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Comparative efficiency of three manners of mixing with media for controlling *Trogoderma granarium* on wheat grain.

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ABSTRACT

The insect infestation of stored grain and their products is a serious problem over the world since it reduces crop yields, causes contamination for stored products, damages seed germs, carries diseases that effect plants, peoples, animals, and changes the quality of stored products. Six compounds, one organophosphorus, pirimiphose methyl, two biocides; ivomic and *Bacillus thuringiensis*, one mineral oil (KZ), one insect growth regulator, cascade and one organic acid, formic acid were evaluated against *Trogoderma granarium* by exposure to treated media and other two ones were layer manner. Based on $LC_{50,s}$ values, data indicated that primiphos-methyl was the most toxic compound with LC_{50} of 0.135 ug/g, while the least toxic one was the mineral oil with LC_{50} value of 4300 ug/g grain at 24 h post-treatment. The efficacy of the biocide ivomic followed the effect of pirimiphos-methyl. Data obtained cleared that cascade had a higher toxic effects on the tested insect than the mineral oil formic acid. Batches of grains (wheat grain) were mixed using the three mentioned manners. Based on the behavior of stored product insects which quickly move between grains where contact with the treated media. Results obtained showed that half and one third of dose had more percent of mortality than the expected values. The down position of treated layer indicated the most effect compared to the other two layers (middle and surface).

Keywords: Pest control - *Trogoderma granarium*- wheat grain.

INTRODUCTION

Wheat suffers heavy losses during storage due to insect pests. According to FAO 10-25% of world harvested food is destroyed annually by insects and rodents pests, Anonymous (1980). Losses caused by khapra (*Trogoderma granarium*) have been reported to range from 0.2 to 2.9% over a period of 1-10.5 months, Irshad and Iqbal (1994). Insect pests cause damage to stored grain and processed products by reducing dry weight and nutritional value.

The khapra beetle is one of the most notorious primary insect pests for stored grain (Banks (1977); Viljoen, 1990 and Sarmamy, *et al.*, (2011).

In addition to direct loss caused by larval feeding, infestation is often followed by colonization of secondry insect pests, Fungi and consequently leading to deterioration in grain characteristics.

Protection of stored grain from insect damage is currently dependant on synthetic pesticides Price and Mills (1988) and Noling and Becker (1994).

These pesticides resulted in potential hazards for mammals, disturbances of the environment, pest resistance to pesticides and lethal effects on nontarget organisms, agroecosystems in addition to direct toxicity to users (Prakash and Rao, 1986 &

1987), therefore, an urgent need to develop environment friendly alternatives with the potentials to replace the highly toxic chemicals.

Some alternatives to synthetic insecticides are biocides, organic acids, mineral oils and insect growth regulators.

Recent development in microbial control of insect pests have indicated that entomopathogenic fungi have potential applications in a variety of environments and some are used commercially.

Beauveria bassiana (Balsamo) Vailemin and species of *Metarhizium anisopliae* are well studied in this context. Ivermectin one of the antibiotics which gave a promising control against *Callosobruchus maculatus* compared to the chemical insecticide (Abo-Arab and El-Hamady, 1998).

A unique feature of plant metabolism, when compared with that of animals and microorganisum, is the ability of plants to accumulate organic acids in the cell vacuole, sometimes in considerable amounts. Many Researchers investigated several of these organic acids against mites and stored product insects (Datta and Banerjee, 1982; Dunkel and Nancy Read, 1986; Daniels *et al*, 1999; and Serag El-Dien and Eissa ,2003).

Insect growth regulators have many advantages over low mammalian toxicity, high specifity, rapid degradation in the environment and are effective at killing all immature stages of wide range of insect species including the internal grain feeders (Moser, *et al*, 1992). Thus the objectives of this study were:

1- To determine the efficacy of six compounds, one organophosphorus, pirimiphos-methyl; two biocides, ivomic and *Bacillus thuringiensis*, one mineral oil, K.Z.; one insect growth regulator, flufenoxuron (cascade) and one organic, formic acid against khapra beetle, *Trogoderma granarium* in the laboratory on wheat grain.
2- To evaluate the mentioned compounds on emergence of offspring.
3- Study the effect of half and one third of dose using the traditional method.
4- Study new manners of mixing compounds with feeding media (layer method) using rat, b of LC_{90}.

MATERIALS AND METHODS

Pest culture technique:

Trogoderma granarium:

T. granarium adults tested in this study were continuously reared free of any insecticidal contamination for several years at 30 ± 2 ºC and 70 ± R.H. at Department of Stored Product Pests, Plant Protection Research Institute, Sakha Agriculture Research Station.

