INSECTICIDAL ACTIVITY OF CERTAIN PLANT ESSENTIAL OIL, PLANT EXTRACTS AND INORGANIC SALTS AGAINST THE GRANARY WEEVIL, *Sitophilus granarius* (LINNAEUS) INFESTING WHEAT CROP IN EGYPT.

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ABSTRACT

Toxicity of six materials, two plant essential oil (clove and flax seed oil), two plant extracts (neem and harmal seed extracts) and two inorganic salts (silica dust and tri-calcium phosphate) mixed with wheat grains at different concentrations against granary weevil was studied. Based on LC_{50}, LC_{90}, both lower and upper values of confidence limits as well as slope values of LCP lines for the six materials, the obtained results indicated, in general, that these compounds could be arranged according to their effectiveness at all tested concentrations as follows: clove oil > flax seed oil > silica dust > tri-calcium phosphate > neem seed extract > harmal seed extract.

Keywords: Pesticide alternatives, Bioassay, *Sitophilus granarius*, Wheat.

INTRODUCTION

Wheat, *Triticum aestivum,* is one of the main sources of food for human and animals. In Egypt wheat occupies an important belt among cultivated area during the winter season. It is essential to increase wheat production to meet the increase in wheat consumption (Abdel-Rahman, 1997). This important crop in Upper Egypt is usually attacked by several species of stored product insects; particularly the granary weevil, *Sitophilus granarius* (L.), which is the most harmful pest of stored grains and other stored products in temperate climates. It can develop on wheat, rye, barley, oats, corn and rice (Andersen, 1934). Adults cause damage by destroying kernels, mainly germs, producing debris, and raising temperature and water contents, facilitating the invasion of secondary insect pests, mites, bacteria and fungi. Larvae develop inside the kernels and consume about 64% of their contents (Campbell and Sinha, 1976). The resulting post-harvest losses are approximately 10-15% worldwide annually (Rajendran, 2002 and Neethirajan *et al.,* 2007). In some countries cereal grain losses during storage can reach 50% of the total harvest, in addition to a reduction in quality and monetary value (Fornal *et al.,* 2007). Therefore, managing insect populations that infest stored commodities is a greater challenge today than previously and prevention of loss due to insects is of most important. Insecticides, however, when properly used will continue to play an important role in reducing storage losses, but the indiscriminate use of insecticides has posed several problems like residual toxicity, resistance development and environmental hazards. So, it has become evident that there an urgent need for the development of safe and environmentally friendly alternative agents, possessing the desirable

properties of chemical pesticides making them highly toxic to the target organisms, which can be mass produced on an industrial scale, have long shelf life and can be safely transported to control insect pests of stored products (Moazami, 2008). For that, the present study was carried out to evaluate the toxic effects of some pesticide alternatives (Plant essential-oil, plant extracts and inorganic salts) in order do protect wheat grains against *S. granarius* attack.

MATERIALS AND METHODS

Test insect:

The weevil individuals used in the present study were obtained from a laboratory colony maintained at the Stored Product Insects Research Section, Ministry of Agriculture and Reclaimed Lands, Dokki, Giza, Egypt. Rearing the pest was always carried out in a maintaining room at 27±2°C and 70±5% R.H. according to Saleh (1990). From this culture the newly emerged weevils were removed every day, reared until the desirable age and used as and when required.

Pesticide alternatives:

Two plant essential-oil, two plant extracts and two inorganic salts were experimented against adult stage to determine their toxic effects. Plant essential oil included clove oil and flax seed oil, both were purchased in crude form from the local market. Each oil was tested at the rate of 5, 10 15 and 20 ml/kg grains. The plant extracts included seeds of neem and harmal plants. The extraction method used in this investigation is essentially similar to that described by El-Sebae *et al.* (2008) with slight modification. From the original stock solution, four concentrations of each organic extract i.e 250, 500, 750 and 1000 ppm were prepared and used as and when needed. The inorganic salts used in the experiment were silica dust and tri-calcium phosphate. Each material was finely ground in a procelain mortar and passed through 200 μm sieve. Four concentrations of each material namely 1.25, 2.50, 3.75 and 5.00 gm/kg grains were prepared.

