Insecticidal Properties of Some Plant Extracts against 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 extract of different solvents (ethanol, acetone and hexane) from 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 Rust-Red Flour Beetle, *Tribolium castaneum* H. The results showed that the aqueous extract of *N. glauca* gave the highest toxicity among the other tested plants with LD50 equal to 0.00086 x 10^5 ppm using ethanol solvent. However, the organic extracts of *M. azedarach* L. showed the best result with LD50 equal to 0.0002 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 0.0016 x 10^5 ppm and 0.00066 x 10^5 ppm, respectively, with acetone solvent. But using hexane solvent, aqueous extract of *C. limon* L. was obviously the most toxic extract compared to the aqueous extracts of the tested plants with LD50 (0.0086 x 10^5 ppm) and in the organic phase *N. glauca* gave the best result with LD50 equal to 0.0007 x 10^5 ppm .

Keywords: Insecticidal activity, *Nicotiana glauca, Melia azedarach, Citrus limon,* Rust-Red Flour Beetle.

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; Karas *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 insecticide 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 botanical 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; Azadbakt *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 Rust-Red Flour Beetle, *Tribolium castaneum* H.

MATERIALS AND METHODS

Rearing methods of the tested insects:

The laboratory culture of the adult stage of the Rust-Red Flour Beetle, *Tribolium castaneum* H. is used in this study for the bioassay test and wheat flour were used for rearing 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 Relative Humidity (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.

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.

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

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

RESULTS

The insecticidal activities of the aqueous and organic extracts of the tested plants against *T. castaneum* are summarized in table (2 and 3). The results indicated that the aqueous extract of *Nicotiana glauca* showed the highest insecticidal activity with LD50 equal to (0.00086 x 10^5ppm) when we used ethanol solvent. *Citrus limon* was the second in toxicity (LD50 = 0.0039 x 10^5ppm) followed with *Melia azedarach* with LD50 (0.0043x10^5ppm) (Table 2). However, organic extract of *Melia azedarach* had the highest in toxicity compared to the other organic extracts of the tested plants. LD50 value for *Melia azedarach* was 0.0002x10^5 ppm. *Citrus limon* was the second in toxicity against adults of *T. castaneum* (LD50 = 0.00027 x 10^5ppm) followed by *Nicotiana glauca* with LD50 equal to (0.0008 x 10^5ppm) (Table 3).

Results showed that 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 (0.0016x 10^5ppm) in aqueous extract and (0.00066x 10^5ppm) in the organic phase extract against the adult stage of *T. castaneum* (Table 2 and 3). Aqueous extract of *Citrus limon* was the second in toxicity in all tested extracts followed by *Melia azedarach* with LD50 equal to (0.0028x 10^5ppm) and (0.0039x 10^5ppm) respectively (Table 2). However, Organic extract of *Melia azedarach* was the second in toxicity with LD50 equal to (0.0019x 10^5ppm). The lowest toxicity was found with *Citrus limon* in the organic extract of acetone solvent with LD50 equal to (0.00066x 10^5ppm) (Table 3).

Data presented in table (2) showed that the aqueous extract of *Citrus limon* was the highest insecticidal activity with LD50 equal to (0.0086 x 10^5ppm) when we used hexane solvent. *Melia azedarach* was the second in toxicity (LD50 = 0.96 x 10^5ppm) and *Nicotiana glauca* was the lowest in toxicity in all tested plant extracts with LD50 equal to (0.087 x 10^5ppm). However, organic extract of Citrus *limon* was the highest in toxicity than other aqueous extracts of the tested plants with LD50 equal to (0.0007 x 10^5ppm). *Melia azedarach* was the second in toxicity against *T. castaneum* with (LD50 = 0.00096 x 10^5ppm) followed by *Nicotiana glauca* with LD50 equal to (0.0033x 10^5ppm) (Table 3).

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

The solvent	Plant	LD50 (ppm)	Slope	Confidence limits of LD50
Ethanol	*Melia azedarach* L.	0.00420 x10^5	0.4776	0.00054 x10^5 - 0.03270 x10^5
	Citrus limon L.	0.00390 x10^5	0.4848	0.00073 x10^5 - 0.02080 x10^5
	Nicotiana glauca G.	0.00086 x10^5	0.6465	0.00022 x10^5 - 0.00329 x10^5
Acetone	*Melia azedarach* L.	0.00390 x10^5	0.4848	0.00045 x10^5 - 0.03409 x10^5
	Citrus limon L.	0.00280 x10^5	0.5120	0.00050 x10^5 - 0.01565 x10^5
	Nicotiana glauca G.	0.00160 x10^5	0.5664	0.00058 x10^5 - 0.00441 x10^5
Hexane	*Melia azedarach* L.	0.00960 x10^5	0.4211	0.00120 x10^5 - 0.07654 x10^5
	Citrus limon L.	0.00860 x10^5	0.4267	0.00108 x10^5 - 0.06832 x10^5
	Nicotiana glauca G.	0.08700 x10^5	0.3879	0.01580 x10^5 - 0.47960 x10^5

