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Insecticidal properties of some plant extracts against granary weevil, *Sitophilus granaries* L. and rust-red flour beetle, *Tribolium castaneum* H.

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ABSTRACT

Laboratory experiments were conducted under controlled conditions to test the insecticidal activity of aqueous and organic extracts of eight plants collected from north-Sinai (Tree tobacco, *Nicotiana glauca* G.; Lavender cotton, *Achillea fragrantissima* F.; Thymelea *Thymelaea hirsute* L.; Rimth, *Hammad elegans* L., Yarnow, *Achillea santolina* L., Chinaberry, *Melia azedarach* L., Olive, *Olea europaea* L. and Lemon, *Citrus limon* L.) against the adult stages of Granary weevil, *Sitophilus granaries* L. and the Rust – red flour beetle, *Tribolium castaneum* H. The results showed that the aqueous extract of *T. hirsute* gave the highest toxicity among the other tested plants against the Granary weevil, *S. granaries* with LD_{50} equal to $0.250x10^{-3}$ ppm. However, the organic extract of *N. glauca* showed the best results with LD_{50} equal to $0.833x10^{-3}$ ppm on *S. granaries*. On the other hand, both the aqueous and organic extracts of *A. santolina* gave the highest toxicity against *T. castaneum* with LD_{50}'s of 0.105×10^5 and 0.085×10^5 ppm, respectively.

INTRODUCTION

Wheat is one of the most strategic crops in Egypt and all-over the world. Storage of wheat and its products is faced by many sources of damage such as different insect pests especially the stored grain pests. Insect infestation of stored grains and their products is a serious problem throughout the world. Chemical insecticides are currently the method of choice to protect stored grains from insect damage (Domeracki and Zpierska, 1982; Karase *et al.*, 2001; Bell *et al.*, 2003 and Drinkall *et al.*, 2005); however, their widespread use has led to the development of resistant strains to most of the known insecticides (Subramanyam and Hagstrum, 1995). As a result, there is a demand for safer new insecticides because of concern about synthetic 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 most importance (Drinkall *et al.*, 2005).

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; Azadbakht, 2004 and Nagahban, 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 granaries* L. and the Rust-red flour beetle, *Tribolium castaneum* H.

MATERIALS AND METHODS

Rearing methods of the tested insects: Two laboratory cultures of the adult stage of the Granary weevil, *Sitophilus granaries* L. and the Rust-red flour beetle, *Tribolium castaneum* H. were used in this study for the bioassay tests. Wheat grains were used for rearing the *S. granaries* culture, while wheat flour was used for rearing the *T. castaneum* culture. The insect breeding was carried out in special containers of 15 cm diameter and 30 cm height and were kept under laboratory conditions within 27±3°C and 65±5 R.H.

Collection and identification of tested plants: The following eight plants were used in the present study; *Nicotiana glauca* G, *Achillea fragrantissima* F., *Thymelea hirsute* L., *Hammad elegans* L., *Achillea santolina* L., *Melia azedarach* L., *Olea europaea* L. and *Citrus limon* L. 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 (2008). Plant samples (Table 1) were air dried for 2-4 weeks until complete dryness, and then were milled in an electric grinder into a fine powder and stored until used.

Table (1). The list of the eight plant species and their extracte parts studied in this investigation from the vicinity of Al-Arish.

No.	Plant	English name	الاسم العربى الشائع	Extract part
1	*Achillea fragrantissima* F.	Lavender cotton	اخيليا حزنبل	Total plant
2	*Achillea santolina* L.	Yarnow	شيح خراسانى- قيصوم	Total plant
3	*Citrus limon* L.	Lemon	أشجار الليمون	Leaves
4	*Hammad elegans* L.	Rimth	عظو	Total plant
5	*Melia azedarach* L.	Chinaberry	اشجار الزنزلخت	Leaves
6	*Nicotiana glauca* G.	Tree Tobacco	مطاط الدخان	Flowers
7	*Olea europaea* L.	Olive	أشجار الزيتون	Leaves
8	*Thymelea hirsute* L.	Thyme	مثنان- صعتر	Total plant

Organic and aqueous extraction: Twenty gram of each dried plant part (Table 1) was soaked in a dark flask containing 50 ml methylene chloride + 50 ml methyl alcohol 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 a 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 or aqueous extracts: Series of dilutions with distilled water for water extracts, or with methyl alcohol for the organic extracts were prepared for each stock solution. The dilutions were 1/10, 1/100, 1/1000, and 1/10.000 of the original stock solution. For the bioassay treatments, five Petri dishes each containing 20 adults of the tested insects and each insect was topically treated with 5 µl with the micro applicator (McCloud *et al.*, 1988). Five replicates were used for each treatment, including the control. Average percentage mortality was recorded for each after 24 hrs. LD_{50} values and the corresponding slopes were deduced from the regression lines (Finney, 1952), and confidence limits were computed using the normal equivalent deviate programme.

