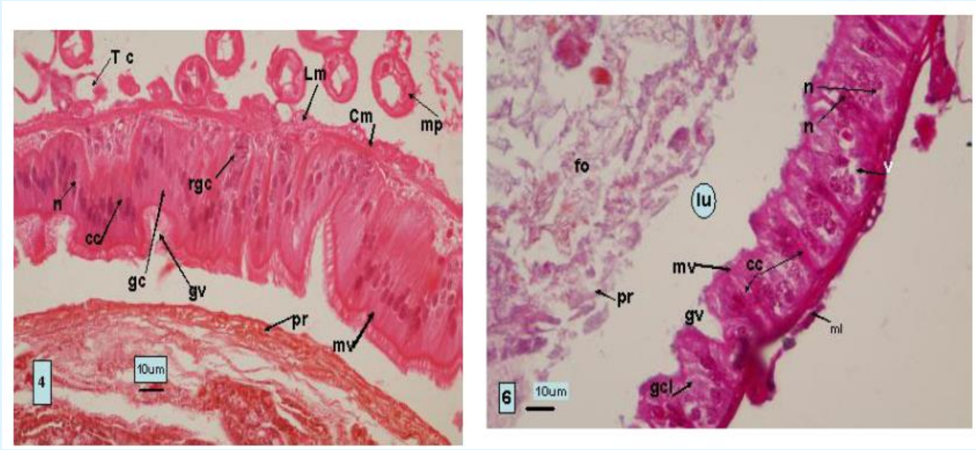


Figure 1: Showing different morphological deformation occurred between pupal and adult stages of H. armigera treated with the different tested. extracts, a: Minute pupa, T, compared with the untreated one C; b: Pupae in intermediate form between papa and adult; c: Deformed moth with twisted legs and minute antennae; d: Deformed moth with twisted wings, and mouth parts with shrinkage abdomen [30].



Aprivations: Md: Midgut; mp: Malpighian tubule; ov: Ovary; ts: Testes; int: Intestine; rc: Rectum; an: Anus; ep: Epithelial cells; cc: Columnar cells; gc: Goblet cells; n: Nucleus; gv: Goblet cavity; rgc: Regenerative cells; mv: Microvilli; pr: Peritrophic membrane; cm: Circular muscles; Lm: Longitudinal muscles; Tc: Trachea; V: Vacuoles; gc: Gastric caeca; In: Intiman; Ml: Muscular layer; fc: Fat cells; Fa: Fat droplets; dep: Degenerated epithelial cells; bm: Basement membrane; hg: hindgut; lu: Lumen; fo: Food particles.

Figure 2: 4: TS section in normal mid-gut, 6 TS section in treated mid gut, showing degeneration and rupture of the pretrophic membrane (Pr). Hypertrophy and hyperplasia of the epithelial cells of the treated mid gut.

Many botanical products have been found to act as oviposition and feeding deterrents, ovicidal, larvicidal agent against diverse range of insect pests [41,42]. At the same time, they have very little or no negative effects on beneficial organisms under field conditions [43]. Mainly, *Azadirachta indica* A. Juss have, substantially, been used in pest management worldwide since years [44]. Also, the seed extract of *Milletia ferruginea* has been observed to be effective in controlling many insects' pests such as *Callasobruchus chinensis*, *Sitophilus zeamais*, *Busseola fusca* (Fuller), *Zebrotes subfaciatus* and mosquito larvae [45-49]. Tremendous research is undergoing to evaluate the efficacy of various plant extracts against agricultural insect pests [50]. However, most of these works have been investigated the effectiveness of these plant derived insecticides under laboratory condition. Botanicals areas inexpensive and readily available tools for insect pest management, and eco-friendly. Moreover, the efficacy of aqueous seed extracts of *A. indica* has been also reported against larvae of *Spodoptera frugiperda* [51]. Combining botanicals with different mode of actions have been reported to be more effective than using them singly which is in correspondence with the previous reports of [52,53]. The seed extract of *M. ferruginea* has also been observed to be effective in controlling various insect pests such as; *Callasobruchus chinensis* [54], *Zebrotes subfaciatus* [48], adult termites, *H. armigera* larvae and *Busseola fusca*, mosquito larvae [49], ticks and other insect pests [55-61].

Essential Oils Formulation by Nanotechnology for Pest Control

Although the promising properties of essential oils based pesticides they have negative effects on their application for examples poor solubility, volatility, degradation. En-capsulation of essential oils inside nano-particle increasing persistence of active ingredients, decrease volatility and their degradation by the weather factors which leading to be active in pest management for long time. Campolo, et al. [7] reported that nano-particles improve the stability and effectiveness of botanical insecticides.