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

The solvent	Plant	LD50 (ppm)	Slope	Confidence limits of LD50
Ethanol	*Melia azedarach* L.	0.00020 x10^5	0.9697	0.00006 x10^5 - 0.00069 x10^5
	Citrus limon L.	0.00027 x10^5	0.8767	0.00006 x10^5 - 0.00117 x10^5
	Nicotiana glauca G.	0.00080 x10^5	0.6598	0.00025 x10^5 - 0.00261 x10^5
Acetone	*Melia azedarach* L.	0.00190 x10^5	0.5470	0.00033 x10^5 - 0.01079 x10^5
	Citrus limon L.	0.03200 x10^5	0.4076	0.00857 x10^5 - 0.11955 x10^5
	Nicotiana glauca G.	0.00066 x10^5	0.6882	0.00023 x10^5 - 0.00191 x10^5
Hexane	*Melia azedarach* L.	0.00096 x10^5	0.6337	0.00034 x10^5 - 0.00274 x10^5
	Citrus limon L.	0.00070 x10^5	0.6809	0.00027 x10^5 - 0.00180 x10^5
	Nicotiana glauca G.	0.00330 x10^5	0.5000	0.00042 x10^5 - 0.0262 x10^5

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 *Melia azedarach* fruits against *Spodoptera littoralis* and *Agrotis ipsilon*, and showed that the percentage of mortality increased with higher concentrations of the methanolic extract of *Melia azedarach* against the two tested insects. Also, Hammad and Mcauslane, (2006) studied the effect of aqueous extract of *Melia azedarach* L. fruit on survival of *Bemisia argentifolii* and found that survival of treated nymphs was significantly lower than survival of untreated control nymphs. In another study, 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. 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, 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 and they may act as attractants, deterrents, phagostimulants, antifeedants or they might modify oviposition. They may retard or 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; Wink *et al*., 2004).

Variations between plants and their effect on tested insects are due to their sensitivity to the tested plant extracts concentrations 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 difference in responses and reactions should be considered (Upitis *et al.*, 1973; Arnaud *et al.*, 2005).

Results in terms LD50 value (Table 2-3) indicated that the organic extract of most tested plants extracts are higher than the aqueous extracts in toxicity. This may be due to variation in the type of active ingredients and its chemical structure and their mode of toxic action exerted by their aqueous and organic extracts (Bell*, et al*., 1990; Liu and Ho, 1999; and Sukmar *et al*., 1991). These results are in agreement with El-Doksh *et al*., (1984) who reported that the LD_{50} values of organic extract were more toxic than LD_{50} values of 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 from some plants and can cause obvious lethal effect, indicating that such plants have certain properties of the 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, methanol extract of the aerial parts of *Synedrella nodiflora* G. was the most toxic to *Spodoptera litura* F. followed by benzene and chloroform, petroleum ether and finally water extract. This 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 concentrations of active ingredients of compounds (Harborne, 1988).

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الملخص العربي

الخصائص الإبادية لبعض المستخلصات النباتية ضد حشرة خنفساء الدقيق الصدئية

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تم إختبار فاعلية وسمية كل من المستخلصات المائية والعضوية باستخدام ثلاثة مذيبات (إيثانول- أسيتون-هكسان) لثلاث نباتات جمعت من صحراء العريش وهي نبات مصاص الدخان *Nicotiana glauca* G. وأشجار الزنزلخت *Melia azedarach* L. ، وأشجار الليمون *Citrus limon* L. وذلك ضد طور الحشرة الكاملة لخنفساء الدقيق الصدئية *Tribolium castaneum* H. وقد تبين من النتائج ما يلي:

أعطي المستخلص المائي لنبات مصاص الدخان أعلي سمية بالمقارنة بباقي النباتات المختبرة، ووصلت قيمة (LD50) الي (0.00086×10^5) جزء في المليون وذلك عند استخدام الميثانول بينما أعطي المستخلص العضوي لبذور أشجار الزنزلخت أعلي سمية ووصلت قيمة (LD50) الي (0.0002×10^5) جزء في المليون. وعلي الوجه الآخر أظهر المستخلص المائي والعضوي لنبات مصاص الدخان اعلي سمية ووصلت قيمة (LD50) الي (0.0016×10^5) و (0.00066×10^5) جزء في المليون علي التوالي وذلك عند استخدام الاسيتون. ولكن عند استخدام مذيب الهكسان، أعطي المستخلص المائي لأشجار الليمون الدخان أعلي سمية بالمقارنة بباقي النباتات المختبرة ووصلت قيمة (LD50) الي (0.0086×10^5) جزء في المليون بينما في الوجه العضوي أعطي المستخلص العضوي لنبات مصاص الدخان أعلي سمية، ووصلت قيمة (LD50) الي (0.0007×10^5) جزء في المليون.