Insecticidal Properties of some Plant Extracts against Granary Weevil, *Sitophilus granarius* L.

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ABSTRACT: Laboratory experiment was conducted under controlled conditions to test the insecticidal activity of aqueous and organic extract of different solvents (ethanol, acetone and hexane) of three plants collected from north-Sinai (Tree tobacco, *Nicotiana glauca* G.; Chinaberry, *Melia azedarach* L. and Lemon, *Citrus limon* L.) against the adult stage of Granary Weevil, *Sitophilus granarius* L. The results showed that the aqueous extract of *C. limon* gave the highest toxicity among the other tested plants with LD50 equal to 0.0013 x 10^5 ppm using ethanol solvent. However, the organic extracts of *M. azedarach* L. showed the best result with LD50 equal to 0.00056 x 10^5 ppm. On the other hand, both the aqueous and organic extracts of *N. glauca* gave the highest toxicity with LD50's of 0.0056 x 10^5 ppm and 0.00086 x 10^5 ppm, respectively, with acetone solvent. But by using hexane solvent, aqueous extract of *N. glauca* was obviously the most superior in toxicity compared to the aqueous extracts of the tested plants with LD50 (0.0017 x 10^5 ppm) and in the organic phase *M. azedarach* had the best result with LD50 equal to 0.00017 x 10^5 ppm .

Keywords: Insecticidal activity, *Nicotiana glauca, Melia azedarach, Citrus limon,* Granary Weevil.

INTRODUCTION

Insect infestation of stored grains and there products is a serious problem throughout the world. Annual worldwide post-harvest losses due to insect damage, microbial deterioration and other factors are estimated to be 10-25% (Matthews, 1993). Chemical insecticides are currently the method of choice to protect stored grains from insect damage (Domeracki and Zpierska, 1982; Karas *et al*., 2001; Bell *et al*., 2003 and Drinkall *et al*., 2005); however, their widespread use has led to the development of pest strains resistant to insecticides (Subramanyam and Hagstrum, 1995). As a result, there is a demand for safer insecticides because of concern about insecticide residues on grain and health hazards to grain handlers. Hence, an alternative to synthetic insecticides especially methyl bromide which depletes the stratospheric ozone layer is of utmost importance (Drinkall *et al*., 2005).

Many natural products are used exclusively as stored-product protectants. Such products have been used to control stored-product insect pests since the dawn of agriculture (Levinson and Levinson, 1998). In addition to being toxic, many natural products are also repellent or attractant to stored-product insects. Growing public concern for the environment has contributed to the change in attitude towards the use of botanicals in pest control. The use of natural products of plant origin is a new trend that preserves the environment from pollution with harmful toxicants. Several studies have suggested the use of plant extracts (Yadova, 1971; Su *et al*., 1972; Schoonhoven, 1978; Singh *et al*., 1978; Nassar *et al*., 1995; Azadbakht *et al*., 2004 and Negahban *et al*., 2007).

On the basis of the above information, the present work was conducted to evaluate the efficiency of aqueous and organic extracts of some wild plants against the adult stage of the Granary weevil, *Sitophilus granarius* L.

MATERIALS AND METHODS

Rearing methods of the tested insects:

Laboratory culture of adult stage of Granary weevil, *Sitophilus granarius* L. was used in the present study for bioassay test, wheat grains were used as rearing media for the tested insect. The insect breeding was carried out in special containers of 15 cm diameter and 30 cm height and was kept under laboratory conditions within 27±3^0 C and 65±5 R.H.

Collection and identification of tested plants:

The following plant samples were collected from the area surrounding Arish Airport (Table 1). Identification of tested plants was based mainly on the taxonomic characters detailed by Boulos and El-Hadidi (1984), and revised through personal communication with Dr. Hameda Bedair (Faculty of Education, Suez Canal University). Plant samples (Table 1) were air dried for 2-4 weeks until complete dryness, and then milled in an electric grinder into a fine powder and stored until used.

