THE EFFICACY OF FOUR SEED POWDERS ON SOME BIOLOGICAL ASPECTS AND MORTALITY OF KHAPRA BEETLE

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ABSTRACT

The seed powders of four plants; harmal *Harmal peganum* L., black pepper *Piper nigrum* L., radish *Raphanus sativus* L. and celery *Apinum graveolen*s L. were tested at the concentrations 2%, 4% and 6% to evaluate their effects on some biological aspects and mortality of *Trogoderma granarium* Everts. The results indicated that the seed powders had toxic effects. They had significant effect on adult mortality. There were significant differences between treatments, concentrations and periods of exposure at 5%. The highest mortality was 96.6, recorded in celery treatment at concentration of 6 % and 6 days of exposure, followed by pepper 83.3% , radish 76.6%, and harmal 73.3%. Seed powders had also moderate repellent action against adult insects. There were significant differences between treatments and concentrations at 5%. The highest repellent percentage was 40%, occurred in celery treatment at concentration of 6% and period of exposure of 24 hours, followed by pepper 34.6 %, radish 33.3% and harmal 30%. No repellency and mortality noticed in control. Seed powders had also significant effect on decreasing average number of F1 progeny. There were significant differences between treatments, concentrations at 5%. The number of F1 progeny found in celery treatment at concentration of 6% was zero, followed by pepper 23, radish 31 and harmal 56. Whereas in the control was 78. The seed powders disrupted the life cycle of the insect resulting in prolonging the period of F1 adult appearance; the periods were 20 days for control, 26 days for harmal and radish, no adult appearance in pepper and celery even after 34 days. The result showed that the seed powders had significant effect on mortality, repellency and reduction of progeny. Thus more studies on these plant species are needed to establish their potential sources which might used for stored product protection.

تأثيرمساحيق اربعة بذور في بعض الاوجه الحياتيه وموت خنفساء الخابرا

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المستخلص

أختبرت مساحيق بذور مطحونة لاربعة نباتات هي الحرمل *Peganum harmala L.*، الفجل Raphanus *sativus* L.، الكرفس *Apinum graveoles* L والفلفل *Piper nigrum* L. وبتراكيز 2 %، 4% و 6% لتقييم تأثيرها على بعض الا وجه الحياتيه والموت في حياة خنفساء الخابرا *Trogoderma granarium* Everts. اوضحت الدراسة ان للبذور تأثيرا ساما قاتلا على البالغات وان نسب القتل ازدادت معنويا حسب نوع البذور، التركيز وفترات التعريض عند مستوى احتمال 5 %، كانت اعلى نسبة قتل في معاملة بذور الكرفس عند التركيز 6 %, و بعد ٦ ايام تعريض اذ كانت 96.6% تلتها معاملة الفلفل ٨٣.٣، الفجل ٧٦.٦ والحرمل ٧٣.٣%. كما اظهرت الدراسة ان للبذور تأثير طارد ضد بالغات الحشرة حيث أن نسبة الطرد لبالغات الخابرا في معاملة الكرفس عند التركيز ٦% و بعد مرور 24 ساعة من التعريض كانت 40% تلتها معاملات الفلفل ٣٤.6%، الفجل ٣٣.٣% والحرمل 30%. وكانت هناك فروق معنوية بين المعاملات والتراكيز تحت مستوى احتمال 5 %. لم يلاحظ موت او طرد في معاملة المقارنة. وجد ايضا ان هناك تأثير للبذور على خفض تعداد افراد الجيل الاول. وأن هذه الخفض يتناسب طرديا مع نوع البذور والتركيز. معدل اعداد الجيل الاول في معاملة الكرفس كان صفرا عند تركيز 6% تلتها معاملة الفلفل 23, معاملة الفجل 31 وفي معاملة الحرمل 56 فرد في حين كانت في المقارنة ٧٨ فرد. أثرت مساحيق البذور ايضا على فترة ظهور بالغات الجيل الاول ، كانت الفترة ٢٠ يوما في المقارنة، ٢٦ في الفجل والحرمل ولم تظهر البالغات حتى بعد 34 يوماً في معاملتي الكرفس والفلفل. اوضحت نتائج الدراسة ان لمساحيق البذور المستخدمة سمية عالية في قتل الحشرات وابعادها عن الحنطة المعاملة بها وخفض اعداد الجيل الاول. نوصي بالحاجة الى اجراء المزيد من الدراسات الخاصة باستخدام البذور ومعرفة محتويا تها الكيماوية لأمكانية استخدامها بشكل فعال في وقاية الحبوب المخزونه.