Bioassay:

Quantities of 20 gm. wheat grains were placed in glass jars 250 ml. capacity and treated with appropriate dosage of the experimental materials. The jars were manually shacked for suitable time to ensure even coating of grains, and then infested with 20 adults of *S. granarius* 14 day-old. Each jar was covered with muslin cloth. Untreated wheat grains (control) were used as described previously. Four replicates were made for each treatment. Treated and untreated jars were held in an incubator at 27±2°C and 70±5%. R.H. Mortality determination was stared after 24 hours of exposure of insects and continued at 24 hours intervals up to 72 hours exposure. The contents of each glass jars were spreaded out in a try and the dead insects were removed and counted. After each count, the grains and the alive insects were returned back to the glass jars. The mortality in the control was also calculated and corrected according to Abbott's formula (1925).

$$\% \text{ Corrected Mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Probit analysis was done to reckon either LC_{50} and LC_{90}, or LT_{50} and LT_{90} and confidence limits using SPSS V. 10 system software (SPSS Inc., 1999).

RESULTS AND DISCUSSION

Table (1) shows the LC_{50} and LC_{90} values and both lower and upper values of confidence limits as well as slope values of LCP lines for, 2 plant essential-oil, 2 plant extracts and 2 inorganic salts which were mixed with the grains. The data cleared that the most effective one was clove oil, which gave 100% mortality after 24 hrs., followed by flax seed oil (LC_{50} = 2.47±0.31; LC_{90} = 4.86±0.44) then by silica dust (LC_{50} = 2.95±0.28; LC_{90} = 21.51±4.20) while both plant extracts, neem seeds and harmal seeds, showed the lowest toxic effect with LC_{50} = 226.78±113.89, 429.15±37.87 and LC_{90} = 879.59±457.25 and 1090.24±250.02, respectively. Flax seed oil was more potent than neem and harmal seed-extracts by 91.81 and 73.89 folds. Meanwhile, silica dust was more potent than neem and harmal seed-extracts by 76.87 and 145.47 folds, respectively. The toxicity order of all tested compounds based on LC_{50} was as follows: clove oil > flax seed oil > silica dust > tri-calcium phosphate > neem seed extract > harmal seed extract.

The arrangement based on LC_{90} did not differ than previous groups, where groups could be arranged as follows: plant oils > inorganic salts > seed extracts. The slope values of the regression lines (Table 1) were fluctuated around 2.00 for almost tested compounds. The least slope (1.52) was observed for silica dust and the highest one (4.34) was recorded for flax seed oil. This indicates that the insect population was relatively homogenous in their susceptibility toward tested compounds.

Data in Tables (2 and 3) show the efficiency of tested materials after 48 hrs (2 days) and 72 hrs (3 days) based on their values of LC_{50} and LC_{90}. After 48 hrs (Table 2), treatment of both oil (clove and flax) gave 100% mortality. The rest four compounds showed that silica dust was the most effective one (LC_{50} = 1.55±0.01 and LC_{90} = 13.46±4.18) and harmal seed extract was the least effective one with LC_{50} = 194.07±96.41 and LC_{90} = 513.70±259.69, respectively. These four materials could be arranged based on their effectiveness (the lowest LC_{50} or LC_{90}) as coming silica dust > tric-calcium phosphate > harmal seed extract > neem seed extract. Slopes of the LC_{50} line ranged between 2 and 3 which mean that the treated insect populations have high homogeny. Results of 72 hrs post treatment (Table 3) did not depict any changes of either the efficiency or the arrangement based on both LC_{50} and LC_{90}. The most effective material was silica dust after 3 days (LC_{50} = 1.20±0.11 and LC_{90} = 3.49±0.46), meanwhile, the least effective one was neem seed extract after 3 days (LC_{50} = 166.41±22.28 and LC_{90} = 317.66±23.24). Slopes of LC lines showed high homogeneity in the insect populations.

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The tested materials which have been used here are belonging to three groups: plant essential oil (clove and flax), plant extracts (neem and These groups could be arranged, based on their toxicity, as plant essential oil, inorganic salts and plant extracts, however, all materials gave 100% mortality after 4 days of treatment except of tri-calcium phosphate.