Table (1): List of the tested plant species and their extract parts.

No.	Tested plants	English name	Extract part
1	*Melia azedarach* L.	Chinaberry	Seeds
2	*Citrus limon* L.	Lemon	Leaves
3	*Nicotiana glauca* G.	Tree Tobacco	Leaves and flowers

Organic and aqueous extraction:

Twenty grams of each dried plant part (Table 1) was soaked in a dark flask containing 100 ml of one from three solvents (Ethanol, Acetone and Hexane) for organic extraction of each sample. The mixture was allowed to stand for 24 hours, and then filtered using whatman No.1 filter paper on Büchnur funnel. The obtained filtrate liquid represents the organic extract for each sample. Simultaneously, the solid deposit on the Büchnur funnel was washed with 100 ml of distilled water for each. The obtained water wash resembles the water extract for each plant sample. Both organic and water extracts were freshly prepared and used for the bioassay purposes.

Bioassay tested for each of the organic and aqueous extracts:

Series of dilutions with distilled water for water extracts, or with (Ethanol, Acetone and Hexane) solvent for the organic extracts were prepared for each stock solution. The dilutions were 1/10, 1/100, 1/1000 and 1/10 000 of original stock solution. For the bioassay treatments, five Petri dishes each containing 20 adults of the tested insect and each insect was topically treated with 5µl with the micro applicator (McCloud *et al*., 1988; Pemonge *et al.,* 1997; Zapata and Smagghe, 2010). Five replicates were used for each treatment, including the control. Average percentage mortality was recorded for each after 24 hrs. LD50 values and the corresponding slopes were deduced from the regression lines (Finney, 1952), and confidence limits were computed using the normal equivalent deviate program.

RESULTS

The insecticidal activities of the aqueous and organic extracts of the tested plants against *Sitophilus granarius* are summarized in table (2 and 3). The results indicated that the aqueous extract of *Citrus limon* showed the highest insecticidal activity with LD50 equal to (0.0013 x 10^5ppm) when we used ethanol solvent. *Melia azedarach* was the second in toxicity (LD50 = 0.0015 x 10^5ppm) followed by *Nicotiana glauca* with LD50 (0.0032x10^5ppm) (Table 2). However, organic extract of *Melia azedarach* was the most superior in toxicity compared to the other organic extracts of the tested plants. LD50 value for *Melia azedarach* was 0.00056x10^5 ppm. *Citrus limon* was the second toxicity against adults of *Sitophilus granarius* (LD50 = 0.0007 x 10^5ppm) followed by *Nicotiana glauca* with LD50 equal to 0.00078 x 10^5ppm (Table 3).

Result proved when we used Acetone solvent, both aqueous and organic extracts of *Nicotiana glauca* were the highest in toxicity than other extracts of tested plant species with LD50 equal to (0.0056x 10^5ppm) in aqueous extract and (0.00086x 10^5ppm) in the organic phase extract against the adult stage of *Sitophilus granarius* (Table 2-3). Aqueous extract of *Melia azedarach* was the second in toxicity among all tested extracts followed by *Citrus limon* with LD50 equal to (0.17x 10^5ppm) and (0.019x 10^5ppm) respectively (Table 2). However, Organic extract of *Melia azedarach* was the second in toxicity with LD50 equal to (0.008x 10^5ppm). The lowest toxicity was found with *Citrus limon* in the organic extract of acetone solvent with LD50 equal to (0.06x 10^5ppm) (Table 3).

Data in table (2) showed that the aqueous extract of *Nicotiana glauca* was the highest insecticidal activity with LD50 equal to (0.0017 x 10^5ppm) when we used hexane solvent. *Melia azedarach* was the second in toxicity (LD50 = 0.002 x 10^5ppm) and *Citrus limon* was the lowest in toxicity among all tested plant extracts with LD50 equal to (0.0032 x 10^5ppm). However, organic extract of Melia *azedarach* was the highest in toxicity than other aqueous extracts of the tested plants with LD50 equal to (0.00017 x 10^5ppm). *Nicotiana glauca* was the second in toxicity against *Sitophilus granarius* (LD50 = 0.0005 x 10^5ppm) followed by *Citrus limon* with LD50 equal to (0.0023x 10^5ppm) (Table 3).