Introduction

Conservation of reserve grain stocks is necessary to ensure a continuous supply at stable prices (27). Losses due to insect infestations are serious problem in the grain storage for the developing countries in particular, where poor sanitation and inappropriate storage facilities are available (28). In general, estimation of yield losses due to insects and diseases is ranging between 50- 100 % for the tropical regions (5). Stored grains in general are a lot infested by different stored insects, for example, by khapra beetle *Trogoderma granarium* Everts which is well known as one of the most overspread among the grain stores of Iraq (20). The losses of stored grains due to this insect in Iraq were estimated by a number of researchers, it was 30% or more of stored grains (3, 19). It is also the widest occurrence in stored grains in tropical and subtropical regions of Asia and Africa (4). It has been ranked one of the 100 "World's Worst" invaders. Khapra beetle is one of the most important pest which can maintain its presence in stores at a very low numbers and is able to survive long period of inactive state (6). It is a synanthropic which occurs for instance in grain stores, food stores, malt houses, seed processing plants, fodder production plant, dried milk factories, merchant stores, stores of packing materials used (sacks , bags, crates) (6). If the beetle is left undisrupted it can cause significant grain weight loss in the store. It may lead to significant reduction in seeds viability too. The larvae feed on different kinds of seeds such as cereals, oil, and legume seeds as well as on other foods. The insect was first recognized in Iraq in 1954 by Derwesh (24).

Trogoderma granarium is a member of the Coleoptera family Dermestidae. The adult males are 1.4- 2.3 mm long, 0.75- 1.1mm wide; adult females are 2.1- 3.4 mm long, 1.7- 1. .9 mm wide, ovate and densely hairy beetles. Adult females are slightly larger than males and lighter in colour. Colour of the Khapra beetle is as follows ; head and pronotum dark reddish-brown, usually with indinstinct lighter reddish- brown fasciae ; venter of thorax and abdomen reddish-brown; legs yellowish-brown. The setae on the dorsal surface are of two types: evenly distributed, coarse, semi-erect, yellowish-brown ones; and, few scattered, dark reddish brown setae, colour of setae follows the colours of cuticles. The pronotum medially and laterally has indistinct patches of yellowish-white, ensiforum setae, and elytra with two or three indistinct band of yellowish-white, ensiform (flattened) setae. The median ocellus on the front is always distinct. Antennae are yellowish-brown, 9, 10 or 11 segmented, with 3-5 segmented clubs (6). Mated females live from four to seven days, unmated females from 20 to 30 days,

and males from seven to 12 days. Mating occurs about five days after emergence, and egg laying begins almost immediately at 40 º C. Egg laying may begin at one or three days at cooler temperatures, Eggs hatch in three to 14 days after the female lays, the female lays an average of 50 to 60 eggs that are loosely scattered in the host material (4). Many insecticide trials were carried out to control it such as the work of Mhemed, et.al, (20) and many others. Present pest control is mainly relying on the use of synthetic insecticides and fumigants (23). Their indiscriminate use led to a number of problems including toxic residues in food grains and to environmental pollution (8,29,30). These problems, together with the development of insect resistance, increasing costs of application, pest resurgence and lethal effects on non-target organisms in addition to direct toxicity to users make the situation much more complicated (1). Today, the use of synthetic insecticides to protect stored grains has significantly been declined to a drastic level. (18). Statistics indicated that there are more than one million poisoning cases (22).The need to find insecticides to protect stored products with minimum harm to the environment is urgent now to turn the attention to plant materials. The use of insecticides of natural origin is therefore an important development in storage pest control. They have short residual action, low mammalian toxicity and more importantly reducing environmental pollution. On the basis of physiological activities on insects, Jacobson (12) conventionally classified the plant component into 8 groups, namely repellents, feeding deterrents, anti-feedants, toxicants, growth retardants, chemosterilan and attractants as well as secondary compounds from plants include alkaloids, terpenoids, flavoroids, chromen and minor chemicals can affect insects in several ways .They may disrupt major metabolic pathways and cause rapid death, act as attractants, deterants, phago-stimulants, anti feeders, or modify oviposition, they may retard or accelerate development or interfere with the life cycle of the insects in other ways (11). Moreover, products from several flora species act as repellents and anti feedants to a number of Coleoptera species that attack stored products (29).Wheat crop is considered as one of the main source of food that the Iraqi nation depends on and subsequently interested in its cultivation. Iraq is main prospective is to urgently increase wheat production level so that to fulfil local consumption and much more for the future.