Table (3): Probit analysis parameters (LC$_{50}$ and slopes) showing the effects of some plant essential oil, plant extracts and inorganic salts against *S. granarius* at 72 hrs. post-treatment.

Compoud	Mean ± SE						
	Slope	LC$_{50}$	Confidence Limits		LC$_{90}$	Confidence Limits	
			Lower	Upper		Lower	Upper
Clove oil	-	-	-	-	-	-	-
Flax seed oil	-	-	-	-	-	-	-
Neem seed extract	4.96 ± 1.23	166.41 ± 22.2	98.94 ± 22.4	202.51 ± 15.8	317.66 ± 23.24	275.51 ± 24.79	403.80 ± 28.86
Harmal seed extract	3.17 ± 0.66	125.6 ± 62.4	73.61 ± 59.72	112.38 ± 90.8	281.01 ± 140.40	170.31 ± 136.51	225.83 ±178.39
Silica dust	3.00 ± 0.51	1.20 ± 0.11	0.39 ± 0.19	1.54 ± 0.20	3.49 ± 0.46	2.78 ± 0.64	9.87 ± 2.96
Tri-calcium phosphate	2.31 ± 0.17	1.40 ± 0.03	1.02 ± 0.03	1.64 ± 0.01	5.13 ± 0.42	4.32 ± 0.29	7.68 ± 1.12

The results of the present investigation could be interpreted on the base that oil may affect the target pests in several ways. Petroleum oil and vegetable oil may block the insects air or breathing holes (Spiracles), so that the insect dies by suffocation. The fatty acid in oil may disrupt cell membranes and interfere with normal metabolism. Zeng *et al.* (2010) reported that the essential oil from clove has a number of bioactive compounds having toxic effects against *S. oryzae*. There are no references available at present concerning the effect of flax seed-oil on the stored product insect pests but this oil may have the same effect as other plant essential oil. Moreover, the toxicity of essential oil to stored-product insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, methods and time of extraction and plant part used (Lee *et al.,* 2001).

The biological activity of some plant extracts against insect pests has been reported by Sekulovi'c *et al.*, (1995) who found that some tested botanical agents deserve to be further investigated for their mortality. In the present study neem and harmal seed extracts did not give satisfactory results comparing with other materials, which have been used, where these two extracts came at the last group which has the least effects on *S. granarius* even though plant extracts have many good biological effects on insect pests. Our results clearly showed that the effeciciency kill of the tested extracts depends on the time of exposure, as the increase in time resulted an increase in mortality percent. Jovanovi'c *et al.* (2007) reported that ethanol extract of *Uritica dioica* L. leaf and *Taraxacum officinals* L. below-ground part extract efficiently killed adults of *Acanthoscelides obtecus* (Say.) and their activity increased with time of exposure. El-Sebae *et al.* (2008) found that the

aqueous extract of *Thymelea hirsute* L. showed the highest insecticidal activity against adults of *S. granarius* with LD_{50} equal to 0.250×10^{-3} ppm, whereas *Nicotina glauca* G. was the lowest in toxicity among all tested extracts against *S. granarius* with LD_{50} equal to 0.944×10^5 ppm.

Inert dusts applied in the present study are considered as strong alternative pesticides in protecting wheat grains against granary weevil attack. Silica dust and tri-calcium phosphate occupied the third and fourth ranks among the six-tested materials respectively. Mortality caused by the inert dust is induced primarily as a result of desiccation body water loss as a consequence of cuticle disruption by removal of epicuticular waxes. Therefore, the US Food and Drug Administration recognized the inert dusts as safe for use in grains (Banks and Fields, 1995). The results on the effect of silic dust and tri-calcium phosphate on *S. granarius* are in close agreement with those reported by several workers (Golob, 1997 and Lorini, 2003), especially there are no previous work to determine either LC_{50} or LC_{90} for these materials.

It may be concluded that results of the present study lead us to believe that some pesticide alternatives such as plant essential oil, plant extracts and inorganic salts may prove to be a distinct class of insecticide only under some conditions against the granary weevil attack and to prevent post-harvest losses as well as to avoid problem results from traditional insecticides currently used against stored product insects.