RESULTS

The insecticidal activities of the aqueous and organic extracts of the tested plants against adults of either *S. granaries* or *T. castaneum* are summarized in Tables (2-5). The results indicated that the aqueous extract of *T. hirsute* showed the highest insecticidal activity against the adults of *S. granaries* with LD_{50} equal to 0.250 x 10^{-3} ppm. *A. fragrantissima* was the second in toxicity (LD_{50} = 0.022 x 10^5 ppm) followed by *C. limon* , *H. elegans*, *A. santolina*, *M. azedarach* and *O. europaea* with LD_{50}'s of 0.100 x 10^5, 0.200 x 10^5, 0.211 x 10^5, 0.272 x 10^5 and 0.500 x 10^5 ppm, in a descending order, respectively. *N. glauca* was the lowest in toxicity among all tested extracts against adults of *S. granaries* with LD_{50} equal to 0.944 x 10^5 ppm (Table 2). However, organic extract of *N. glauca* was the most superior in toxicity compared to the other organic extracts of the tested plants. LD_{50} value for *N. glauca* was 0.833 x 10^{-3} ppm (Table 2). *T. hirsute* was the second toxicity against adults of *S. granaries* (LD_{50} = 0.017 x 10^5 ppm) followed by *C. limon*, *A. fragrantissima*, *O. europaea*, *A. santolina* and *M. azedarach*, in a descending order with LD_{50} of 0.060 x 10^5, 0.190 x 10^5, 0.198 x 10^5 and 0.253 x 10^5 ppm, respectively. *H. elegans* was the lowest in toxicity among all tested plants extracts with LD_{50} equal to 0.320 x 10^5 ppm (Table 3).

Table (2). LD_{50}, slope and confidence limits values of aqueous extract of the tested plants against the adults of *Sitophilus granaries* L.

No.	Plant	LD_{50} (ppm)	Slope	Confidence limits of LD_{50}
1	*Achillea fragrantissima* F.	0.022 x 10^5	0.593	0.007 x 10^5 -0.065 x 10^5
2	*Achillea santolina* L.	0.211 x 10^5	0.492	0.098 x 10^5 -0.456 x 10^5
3	*Citrus limon* L.	0.100 x 10^5	0.627	0.053 x 10^5 -0.189 x 10^5
4	*Hammad elegans* L.	0.200 x 10^5	0.780	0.076 x 10^5 -0.525 x 10^5
5	*Melia azedarach* L.	0.272 x 10^5	1.000	0.117 x 10^5 -0.633 x 10^5
6	*Nicotiana glauca* G.	0.944 x 10^5	0.640	0.686 x 10^5 -1.297 x 10^5
7	*Olea europaea* L.	0.500 x 10^5	0.418	0.316 x 10^5 -0.790 x 10^5
8	*Thymelea hirsute* L.	0.250 x 10^{-3}	0.744	0.104 x 10^{-3}-0.599 x 10^{-3}

Table (3). LD_{50}, slope and confidence limits values of organic extract of the tested plants against the adults of *Sitophilus granaries* L.