This experiment specifically aimed to determine the toxicity action on adult mortality, repellency and growth inhibitory effect of powdered seeds of four plants to control *Trogoderma granarium* Everts.

Materials and methods

1. Rearing of test insects

The culture of the *Trogoderma granarium* was originally taken from the laboratory of plant protection, College of Agric., Karbala University, Iraq .It was reared on local grain variety (Abaa 99) of wheat *Triticum aestivum* L. at 34º C ±2 & 60 ± 5 % Relative Humidity (R.H). The culture was maintained in a room, good aerated of (6.25x5.5 m) fitted with air cooler which is operated 24 hours a day, (using National Electricity supported with electric generator working day and night), maintaining the room temperature at 33º C ± 3& 65% ± 5 R.H. The temperature and relative humidity was measured by the means of four readings throughout the day and night using Anymetre in-out door Thermo-Hygrometer model; TH6008. Four clean glass containers, each of (16 cm long x 8 cm diameter width), were prepared, 200g of non infested wheat grains were placed in each container, then 20 pairs of one day old (20 males+ 20 females) of adult insects were introduced to each container, they were covered with muslin cloth fastened with rubber bands. The studies were carried out at similar conditions.

2. Preparing the seed powders

Seeds of four plants*;* harmal *Peganum harmala* L., black pepper, *Piper nigrum* L., radish *Raphnus sativus* L. and celery *Apinum graveolens* L. were obtained from the local market of Al- Kadhimyah, Baghdad, Iraq. The seeds were washed under tap water and then shed to dry out. The seeds of each plant were grounded by moulinex electric grinder into very fine powder. The seed powder of each plant were kept in a separate small container covered air tightly which then kept into a refrigerator at a freezing point degree.

3. Mortality tests

Mixtures of both seed powders and wheat grains were made at concentrations 2%, 4%, 6%. Each concentration has been placed in a separate container, well shaken to homogenize the mixture and subsequently kept in refrigerator .Ten g of each concentration were placed in plastic petri dish (8.5cm diameter x 1.2 cm depth) lined with whatmann no.1 filter paper. Ten adult beetles one day old (5 males + 5 females) were introduced to each petri dish. For the control treatment, only 10 g of wheat grains and 10 adult beetles one day old (5 males+5 females) were placed in the dishes in the same way as previously mentioned. Mortality was recorded at 3 and 6 days after treatment. Each test was replicated three times.

4. The repellency test

Following the modified test described by Helen (10), a 4 cm diameter circle was drawn in the centre of the whatmann paper no.1 which fitted in the dishes described in the mortality test. Ten g of each concentration and 10 adult insects one day old (5males + 5

females) were placed on the circle. For the control treatment, only 10 g of wheat grains and 10 adult (5 males + 5 females) were placed on the circle, each treatment was replicated three times. The insects which were found outside the circle 24 hours after treatment were regarded as repelled insects.

5. Effect of the seed powder on the life cycle of f1 generation

A total 20 gm of each concentration were added into cylindrical glass container (16 cm long x 8 cm diam.). Five pairs of adult khapra (5 males +5 females) one day old were introduced to each container. For the control treatment, 20 gm of wheat grains were placed in each container, five pairs of adult insects (5males +5 females) of one day old were introduced to each container. Each treatment was replicated three times.

6.Statistic analysis

The experiment was subjected to the Complete Randomised Design (CRD). Analysis the main variables and their interactions were counted according to Steel and Torrie (26) to determine the analysis of variance, then means were compared by using L. S. D. at 5% probability.

