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Combined Effect of Diatomaceous Earth and Insect Growth Regulators Against *Trogoderma granarium* (Coleoptera: Dermestidae)

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Abstract

Diatomaceous earth (DE) and two insect growth regulators (IGRs) were tested under laboratory conditions against khapra beetle (*Trogoderma granarium*). Khapra beetle is a severe pest of stored products and causes heavy losses. Three dose levels of both IGRs (lufenuron and tebufenozide) and DE were used alone and in combinations. Twenty-three instar larvae of khapra beetle were released on treated and untreated wheat grains. Data of larval mortality was recorded after 7, 14 and 21 days, while adult emergence was observed after 14 and 30 days. The progeny production was observed after 60 days. The mortality rates of 84.9%, 66.4%, 41.5% and 91.6% were recorded in case of DE, lufenuron, tebufenozide and combined treatment (lufenuron + DE), respectively, at their highest dose rates compared to control, revealing the highest mortality rate in combined treatment. Similarly, minimum pupation of 1.22 and minimum adult emergence of 0.22 were also observed in combined treatment of lufenuron and DE at their maximum dose rates. Adult progeny production data showed that the highest reduction of 64% was determined with combined application of lufenuron and DE compared to control. The lufenuron and DE were found most appropriate against the khapra beetle, not only alone but also combined. Overall, the outcomes of the present work prominently specify that the combined use of DE and IGRs is highly operative and beneficial against stored grain insect pests.

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Introduction

Khapra beetle [*Trogoderma granarium* (Coleoptera: Dermestidae)] is a severe insect pest of stored wheat grains, cereals and their products [1]. It is included in the hundred worst invasive species of the world [2]. Khapra beetle has also got the importance of quarantine pest because of its high economic significance [3]. The origin of this insect pest is India, but now it has been spread to different parts of the world like USA, Africa and East Asia [4]. Khapra beetle causes damage to stored cereals like rice, maize, sorghum, barley and other dried fruits. Due to its ability to survive without food for a long time, it is difficult to control [5]. It remains active from March to October and hibernates from November to February. Wheat is a major staple food and grain crop in Pakistan, during storage it suffers heavy losses due to stored insect pest complex. According to recent studies, in Pakistan, the post-harvest losses have been assessed to be a varying almost 10-15% and 20-30%, respectively [6-8]. Khapra beetle is the primary insect pest and only at the larval stage causes serious damage to grains [9] while at the adult phase, it only lays the eggs and does not damage the food [10]. In dry and hot conditions, the populations of khapra beetle increase rapidly and cause 30-70% grain damage [11]. It is difficult to control khapra beetle because of its ability to develop resistance against many conventional pesticides [12]. Nowadays, different geographical strains of Khapra beetle become resistant against phosphine, which was an effective fumigant to control stored pests for a long time [13]. The use of different types of pesticides to control insect pests has a lot of drawbacks, for example; many pesticides have a very drastic effect on human health as well as on livestock and cause many diseases in them. Different alternatives of these pesticides like plant derivative, diatomaceous earth and insect growth regulators having chitin synthesis inhibitors, ecdysteroids and juvenile hormone have been recommended [14-16].

Insect growth regulators (IGRs) are the compounds that affect the growth, development and metamorphosis of the insects. They are also called third-generation insecticides because they cause hormonal imbalance in insects. IGRs interfering with the molting process and stops the growth of insects to reach its maturity. Juvenoids, ecdysone inhibitor and chitin synthesis inhibitor (CSIs) are the three groups of IGRs [17]. IGRs are target specific and have no harmful effect on the beneficial insects. They interrupt the normal growth of insects by inhibiting the chitin synthesis hormone or by mimic the molting hormone. IGRs are also called “reduced-risk insecticides” because they have low mammalian toxicity and thus used in stored grain pest management [18]. As compared to conventional insecticides, IGRs are less toxic to humans and other vertebrates [19]. Diatomaceous earth (DE) is inert dust harvested from sediments at the bottom of oceans, lakes, and rivers around the globe. It comes from diatoms, a type of single-celled algae with microscopic, beautiful geometric shells made of silicon dioxide. Over millennia, diatoms accumulate in aquatic sediments as fossils that can be harvested and dried into fine white dust [20]. Silica (a component of DE) has a lethal effect on the insects and usually used in pest management. DE is widely used as alone or in combination with different insecticides to control different types of insect pests of stored products [21]. DE is gently mixed with grains and most useful to control adults as well as larvae of external insect pests. When the insects move from the grains, their bodies rub with the DE dust, which causes an abrasive action on the cuticle (a layer that stops the dehydration) of the insects [22]. Due to its abrasive properties, it is used in insect pest management. It causes dehydration in insects by absorbing lipids from the exoskeleton (the outer waxy layer of the insects). Dehydration causes severe loss of water (60%) from the insect body [23]. Due to loss of water from the epicuticle, the body of insect shrinks and ultimately insect mortality occurs [24]. It is widely used in many regions of the world for the control of insect pests [25]. Diatomaceous earth has no toxic effect on stored products and can easily remove from the grains by washing [26].