Table (2): LD50, slope and confidence limits value of aqueous extract of the tested plants against the adult stage of *Sitophilus granarius*

The solvent	Plant	LD50 (ppm)	Slope	Confidence limits of LD50
Ethanol	*Melia azedarach* L.	0.0015 x10^5	0.5766	0.00048 x10^5 - 0.00468 x10^5
	Citrus limon L.	0.0013 x10^5	0.5926	0.00032 x10^5 - 0.00535 x10^5
	Nicotiana glauca G.	0.0032 x10^5	0.4812	0.00089 x10^5 - 0.01156 x10^5
Acetone	*Melia azedarach* L.	0.0170 x10^5	0.3879	0.00576 x10^5 - 0.05017 x10^5
	Citrus limon L.	0.0190 x10^5	0.3809	0.00508 x10^5 - 0.07107 x10^5
	Nicotiana glauca G.	0.0056 x10^5	0.4539	0.00174 x10^5 - 0.01798 x10^5
Hexane	*Melia azedarach* L.	0.0020 x10^5	0.5470	0.00055 x10^5 - 0.00724 x10^5
	Citrus limon L.	0.0032 x10^5	0.5000	0.00094 x10^5 - 0.01094 x10^5
	Nicotiana glauca G.	0.0017 x10^5	0.5614	0.00055 x10^5 - 0.00527 x10^5

Table (3): LD50, slope and confidence limits value of organic extract of the tested plants against the adult stage of *Sitophilus granarius*

The solvent	Plant	LD50 (ppm)	Slope	Confidence limits of LD50
Ethanol	*Melia azedarach* L.	0.00056 x10^5	0.7273	0.00014 x10^5 - 0.00232 x10^5
	Citrus limon L.	0.00070 x10^5	0.6809	0.00024 x10^5 - 0.00204 x10^5
	Nicotiana glauca G.	0.00078 x10^5	0.6667	0.00031 x10^5 - 0.00199 x10^5
Acetone	*Melia azedarach* L.	0.00800 x10^5	0.4324	0.00284 x10^5 - 0.02253 x10^5
	Citrus limon L.	0.06000 x10^5	0.3316	0.01750 x10^5 - 0.20570 x10^5
	Nicotiana glauca G.	0.00086 x10^5	0.6465	0.00020 x10^5 - 0.00367 x10^5
Hexane	*Melia azedarach* L.	0.00017 x10^5	0.6809	0.00004 x10^5 - 0.00082 x10^5
	Citrus limon L.	0.00230 x10^5	0.5333	0.00068 x10^5 - 0.00776 x10^5
	Nicotiana glauca G.	0.00050 x10^5	0.7356	0.00019 x10^5 - 0.00132 x10^5

DISCUSSION

Bioassays with aqueous extract of fruit and leaves of the Chinaberry tree, *Melia azedarach* against adults of *Bemisia tabaci* showed significant repellent activity and decreased the oviposition rate of the insect (Hammad *et al*., 2001).

In another study, Chinaberry tree, *Melia azedarach* has a potent mosquito larvicidal activity, and can be used for the control of mosquito, *Anopheles stephensi* populations (Pandey and Verma, 2002). Also, Su (1991) reported that the topical application of chenopodium (*Chenopodium ambrosioides* L.) oil to wheat seeds at 2000 ppm reduced the infestation of *Sitophilus oryzae* L. While, Bodnaryk *et al.* (1999) found that extracts of pea (*Pisum sativum* L.) resulted in adult mortality and reduced the reproduction rate of several stored-product insect pests at a concentration as low as 0.01%. The mortality was happened because of active ingredients found in the tested extracts which have potential insecticidal activities against the tested insects.