The subculture was maintained under the same conditions, 200-400 adults from the previous culture were added in 850 ml glass jars containing 400 gm of crushed and whole wheat grain (*Triticum aestivumL.*) (variety of Sakha 69 initial moisture content of 14%) as a culture medium, the jars were covered with muslin cloth, to be tightly closed it was ringed by rubber bands. Newly emerged adults (0-24 hours old) wer used in the experiments.

Chemicals used:

Organophosphorus compound:

Common name:	Pirimiphos-methyl
Trade name:	Actellic
Chemical name:	0-2 diethyl amino -6- methyl - pyrimidin -4- yl- 0.0 - dimethylphosphrothioate.
Formulation:	50% emulsifiable concentrate (EC).

Insect growth regulator (IGR):

Common name: Flufenoxuron (BSI/ISO) (10.0 EC)

Trade name: Cascade

Chemical name: 1- [4- [2- chloro -4- (trifluormethyl) phenoxy] 2- fluorophenyl] - 3- (2,6 – difluorbenzoyl) urea.

Mineral oil:

KZ

Emulsifiable mineral oil

Essential mineral oil (95%) (W/V.)

Emulsified material (5%)

Biocides:

a- Ivomic 1% W/V ivermectin and 10% W/V clorsulon in a sterile solution.

b- B.t. (Bacillus thuringensis) var. kurstaki, 32000 international units of potency per mg. (14.52 Billion International units per pound) 6.4%

Organic acid:

Formic 60% (MODERN EST. & EXP)

Egypt – Cairo.

All tested compounds were in the formulated form and dosages were calculated on the basis of ppm of active ingredient.

Serial concentrations of the tested materials were prepared by dissolving the amount of any tested toxicant to obtained the desirable concentrations.

Bioassay of the tested compounds

Mixing with media

Mixing with media using different concentrations:

The desired considerable concentrations of each tested compound were diluted with water except ivomic which diluted with acetone. 20gm sample of wheat grain was placed in small glass jar (11.5 by 6cm diameter), one ml of each concentration was placed in each glass jar above the surface of grain using micropipette. The jars were shaken by hand to mix the grain with the tested concentration. The treated sample were left on jars a convenient time until the solvent evaporated, each concentration replicated three times. Other jars were left without toxicants to serve as control treatment. Ten of newly emerged adults of *T. granarium* were transferred to each jar, covered with muslin cloth and kept under laboratory conditions. Mortality counts were recorded after 24 and 48 hr. all results were corrected according to **Abbot's formula (1925).**

Mixing with media using LC_{90}:

Traditional method

In this experiment three types of mixing were used:

a- Batches of grains each of 30 gm wheat treated with the LC_{90} of the used toxicants.

b- Batches of 15 gm of wheat grains were treated with the appropriate LC_{90} of the used toxicants and thoroughly mixed with equal untreated amount of grains.

c- Batches of wheat grains were divided into 3 groups each of 10 gm, one of the three groups was treated with the desired LC_{90} amount of the used toxicants and carefully mixed with untreated two groups.

Five pairs of *T. granarium* were posed to the batches of treated grains in the three cases with the same mentioned in the type (a). The corrected percent of mortality was estimated. In the all types three replicates were used beside the control.

Modified method:

Treated layers method:

1- 30 gm of wheat grain were divided into three groups each of 10 gm. One of them treated with the appropriate LC_{90} of the tested toxicants. This treated group used as a layer of grain in three different places, the first place was above the remaining of untreated wheat grain (20 gm), the second one was between the two untreated layers (each of 10 gm) and the later was under the untreated grains (20 gm) on the bottom of the used jars (250 ml in capacity). Five pairs of *T. granarium* were exposed and the corrected percent of mortality determined. In all cases three replicates were used beside the control.

2- 15 gm of wheat grain were individually treated with the appropriate LC_{90} of the tested toxicants and used as a layer, one was the higher and the another was on bottom of the container jar. 5 pairs of *T. granarium* were exposed to the layers and corrected mortality percentages were countend and recorded.

Biological effect of the tested compounds:

T. granarium

LC_{50} concentrations of the tested compounds were diluted with water except ivomic which diluted in acetone. 20 gm wheat grains was placed in small glass jar (11.5 by 6 cm diameter), one ml of each concentration placed in each glass jar above the serface of grain using micropipette. The jars were shaken by hand to mix the grain with the compound, the treated samples left on jars for a convenient time until the solvent evaporated, each treatment was replicated three times. Other jars were left to serve as control treatment. Five pairs of newly emerged adults of *T. granarium* were transferred to each jar covered with muslin cloth and kept under laboratory conditions. Mortality counts were recorded after 24 and 48 hr. All results were corrected according to Abbot's formula (1925). Survival of parent insects were removed. The number of adults was counted. Percent of reduction of the studied stage compared with untreated check was calculated as mentioned before.