Results and Discussion

The effect of seed powder on average mortality percentages of *Trogoderma granarium* of the present study showed that all seed powder treatments had a moderate effect on adult mortality after 3 days of treatment, whereas the insects did not effected in control treatment. At 6 days after treatment all seed powders had high effect on adult mortality (Table 1). It was clear that celery had the lead in mortality action followed by pepper, radish and harmal with different variations. The mortality percentages were increased with increased of concentration. The Statistical analysis indicated that there were significant differences between the seed powders of plant species (s), concentrations (c), period of exposures (p), interaction of s x c and s x p, but there were no significant differences of the interaction of c x p. At p.5% .The least significant differences (L.S.D) between seed powder treatments = 0.26,

Table1. The effect of botanical seed powders (s) with different concentrations (c) on Adult mortality percentages of *Trogoderma granarium* Everts at two periods of exposures (p).

C	p /days	s/ mortality %					
		Harmal	Radish	Celery	Pepper	Control	C x p
2 %	3 6	1.33 7.00	1.33 6.66	2.00 7.66	1.66 7.66	0.00 0.00	1.60 5.80
4 %	3 6	1.33 7.33	1.66 7.33	2.33 8.00	2.33 8.00	0.00 0.00	1.53 6.13
6 %	3 6	1.33 7.33	1.66 7.66	2.66 9.66	3.00 8.33	0.00 0.00	1.73 6.60
l. s. d. 5% c x p = no significant differences (N.S.) l. S. d. 5% c x p s= N. S.							
C x s	2 % 4 % 6 %	4.16 4.33 4.33	4.00 4.5 4.66	4.83 5.16 6.16	4.66 5.16 5.66	0.00 0.00 0.00	3.53 3.83 4.16
l. s. d. 5 % c = 0.20 l. s. d. 5% s x c = 0.45							
S x p	3 6	1.33 7.22	1.55 7.22	2.33 8.44	2.33 8.oo	0.00 0.00	1.51 6.17
l. s. d. 5 % p = 0.16 l. s. d. 5% s x p = 0.37 l. s. d. 5% S = 0.26							
S		4.27	4.48	5,38	5.16	0.00	

A lot of previous work were done on the effect of harmal and pepper on stored insects and others such as the works of (14, 15, 16, 17), on *T. castaneum*. Jubilow et al. (15) revealed that the effect of harmal crude extract had significant insecticidal activity against *T. castaneum* larvae and adults, they also mentioned that harmal reduced significantly larval growth just 2 days after the treatment. Our results have shown that all seed powders used, possess high insecticidal activity on *Trogoderma granarium*. Saljoki et al. (25) found similar effect on *S. oryzae* , however the results of previous work are comparable with the present findings. A little work had been done on radish and celery effects, such as the work of Almoagel (2). She stated that the radish seed powder at high concentrations had significant effect against adult mortality of khapra beetle. Mohammad and Abdullah (21) indicated that slices of garlic, celery seeds and three others plant extracts showed high significant effect on adult mortality of *C. maculates* at 24 hours after treatment.