No.	Plant	LD_{50} ppm	Slope	Confidence limits of LD_{50}
1	*Achillea fragrantissima* F.	0.160 x 10^5	0.500	0.058 x 10^5 -0.444 x 10^5
2	*Achillea santolina* L.	0.198 x 10^5	0.842	0.118 x 10^5 -0.332 x 10^5
3	*Citrus limon* L.	0.060 x 10^5	0.865	0.033 x 10^5 -0.110 x 10^5
4	*Hammad elegans* L.	0.320 x 10^5	0.696	0.071 x 10^5-1.433 x 10^5
5	*Melia azedarach* L.	0.253 x 10^5	1.208	0.156 x 10^5 -0.408 x 10^5
6	*Nicotiana glauca* G.	0.833 x 10^{-3}	0.681	0.516 x 10^{-3} -1.347 x 10^{-3}
7	*Olea europaea* L.	0.190 x 10^5	0.696	0.121 x 10^5 -0.299 x 10^5
8	*Thymelea hirsute* L.	0.017 x 10^5	0.901	0.005 x 10^5 -0.048 x 10^5

Both aqueous and organic extracts of *A. santolina* were the highest in toxicity than other extracts of tested plant species with LD_{50} equal to 0.105 x 10^5 ppm in aqueous extract and 0.085 x 10^5 ppm in the organic phase extract against the adult stage of *T. castaneum*, respectively. Aqueous and organic extracts of *T. hirsute* were the second in toxicity among all tested extracts followed by other extracts of the tested plants (Tables 4 and 5). The lowest toxicity was found with *O. europaea* in the aqueous extract and *A. fragrantissima* in the organic extract with LD_{50}'s equal to 1.888 x 10^5 ppm and 2.066 x 10^5 ppm, respectively.

Table (4). LD_{50}, slope and confidence limits values of aqueous extract of the tested plants against the adults of *Tribolium castaneum* H.

No.	Plant	LD_{50} (ppm)	Slope	Confidence limits of LD_{50}
1	*Achillea fragrantissima* F.	1.222 x 10^5	0.853	0.889 x 10^5-1.679 x 10^5
2	*Achillea santolina* L.	0.105 x 10^5	1.016	0.039 x 10^5 -0.281 x 10^5
3	*Citrus limon* L.	1.311 x 10^5	0.688	1.156 x 10^5-1.485 x 10^5
4	*Hammad elegans* L.	1.066 x 10^5	0.571	0.815 x 10^5 -1.393 x 10^5
5	*Melia azedarach* L.	1.555 x 10^5	0.711	1.203 x 10^5 -2.008 x 10^5
6	*Nicotiana glauca* G.	1.111 x 10^5	0.955	0.808 x 10^5 -1.525 x 10^5
7	*Olea europaea* L.	1.888 x 10^5	0.640	1.464 x 10^5 -2.434 x 10^5
8	*Thymelea hirsute* L.	0.877 x 10^5	0.667	0.467 x 10^5 -1.644 x 10^5

Table (5). LD_{50}, slope and confidence limits values of organic extract of the tested plants against the adults of *Tribolium castaneum* H.

No.	Plant	LD_{50} (ppm)	Slope	Confidence limits of LD_{50}
1	*Achillea fragrantissima* F.	0.855 x 10^{-3}	0.674	0.498 x 10^{-3}-1.466 x 10^{-3}
2	*Achillea santolina* L.	0.085 x 10^5	0.889	0.045 x 10^5 -0.161 x 10^5
3	*Citrus limon* L.	0.966 x 10^5	0.634	0.646 x 10^5-1.443 x 10^5
4	*Hammad elegans* L.	0.572 x 10^5	0.914	0.388 x 10^5 -0.842 x 10^5
5	*Melia azedarach* L.	1.733 x 10^5	0.667	1.334 x 10^5 -2.250 x 10^5
6	*Nicotiana glauca* G.	0.833 x 10^5	0.681	0.590 x 10^5 -1.175 x 10^5
7	*Olea europaea* L.	2.066 x 10^5	0.571	1.488 x 10^5 -2.868 x 10^5
8	*Thymelea hirsute* L.	0.250 x 10^5	0.744	0.118 x 10^5 -0.528 x 10^5

DISCUSSION

The present results are in agreement with the results found by Schmidt *et al.*, (1997), who tested different concentrations of the methanolic extract of *M. azedarach* fruits against *Spodoptera littoralis* and *Agrotis ipsilon*, and showed that the percentage of mortality increased with higher concentrations of the methanolic extrasct of *M. azedarach* against the two tested insects. Also, Hammad and Mcauslame (2006) studied the effect of aqueous extract of *M. azedarach* L. fruit on the survival of *Bemisia argentifolii* and found that survival of treated nymphs was significantly lower than survival of untreated nymphs. In another study, Su (1991) reported that the application of chenopodium (*Chenopodium ambrosioides* L.) oil to wheat seeds reduced the infestation of *S. 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. Meanwhile, 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, gave plant materials which extracts have an anti-insect activity. Secondary metabolite compounds including 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 and they may act as attractants, deterrents, phagostimulants, antifeedants or they might modify oviposition. They may retard, accelerate development or interfere with the life cycle of the insect in other ways. This can explain the high mortality by using such plants as potent insecticides (Lloyed, 1973; Huang *et al.*, 1997; Asgary *et al.*, 2000 and Wink *et al.*, 2004).