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النشـاط الإبـادى لـبعض الزيـوت النباتيـة الأساسـية، المستخلصـات النباتيـة والأمـلاح الغير عضوية ضد حشرة سوسة الحبوب (.L) *Sitophilus granarius* التى تصيب محصول القمح فى مصر

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تم دراسة التأثير السام لستة مركبات شملت اثنين من الزيوت النباتيـة الأساسـية همـا زيـت القرنفل وزيت بذور الكتان واثنين من المستخلصات النباتية هما مستخلص بذور النيم ومستخلص بذور الحرمل واثنين من الأمـلاح الغيـر عضـوية (المسـاحيق الخاملـة) همـا مسـحوق السيليكا ومسحوق الفوسفات ثلاثى الكالسـيوم، حيث تم خلط هذه المركبات مع حبوب القمح بتركيزات مختلفة .

بناء على قيم LC$_{50}$، LC$_{90}$ وكذا قيم حدود الثقة العليا والدنيا بالإضافة إلى قيمة ميل خطوط السمية للمركبات الستة المختبرة، أشارت النتائج بصفة عامة إلى امكانية ترتيب هذه المركبات طبقاً لفعاليتها عند جميع التركيزات المختبرة علـى النحـو التـالى : زيـت القرنفل > زيت بـذور الكتـان > مسـحوق السيليكا > مسـحوق الفوسفات ثلاثى الكالسيوم > مستخلص بذور النيم> مستخلص بذور الحرمل .

قام بتحكيم البحث

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Table (1):Probit analysis parameters (LC_{50} and slopes) showing the effects of some plant essential oil, plant extracts and inorganic salts against *S. granarius* at 24 hrs. post-treatment.

Compound	Mean ± SE						
	Slope	LC_{50}	Confidence Limits		LC_{90}	Confidence Limits	
			Lower	Upper		Lower	Uppe
Clove oil	-	-	-	-	-	-	-
Flax seed oil	4.34 ± 0.27	2.47 ± 0.31	0.86 ± 0.35	3.66 ± 0.16	4.86 ± 0.44	4.12 ± 0.55	6.35 ± 0.32
Neem seed extract	2.25 ± 0.24	226.78 ± 113.8	155.3 ± 127.01	208.07 ± 168.4	879.59 ± 457.25	458.39 ± 372.43	1681.88 ± 220.25
Harmal seed extract	2.31 ± 0.19	429.15 ± 37.87	190.21 ± 98.54	745.92 ± 271.20	1090.24 ± 250.02	913.98 ± 30.38	1412.45 ± 41.38
Silica dust	1.52 ± 0.10	2.95 ± 0.28	2.20 ± 0.23	3.48 ± 0.49	21.51 ± 4.20	11.91 ± 1.82	108.77 ± 55.50
Tri-calcium phosphate	1.56 ± 0.22	3.72 ± 0.35	2.78 ± 0.12	4.34 ± 0.21	28.20 ± 6.73	13.04 ± 2.45	208.77 ± 120.67

Table (2): Probit analysis parameters (LC_{50} and slopes) showing the effects of some plant essential oil, plant extracts and inorganic salts against *S. granarius* at 48 hrs. post-treatment.

Compoud	Mean ± SE						
	Slope	LC_{50}	Confidence Limits		LC_{90}	Confidence Limits	
			Lower	Upper		Lower	Upper
Clove oil	-	-	-	-	-	-	-
Flax seed oil	-	-	-	-	-	-	-
Neem seed extract	3.45 ± 0.15	138.54 ± 69.79	0.004 ± 0.00	148.71 ± 119.8	314.27 ± 158.15	117.56 ± 94.33	523.11 ± 72.54
Harmal seed extract	2.94 ± 0.22	194.07 ± 96.41	26.55 ± 21.5	212.42 ± 171.7	513.70 ± 259.69	232.53 ± 187.66	1994.56 ± 1591.78
Silica dust	1.56 ± 0.33	1.55 ± 0.01	0.55 ± 0.34	2.21 ± 0.16	13.46 ± 4.18	5.95 ± 1.96	212.60 ± 134.53
Tri-calcium phosphate	1.84 ± 0.08	2.21 ± 0.18	1.66 ± 0.19	2.48 ± 0.21	10.99 ± 0.72	7.65 ± 0.55	22.51 ± 2.84