Concerning the work on repellent effect of seed powders against adult of *T. granarium*, all the plants had moderate repellent action after 24 hours exposure (Table 2). All seed powders had an effect on average repellency percentages on *T*rogoderma *granarium*, the celery had the lead in repellent action followed by pepper, radish and harmal with different variations. It was increased with increased of concentration. The statistical analysis indicated that there were significant differences between the plant species treatments, concentrations, but it was not in the interaction of treatments and concentrations at p. 5%. The (L.S.D) between seed powders was 0.574, between concentrations was 0.447. No previous work have been found in literature concerning the repellency of celery seed powders against *Trogoderma granarium* yet, however the results of some previous work of harmal and pepper on other insects such as the work on *Tribolium castaneum*, is supported our present findings (17). Kanvil et al. (16) reported that all concentrations of harmal were more effective initially, but with passage of time, repellency was decreased. Majority of previous workers also reported in the same trend (12, 13). Harish et.al. (9) confirmed the present finding . It was mentioned that harmal extract was effective on S*itophilus. oryzae,* (23).These results were confirmed the present work. Harmal possess high insecticidal activity on *Tribolium castaneum*. It is rich source of carboline, alkaloids as harmol, harmine and harmeline. These alkaloids of this plant may explain the toxic effect on the insects (l4). The effect of seed powders on F1 generation; number of progeny and the period needed for appearance of F1 adults were recorded. The results presented in tables (3, 4) clearly indicated that the most striking effect was taken place on both celery and pepper seed powder treatments. It was found reduction in fecundity; there were significant effects on reduction in progeny, the average number of the progeny of F1 generation was declined to zero in celery treatment at concentration of 6 %, followed by pepper, radish and harmal with different variations compared with the control group which had no repellent effect was seen. The statistical analysis indicated that there are significant differences between the treatments, concentrations, and in the interactions of treatments and concentrations at level 5 %. Celery had the lead in reduction the number of the progeny followed by pepper, radish and harmal. The least significant difference between seed powder of plant species was 5.52, between concentrations was 4.27, the interaction of seed powder treatments and concentrations = 9.56 at p 5 %. The weakening of adults by seed powder may cause them to lay few eggs.

Table 2. Average repellency percentages of adult *Trogoderma granarium* in seed powders..

Treatments	S. C. (%) Repellency %			Means
	2%	4%	6%	
Harmal	1.33	2.000	3.000	2.111
Radish	2.33	2.333	3.333	2.556
Celery	3.000	3.667	4.0000	3.556
Black pepper	2.667	2.667	3.4662	3.000
Control	0.000	0.000	0.000	L. S. D. 5% S = 0.574
Means	1.87	2.73	2.13	
L. S. D 5 % S x c = N. S. L. S. D 5% C=0.447				

High concentrations of seed powders in the present study had significant effect in reducing the fecundity of the *Trogoderma granarium*. Work of Almoagel (2) was in the same trend. This study has shown that all seed powders applied to wheat grains significantly either reducing the progeny by killing the adults or weakening the larvae resulted in decrease the number of eggs laid down by females. In the same trend Epidi (7) stated, if death does not occur immediately, the factor (s) have debilitating effect on the larvae and their life cycle may be prolonged. The result in table 4 showed that the differences of length of the periods for adult F1 generation appearance of *T. granarium*. The periods were 20 days for the control and 26 days for each harmal and radish treatments. In pepper treatments no adults were appeared even at 34 days after the treatment. The same result were recorded in celery treatment at concentrations 2% and 4% but no live insects were appeared at the concentration of 6%. Most of the botanical seed powders applied against *Trogoderma granarium* did not reveal similar activity on adults, larvae and on population reduction, there were significant effect on both mortality and reduction of progeny, so the toxicity action of the powders was on larvae, especially on the first instar larvae, additionally, the weakening of the adults by the powder may cause them lay a few eggs. The previous work of Al moagel (2) was supported the finding of the present study.

Table3. Number of the progeny of F1 generation in seed powder treatments.

s	c % Average number of F1 progeny			Mean T x C
	2	4	6	
Harmal	69.00 d	66.00 d	56.00 c	63.67
Radish	54.00	45.00 c	31.00	43.00
Celery	21.00	07.33	0.00	09.44
Black pepper	46.00	38.00	23.00	35.67
Control	78.00	7 8.00	78.00	78.00
Ls d at 5 % Treatments = 5.52, Concentrations =4.27, C x T = 9.56				
Mean	53.60	46.87	37.60	

Table 4. Average length of periods for adult insects of F1 generation in seed powders.

Plants	c x p		
	2	4	6
Harmal	26	26	26
Radish	26	26	26
Celery	*	*	**
Pepper	*	*	*
Control	20	20	20

* no adult were seen even at 34 days after treatment . * * No live insects were seen at 34 days after treatments

Results reported in this study show food repellency and toxicity effects of all plant species on each of adult mortality and progeny reduction. The effect of these botanical seed powders on the insects can be explained by several hypothesis as following; ovicidal effect on the eggs, the toxic effect on early or first instar larvae, the toxic effect on the adults, the oviposition repellency to the insects. Thus more studies are needed on theses plant species to establish their potential sources might lead to their improvement as protectants in direct application assay.

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