The quality of the grains is not affected by DE when it is properly used [27]. DE has a lot of advantages like less toxic to the environment, less toxic to mammals, have no harmful effect on the treated commodities like grain cereals, very effective against those insect species, which produce resistance against many insecticides and have a lot of stability at low and high temperature [28]. Indiscriminate use of insecticides and fumigants to control stored grain insect pests create a problem of insecticide resistance and give rise to serious health effects in human beings [29, 30]. We can reduce stored grain losses by using bio rational approaches. In this regard, the aims of the current study were to determine the possible potential of diatomaceous earth and insect growth regulators for the management of khapra beetle.

Materials and Methods

Diatomaceous earth (DE)

The DEs formulation (Concern) contained 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O with an average particle size between 8-12 mm was imported from Wood Stream^{TM} Corporation, USA. The dose rates of DE used in the bioassay were 250 ppm, 500 ppm and 750 ppm.

Insect growth regulators

Two synthetic insect growth regulators (lufenuron and Tebufenozide) were used in bioassay studies at the dose rate of 0.25 ppm, 0.50 ppm and 0.75 ppm. Lufenuron (Match) was purchased from Syngenta, Pakistan, while Tebufenozide (Confirm) was purchased from Rohm and Haas Corporation, Spring House, PA, USA.

Insect rearing

The khapra beetle samples were collected from the grain market and godown of Punjab Food Department, Faisalabad, Pakistan. The culture of khapra beetle was raised in an incubator on wheat grains at 30°C±2 and 65%±5 relative humidity. All wheat grains were sterilized at 70°C for 15 minutes and then put in separate glass jars, each jar has a capacity of 250 g. Fifty adults were released on the wheat grains inside the jars. The mouth of each jar was tightly covered with muslin cloth by using a rubber band to prevent the escape of adults. After three days, these adult beetles were sieved out from the culture and wheat grains having freshly laid eggs were put into the same jars and kept in the incubator to get a uniform population of khapra beetle.

Toxicity of diatomaceous earth and insect growth regulators against khapra beetle

Three bioassays were performed for the study of both IGRs (0.25, 0.50 and 0.75 ppm) and DEs (250, 500 and 750 ppm) against khapra beetle. The sterilized wheat grains having a weight of 40 g were taken into the plastic jars. Different concentrations of IGRs and DEs were uniformly mixed with the wheat grains alone and in combined forms. In alone form, both IGRs were prepared in 30 ml water separately and sprayed over 40g wheat grains using a small handheld spray gun, except control treatment (no IGR). The mixture of IGR and wheat grains was mixed for ten minutes in a rotating flask. After mixing well, the mixture was placed under sunlight for drying and then shifted into plastic vials. After application, jars were shaken for one minute to ensure uniform treatment. The DE in alone form was applied directly to the wheat grains. These vials were shaken manually for 5 minutes to achieve the equal distribution of DE particles in the grain mass. There also was an untreated check (control) for comparison. In this experiment, DE and the most effective IGR (lufenuron) from the above bioassays were selected to check the combined effect of both on the khapra beetle. The lufenuron (0.25, 0.50 and 0.75 ppm) and DEs (250, 500 and 750 ppm) were applied to the weighed amount of wheat grains in the plastic vials, respectively. In case of the combined application experiment, IGR which showed good results alone was selected. Lufenuron performed best in alone form, hence, first of all, the grains were treated with lufenuron as described above and after drying the treated grains, the DEs dust was applied and mixed well. There was also an untreated control for combined (IGR + DEs) treatments. Twenty 3rd instar larvae of Khapra beetle were released in each plastic jar containing IGRs, DEs and IGRs + DEs treated and untreated wheat grains. Jars were kept in the incubators at 30°C±2 and 65%±5 relative humidity. Mortality data were observed after 7, 14 and 21 days of treatment application and pupal and adult emergence was recorded after 14 and 30 days, respectively. The progeny production in treated and untreated wheat grains was observed after 60 days. Corrected mortality was calculated from observed mortality data using Abbott's formula. The data were evaluated statistically using Statistix analytical software, Tallahassee, FL. The means of significant treatments were compared by using Tuckey's HSD test.

Results and Discussion

The experiment was conducted in order to evaluate the efficacy of two insect growth regulators (lufenuron and tebufenozide), DE and the combination of most effective IGR with DE against khapra beetle. The results showed that the mortality of khapra beetle was increased as the concentration of IGRs and DE was increased. Lufenuron and tebufenozide at the highest dose rate (0.75 ppm) showed maximum mean mortality of 66.44% and 41.47%, respectively. These results are in line with Kostyukovsky and Trostanetsky [31] who reported an increase in the death rate of 3rd instars larvae of

Table 1 Comparison of mortality of khapra beetle after different time intervals by using different concentrations of diatomaceous earth (DE), lufenuron, tebufenozide and combined application of lufenuron + diatomaceous earth.