RESULTS AND DISSCUSION

Insecticidal activity of the tested toxicants:

To evaluate the toxic efficacy of the tested materials, *T. granarium* adults of laboratory strain were exposured to wheat grain prior treated with the desirable concentrations of the tested compounds. LC_{50} values with their confidence limits and slope values were tabulated in table (1). Data obtained indicated that pirimiphos-methyl was the most toxic compound with LC_{50} value of 0.135 ug/g at 24h post treatment, while the least toxic compound was the mineral oil (KZ) with LC_{50} value of 4300 ug/g. Based on the LC_{50} values the activity of the biocide ivomic followed the effect of pirimiphos-methyl. Data also cleared that cascade had a higher toxic effect than the mineral oil and formic acid. As a conclusion, pirimiphos-methyl was the most effective compound followed by the biocide, ivomic, insect growth regulator, cascade, formic acid and mineral oil. The effect of *B. thuringiensis* did not appear before 48 hrs. after treatment where the LC_{50} value was 320 ug/g wheat grain table (1). Concerning the tested OP compound, these findings are in good agreement with Zettler and Janes (1977) who found that pirimiphos-methyl was the most toxic to both the susceptible and malathion resistant strains of *T. castaneum* and was more toxic than malathion. The potencies of plant extracts and powders were confirmed by many authors as stored product protectants (Pereira, 1983; Arnason *et al*, 1989; Shaaya *et al*, 1991; El-Aidy and Helal, 1997; Abo-Arab *et al*, 1998 and Umoetok and Gerard, 2003). Geuirguis *et al.*, 1991 and Khalequzzaman and Sadia, 2010, studied the toxic action of 12 citrus oils extracted from peels of citrus fruits and three essential oils and one conventional insecticide, pirimiphos-methyl to adults of *S. oryzae* and *C. maculatus*, they found that pirimiphos-methyl was the most toxic compound compared to the tested oils.

Table1: Toxicity of the tested toxicants to *T. granarium* by exposure to treated media (after 24 hours).

Toxicant	LC_{50} ug/g	Confidence limits		Slope	Toxicity index
		Lower	Upper		
Organophosphorus					
Pirimiphos-methyl	0.135	0.105	0.175	2.5	100
Biocide					
Ivomic	0.95	0.68	1.33	2	14.2
Insect growth regulator					
Cascade	290	207.1	406	2	0.05
Organic acid					
Formic acid	850	531.25	1360	2.5	0.016
Oil					
Mineral oil (KZ)	4300	2866.7	6450	1.7	0.0031
After 48 hrs.					
Biocide					
Bacillus thuringiensis	320	246.16	416	2	0.042

Insect growth regulators were widely used for controlling many various insects of stored grain (Edward *et al*, 1991; Eisa and Ammor, 1992; Letellier, *et al*, 1995; and Daglish and pulvirenti, 1998).

Cakmakci *et al*, (1987) tested *B. thuringiensis* isolated from different sources in Turkey, they found that some of isolates were more effective than others giving 84-100% mortality against larvae of *Ephestia Kuehniella*. They also mentioned that extention of the use of microbial preparations would results in a considerable reduction of the side effects of chemical insecticides. Abo Arab and El-Hamady (1998) studied the efficiency of ivermectin as a protectant against certain stored grain insects using the technique of exposure to treated feeding media, ivermectin exhibited considerable toxicity nearly equal to that of malathion. Many researchers studied the effect of formic acid on varrow mite (Shawer, *et al*, 1993; Ghoniemy, 1998; Abou El-Enain, *et al*. 1999; Colderon et *al*, 2000; Mansour, 2003, and serag El-Dien and Eissa, 2003), results obtained showed that bee keepers can use formic acid 60% safety during winter and summer seasons for controlling varroa mite.

Effect of the tested compounds on *T. granarium* progeny:

Data in table (2) indicated that mean number of *T. granarium* emerged adults were highly effected by the different tested toxicants.

Results in table (2) are distinguished into four groups based on the percentage of reduction of adult stage. There were significant differences between the efficiency of used compounds aginst the tested insect, compared to control. Pirimiphos-methyl, ivomic and cascade had the highest effect with reduction value of 100%. KZ oil had the second rank with 75.67 followed by *B. thuringiensis* and the formic acid. The differences of efficiency may due to the different chemical composition of the tested toxicants which have varied modes of action.