Meanwhile and by throwing more light, Bell *et al*., (1990) reported that the presence of so-called secondary metabolite compounds, which have no known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts their anti-insect activity. Secondary metabolite compounds include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals can affect insects in several different ways, they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phagostimulants, antifeedants or modify oviposition. They may retard or accelerate development or interfere with the life cycle of the insect in other ways. So that it can explain the high mortality by using such plants as potent insecticides (Lloyed, 1973; Huang *et al*., 1997; Asgary *et al*., 2000; Wink *et al*., 2004).

Also it can be mentioned that there are certain concentrations of aqueous or organic extracts of each plant, which could be named by the optimum and suitable concentrations which causing the best effect. Besides, the variations between each plant and its response and insect target sensitivity to the tested concentrations at each tested phase, i.e. the presence of polar and non polar compounds in the media of testing. So that it is offering a kind of physiological selectivity which occurred due to difference in type of mode of action showing a variability in type of toxic materials, its concentrations and its response. Also the role of genetic factor in elucidating difference in responses and reactions (Upitis *et al.*, 1973; Arnaud *et al.*, 2005)

In conclusion, and by focusing on the nature and body composition of the tested insect, Rynolds (1987) reported that the insect cuticle is a layered structure and the functions of the cuticle that are most vulnerable to insecticidal action are mechanical .These properties of the cuticle stiffness, strength, and hardness are largely due to the major part of the cuticle thickness. Cuticle is a composite material, made of proteins, lipids, phenolics and tannins. They confer chemical and mechanical stability to the cuticle by increasing the hydrophobicity of the cuticle matrix. And by more focusing on the nature and composition of the membranes and its effect by the used extracts on these membranes, Hamburger and Hostellman (1991) reported that the drug affects integrity of membranes and localized these membranes due to its highly lipophilic nature.

In the other side, chemical characteristics of the effective compounds such as charge and polarity of natural compound affecting rates of interchange especially across membranes and cuticles to determine whether it reaches that tissue or target at intoxicating concentrations (Gilpy, 1984).

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الملخص العربي

الخصائص الإبادية لبعض المستخلصات النباتية ضد حشرة سوسة القمح

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تم اختبار فاعلية وسمية كل من المستخلصات المائية والعضوية باستخدام ثلاثة مذيبات (ايثانول- اسيتون-هكسان) وذلك لثلاث نباتات جمعت من صحراء العريش وهي: نبات مصاص الدخان وأشجار الزنزلخت وأشجار الليمون وذلك ضد طور الحشرة الكاملة لسوسة القمح وقد بينت النتائج أن:

أعطي المستخلص المائي لأوراق الليمون أعلي سمية بالمقارنة بباقي النباتات المختبرة ووصلت قيمة (LD50) إلي (0.0013 x 10^5) جزء في المليون وذلك عند استخدام الميثانول بينما أعطي المستخلص العضوي لبذور أشجار الزنزلخت أعلي سمية ووصلت قيمة (LD50) الي (0.00056 x 10^5) جزء في المليون. وعلي الوجه الآخر أظهر المستخلص المائي والعضوي لنبات مصاص الدخان اعلي سمية ووصلت قيمة (LD50) الي (0.0056 x 10^5) و (0.00086 x 10^5) جزء في المليون علي التوالي وذلك عند استخدام الاسيتون. ولكن عند استخدام مذيب الهكسان ، أعطي المستخلص المائي لنبات مصاص الدخان أعلي سمية بالمقارنة بباقي النباتات المختبرة ووصلت قيمة (LD50) الي (0.0017 x 10^5) جزء في المليون بينما في الوجه العضوي أعطي المستخلص العضوي لبذور أشجار الزنزلخت أعلي سمية ووصلت قيمة (LD50) الي (0.00017 x 10^5) جزء في المليون.