Variations between plants and their effect on tested insects are due to their sensitivity to the tested plant extract concentration at each tested phase, i.e. the presence of polar and non polar compounds in the media of testing. Thus, showing a kind of physiological selectivity which takes place due to variations in the mode of action leading to variability in type of toxic materials, its concentrations and its response, also, the role of genetic factor in elucidating differences in responses and reactions should be considered (Upitis *et al.*, 1973 and Arnaud *et al.*, 2005).

Results in terms of LD_{50} values (Tables 2-5) indicated that the organic extracts of most tested plants are higher than the aqueous extracts in toxicity. This may be due to variations in the type of active ingredients and its chemical structure and their mode of toxic action exerted by their aqueous or organic extracts (Bell *et al.*, 1990; Sukmar *et al.*, 1991 and Liu and Ho, 1999). These results are in agreement with El-Doksh *et al.*, (1984) who reported that the LD_{50} values of the organic extract were more toxic than LD_{50} values of the aqueous extract, and that was due to the increasing of effective compounds in organic extract in most plants. However there are some compounds which can be soluble in aqueous extract for some plants and can cause obvious lethal effect, indicating that such plants have certain properties of selectivity and specificity. In addition, natural selection pressure can often negatively affect the other species (Keeler and Tu, 1991). Moreover, Rathi and Krishnan (2005) indicated, on the basis of LD_{50} values, that methanol extract of the aerial parts of *Synedrella nodiflora* G. was the most toxic to *S. litura* F. followed by benzene, chloroform, petroleum ether and finally the water extract. The ecological and physiological selectivity has appeared in all tested plants and insects (Wilkinson, 1976). Besides, Suffness and Douros (1982) reported that sensitivity must be very high in order to detect the low concentration of the active ingredients of some compounds (Harborne, 1988).

Finally, in most cases, there were variations between the two insect species in their respond to the different plant extracts. Also, the organic extract gave in general higher potencies than the aquatic extracts. However, there are other obvious examples of specificity and selective toxicity of the compared plant extracts. Thus, it can be concluded that extracts can lead to newly alternative plant pesticidal molecules which can replace the known hazardous conventional pesticides as much safer, selective, and effective insecticides. Further detailed studies are still needed.

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الخصائص الابادية لبعض المستخلصات النباتية ضد حشرة سوسة القمح وخنفساء الدقيق الصدنية

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تم اختبار فاعلية وسمية من المستخلصات المائية و المستخلصات العضوية لثمانية نباتات جمعت من صحراء العريش وهى : نبات مصاص الدخان ونبات القيسوم ونبات المثنان ونبات العظو ونبات الشيح الخراسانى واشجار الزنزلخت واشجار الليمون واشجار الزيتون وذلك ضد طور الحشرة الكاملة لسوسة القمح وخنفساء الدقيق الصدنية وقد بينت النتائج ان :

- المستخلص المائى لنبات المثنان اعلى سمية بالمقارنة بباقى النباتات المختبرة ضد طور الحشرة الكاملة لسوسة القمح ووصلت قيمة (LD_{50}) الى (0.250×10^{-3}) جزء فى المليون بينما اعطى المستخلص العضوى لنبات مصاص الدخان اعلى سمية بالمقارنة بباقى النباتات المختبرة ضد طور الحشرة الكاملة لسوسة القمح ووصلت قيمة(LD_{50}) الى (0.833×10^{-3})جزء فى المليون.

- واظهر كل من المستخلص المائى العضوى لنبات الشيح الخرسانى اعلى سمية بالمقارنة بباقى النباتات المختبرة ضد طور الحشرة الكاملة لخنفساء الدقيق الصدنية ووصلت قيمة (LD_{50}) الى (0.105×10^{-5})و (0.085×10^{-5}) جزء فى المليون على التوالى.