Longstaff (1991) reported that the effects upon fecundity and development period were more complex and varied with insecticide, dose and strain. He found that no progeny were produced at the higher doses of pirimiphos-methyl compared to deltamethrin. Lale and Mustapha (2000) compared the efficacy of pirimiphos-methyl

(EC) at 175 mg/kg seed with dosages of neem oil (25, 50, 75 and 100 mg). They found no significant differences in reducing oviposition, adult emergence or seed damage.

Table 2: Percentage of reduction and mean numbers of offspring, in adult stage of *T. granarium* for the tested toxicants.

Treatment	Mean adults	% reduction
Control	12.33 e	0.00 f
Pirimiphos-methyl	0.00 a	100.00 a
Ivomic	0.00 a	100.00 a
Cascade	0.00 a	100.0 a
KZ	3.00 b	75.67 b
B. thuringiensis	4.67 c	62.12 c
Formic acid	6.67 d	45.90 d

Means followed by the same letter in the column are not significantly different ($P<0.05$).

Eisa *et al*. (1986) studied the effect of two insect growth regulators, chlorfluazuron and triflumuron besides the organophosphorus ovicide isoxathion (Karphos) on the eggs of *T. castaneum* under laboratory conditions by mixing with media. The results showed that chlorfluazuron was the most effective of the three compounds, where no adults of *T. castaneum* emerged at 1.0 and 10 ppm. Elek (1994) concluded that chitin synthesis inhibitor chlorfluazuron showed promise as grain protectant not only because it was effective as preventing progeny of *R. dominica* but also it killed immature stages early in its development, thus minimizing damage to commodity. Datta and Banerjee (1982) studied the effect of 2% ascorbic acid and 1% acetic acid on the infestation of wheat grain during storage by *S. oryzae*. No appreciable infestation occurred till 10 weeks in the acetic acid treated seeds.

Effect of mixing grain:

The tested compounds in this study exhibited promising control against *T. granarium* either with contact toxicity or with progeny bioassay, since the later highly effected with the rate value of LC_{50} compared to chick treatment. Therefore, further study was carried out to evaluate modified method of mixing with media to obtain the admixing manner with feeding media which causes the highest protection against stored product insects which attack cereal grains in storage to minimize the used amount of an insecticide, cost and pollution of users and stored products.

Mixing with feeding media is one of the main methods of bioassay. Researchers evaluated many chemical compounds included numerous formulations which belong to different insecticide groups, besides several bioassay methods to get high protection against insect pests either in field or storage.

In the present experiment based on the behavior of stored product insects which quickly move between grains where contact with the treated media, traditional method and modified technique of mixing with media were investigated.

Traditional method:

LC_{90} values of the different toxicants obtained after 24 hrs of exposing *T. granarium* to treated media were used at three rates, LC_{90}, ½ LC_{90} and ⅓ LC_{90}, where the total dose (LC_{90}) mixed with the total grain (30 g) while the half and one third dose (of LC_{90}) mixed with the half and one third of grain amount then mixed with the untreated grain (15 and 20 gm grain), respectively.

Modified method (treated layer method)

Where the treated layer was placed in three positions upon, under and between the untreated layers. Results obtained in Tables (3, 4 and 5) revealed that the half dose using the traditional manner had more percent of mortality than the expected nearly equal that of the total dose with mortality values of 90, 85 and 80 with pirimiphos-methyl, ivomic and cascade, respectively.

Table 3: Toxicity (% mortality) of the tested toxicants at different level of LC_{90} values using treated grain mixed with untreated grain *T. granarium*

Toxicant	Dose	1/2 dose	1/3 dose
Pirimiphos-methyl	100	90	85
Ivomic	95	85	70
Cascade	95	80	60
Formic acid	95	45	25
Mineral-oil (KZ)	90	80	65
Bacillus thuringiensis (48 hrs.)	55	35	20
Bacillus thuringiensis (72 hrs.)	90	50	40

Table 4: Toxicity (% mortality) of the tested toxicants at 1/3 level of LC_{90} values using treated grain layer manner against *T. granarium*

Toxicant	1/3 dose		
	Down	Middle	Up
Pirimiphos-methyl	100	33	25
Ivomic	60	25	15
Cascade	50	30	15
Formic acid	45	25	5
Mineral-oil	45	30	30
Bacillus thuringiensis 48 hrs.	40	30	0
72 hrs.	65	50	40