Botanical pesticides based on essential oils and their constituents of Terpenes became to be alternative tool in IPM program Tripathi AK [62] showed toxic, repellent, antifeedants, retardation in development, caused sterilizing effect and affect the reproduction in insect pests [63]. There are some negative properties of the botanical materials such as poor solubility, fast volatility, degradation by weather factors, so en-capsulation inside nano-particles is a good strategy to overcome the previous drawbacks [7] that formulation making it to be more stable and effective than if apply as bulk free substances. Beside that they are increasing persistence of the active ingredients. There are some of essential oils were encapsulated in nano-emulsion, to achieves high stability of efficacy, which used as delivery systems and considered as a promising strategy to deliver essential oils in agriculture and particularly in pest management [64].

Genus *Achillea* is one of the most important genera of family Asteraceae comprising more than 110 species, widespread over the Middle East [65]. The obtained oils Nano-emulsion formulation of *Achillea biebersteinii, A.santolina* and *A.mellifolium* considerable toxic and growth inhibitory activities against the red flour beetle, *Tribolium castaneum.* The oil of *A. santolina* showed the strongest activity introducing malformation in larvae and pupae of *T.castaneum*. Many of these larvae were failed to shed their soft larval skin and shortly died. As a result, the resulted pupae were smaller in size and the developed adults failed to shed pupal exuviae resulting in the formation of larval-pupil and pupil-adult intermediates as recorded in Figure 1, which were shortly died inside their abnormal integument. The life span and F_1 progeny of *T castaneum* were significantly affected, where the oil of *A.sanotolina* showed the strongest activity, all of these developmental disruptions led to a great reduction in the number of adults that undergo successful emergence [65].

Neem oil based nanoemulsion has showed good efficiency as compared to synthetic pesticides. The insecticidal activity of the nano-emulsion formulation of neem oil was studied against *Sitophilus oryzae* and *Triboleum castaneum* adults. The toxicity effects were significantly more pronounced for the nano-emulsion formulation compared with the crude extract of Neem oil and Neemix under all conditions. The nano-emulsion formulations os neem oil not only caused great mortality of the insects but also increased the speed of azadirachtin action to obtain 100% mortality. The increase in azadirachtin׳s speed of action probably is because of the great bioavailability of the active compound of azadirachtin present in the nano-emulsion formulations. The neem oil nano-emulsion formulation for this work increased the effectiveness of azadirachtin with the help of surfactants as compared with a simple, non-formulated crude extract of this compound. A crude extract of neem showed only 30% mortality at 1% concentration after 72 hr. of exposure time against *T. castaneu*.

The citrus peel essential oils (Lemon, Mandarin, and sweet Orange) have the good insecticidal activity against the tomato borer *Tuta absolita*. While the three formulations of pepper mint oil (bulk, nano-emulsion and nano-encapsulation) showed significant effects on most items of *Spodoptera littoralis*. Larval duration were prolonged, percentage mortalities were increased as well as larval malformations. Also, pupal duration was prolonged, percentage pupal mortality and pupal malformation [63]. Formulating essential oils into nano-emulsion which is transparent and can be used in food and beverage products, thereby, decreasing the amount of the oil required. Nano-purslane caused complete inhibition of *Ephestia cautella* and *E. kuehniella* moth emergence. Geranium essential oil loaded-solid lipid nanoparticle increased insecticidal effect on the development of larval stage of *S. littoralis* and *Phthorimaea operculella* well as adult longevity and fertility. Oil nano-particles have a much high chemical activity promoting penetration into insect body and enhancing pesticidal activity.

Conclusion

The plant kingdom offers a rich source of a wide range of structural biodiversity of natural secondary metabolites. One of the most recent trends in fungal and insect pest control is to reduce heavy reliance on synthetic pesticides and to move towards biodegradable substances. Synthetic pesticides of broad spectrum have been widely used as the main tools for controlling weeds, and fungal and insect pest, which are highly toxic to many living organisms as well as to the environment. Hence, new secondary plant metabolites such (plant extracts &volatile oils) and specific trends to pest control should be developed. In this article, an update of findings in the field were presented, those approaches resulting from the studies on inhibition of growth (including larval growth, pupation, toxicants, and emergence) and of the enzymes involved in key processes of insect life, specially modifying the apolysis during molting, sclerotization, pupation, and emergence. Since these approaches refer to control of insect pests, many of them can be extrapolated and also considered suitable for medicinal chemistry studies, because the mode of action of these inhibitors is similar to that from human and other animals. Therefore, there is an increasing expectation about the research on enzymes inhibition by those compounds of botanical origin that could serve as lead compounds for the development of important substances with agrochemical and pharmacological properties. Thus, by studying the plant secondary metabolites that protect plants against the pest attack, we can learn to control this attack in an ecological way and in addition can get pesticide with natural active substances. Their classes are offered good alternative for selective insect pest control that is in harmony with existing integrated pest management program. Nano-encapsulation is currently the most promising technology for protections plants against pests, therefore .it will be revolutionize agriculture including pest management in the future. The agriculture would be improved by means of nanotechnology. Several researches on nano-formulations from the secondary plant metabolites open the way for commercialization of nano pesticides which are more targeted delivery of pesticides on the specific can used in IPM of the insect pests.

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