Table 5: Toxicity (% mortality) of the tested toxicants at 1/2 level of LC_{90} values using treated grain layer manner against *T. granarium*

Toxicant	1/2 dose	
	Down	Up
Pirimiphos-methyl	100	40
Ivomic	65	15
Cascade	70	40
Formic acid	55	20
Mineral-oil	65	35
Bacillus thuringiensis 48 hrs.	45	20
72 hrs.	60	40

Also one third of dose with the mentioned toxicants had high percent of mortality more than the expected value. In the modified manner (layer manner), the down position of treated layer showed the most effect where the mortality percentages had higher values than that of the other position of treated layer (middle and surface). The treated surface layer of grain had the least effect compared to the other two layers.

Generally, one third dose with layer manner had the best effect compared with the traditional method. The differences of results in different positions of the treated layers depend on the behavior of the tested insect where the beetles prefer warmer areas and increasing temperature causes an increased number of insects to move tworards the warmer areas in both vertical and horizontal columns (Jian, *et al*, (2002). Arthur (1992) studied the efficacy of chlorpyrifos-mrthyl for the control of maize weevils and red flour beetles in mixtures of treated and untreated corn after 0, 2, 4, 6, 8 and 10 months of storage. He found that, as the percentage of treated corn in the mixtures increased, insect populations, damaged, kernels decreased. A ratio of 2:3 treated/untreated corn controlled maize weevils and red flour beetles for 6 and 10 months, respectively. The surface treatments were not as effective as the mixture treatments.

CONCLUSION

The khapra beetle is one of the most notorious primary insect pests for stored grain.

Application of chemical insecticides caused health problems to human, animals and environment.

Insecticides are frequently unavailable or too expensive for subsistence farmers. Therefore, cheap and effective methods for reducing damage of stored product insects are needed. Relatively safe alternatives were investigated in this study which aimed to minimize the used amounts of an insecticide, cost and pollution of users and stored products using modified method of mixing with media at reduced ratios, half and one third dose based on behavior of stored product insect pests which quickly move between grains where contact with the treated media. The materials and layer manners in the present study exhibited a considerable control against khapra beetle, but further studies are need to shure these findings.

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ARABIC ABSTRACT

مقارنة كفاءة ثلاثة أساليب للخلط مع البيئة فى مكافحة حشرة خنفساء الصعيد على حبوب القمح

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تسبب الإصابة الحشرية للحبوب المخزونة ومنتجاتها مشاكل خطيرة على مستوى العالم تتمثل فى خفض المحصول ، تلوث المنتجات المخزونة وإصابة البذور كما أنها تحمل مسببات الأمراض التى تؤثر على النباتات ، الإنسان و الحيوان وتحدث تغيرات فى جودة المواد المخزونة.

قيمت ست مركبات واحد منها من المركبات الفسفورية العضوية وهو الأكتيلك (البيريمفوس- مثيل) اثنان من المبيدات الحيوية هما الأيفوميك و الباسيلس ثورينجينسس، حامض عضوى (الفورميك) وزيت معدنى KZومنظم نمو(كاسكيد) كواقيات للحبوب ضد الحشرة المذكورة بطريقة التعريض للبيئة المعاملة باستخدام ثلاثة أساليب ، الأسلوب الأول هو الخلط مع البيئة بالطريقة التقليدية والثانى خلط نصف الجرعة بنصف كمية الحبوب ثم خلط هذه الكمية بكمية مماثلة غير معاملة وكذا مع ثلث الجرعة، والثالث خلط ثلث الجرعة مع ثلث كمية الحبوب ثم وضع هذه الكمية المعاملة فوق أو تحت أو بين طبقتين غير معاملتين.

- أظهرت قيم الجرعة المميتة لنصف عدد الحشرات أن المبيد الكيماوى بيريمفوس – مثيل كان الأشد سمية (0.135 ميكروجرام/ جرام) بينما الزيت المعدنى كان اقلها تأثيرا بقيم جرعة نصفية (4300 ميكروجرام / جرام) بعد 24 ساعة من المعاملة.
- المبيد الحيوى ايفوميك كان التالى فى التأثير للمبيد الكيماوى .
- أظهرت النتائج أن منظم النمو(الكاسكيد) كان له تأثير سام على الحشرة أعلى من الزيت المعدنى والحامض العضوى .
- أظهرت النتائج أن نصف وثلث الجرعة أدت الى نسبة موت أعلى من المتوقع .
- أدى وضع الطبقة المعاملة تحت الطبقات الغير معاملة الى أفضل النتائج حيث حقق نسبة وقاية أفضل منها.