Treatment	Concentration (ppm)	Time interval		
		7 Days	14 Days	21 Days
Diatomaceous earth	250	0.00 ± 0.00 f	6.66 ± 1.66 e	38.16 ± 1.67 c
	500	5.00 ± 0.00 ef	20.00 ± 2.88 d	63.93 ± 4.42 b
	750	11.66 ± 1.66 de	31.66 ± 1.66 c	84.95 ± 2.89 a
	Control	0.00 ± 0.00 f	0.00 ± 0.00 f	0.27 ± 1.23 d
Lufenuron	0.25	0.00 ± 0.00 e	10.00 ± 0.00 de	29.93 ± 2.90 c
	0.50	5.00 ± 0.00 de	18.33 ± 1.66 cd	44.69 ± 2.90 b
	0.75	11.66 ± 1.66 d	25.00 ± 2.88 c	66.44 ± 4.43 a
	Control	0.00 ± 0.00 e	0.00 ± 0.00 e	0.30 ± 1.29 d
Tebufenozide	0.25	0.00 ± 0.00 e	6.35 ± 1.67 de	19.73 ± 2.89 c
	0.50	1.66 ± 1.66 de	11.37 ± 1.67 d	33.11 ± 1.67 b
	0.75	5.00 ± 0.00 d	21.40 ± 1.67 c	41.47 ± 1.67 a
	Control	0.00 ± 0.00 e	0.00 ± 0.00 e	0.29 ± 1.27 d
Lufenuron + Diatomaceous earth	0.25 + 250	9.70 ± 2.89 d	29.76 ± 2.89 d	39.80 ± 2.89 c
	0.50 + 500	21.40 ± 1.67 cd	44.81 ± 2.89 c	66.55 ± 4.42 b
	0.75 + 750	36.45 ± 1.67 ef	59.86 ± 2.89 b	91.63 ± 3.34 a
	Control	0.00 ± 0.00 f	0.00 ± 0.00 e	0.33 ± 1.33 d

Value ± standard deviation; Means sharing similar letters are not significantly different at *P*<0.05.

Table 2 Effect of different concentrations of diatomaceous earth (DE), lufenuron, tebufenozide and combined application of lufenuron + diatomaceous earth on pupal and adult emergence of khapra beetle after 14 and 30 days.

Treatments	Concentration (ppm)	Time interval	
		After 14 days	After 30 days
		Pupal emergence	Adult emergence
Diatomaceous earth	250	7.33 ± 0.47 c	6.77 ± 0.46 b
	500	5.66 ± 0.40 c	5.11 ± 0.45 bc
	750	3.88 ± 0.48 b	2.77 ± 0.52 c
	control	10.66 ± 0.44 a	13.22 ± 1.25 a
Lufenuron	0.25	3.00 ± 0.57 b	3.11 ± 0.71 b
	0.50	2.11 ± 0.45 b	1.88 ± 0.51 b
	0.75	1.55 ± 0.47 b	1.11 ± 0.51 b
	control	7.22 ± 0.32 a	10.11 ±1.25 a
Tebufenozide	0.25	6.11 ± 0.38 d	5.44 ± 0.50 c
	0.50	4.55 ± 0.37 c	4.22 ±0.32 c
	0.75	2.66 ± 0.40 b	2.11 ± 0.51 b
	control	9.44 ± 0.24 a	8.44 ± 0.24 a
Lufenuron + diatomaceous earth	0.25 + 250	5.88 ± 0.20 d	3.88 ± 0.26 c
	0.50 + 500	4.44 ± 0.24 c	2.66 ± 0.28 c
	0.75 + 750	1.22 ± 0.22 b	0.22 ± 0.14 b
	control	10.44 ± 0.29 a	11.88 ± 0.80 a

Value ± standard deviation; Means sharing similar letters are not significantly different at *P*<0.05.

khapra beetle as the dose of IGRs was increased up to 1 ppm. DE showed mean mortality of 84.95% at the dose rate of 750 ppm after the exposure time of 21 days (Table 1). These results are similar to that of Kavallieratos et al. [32], who reported that 125 ppm dose of silicosec (DE) caused 77% mortality of *Sitophilus oryzae* after the exposure of 14 days on barley grains. The mortality of all insects was increased as the dose rate and exposure time of DE increased [33, 34]. Differences in toxicity of DE were dependent upon the type of the commodity. DE formulations showed differences in toxicity when applied alone on different grains. In a separate experiment, it was observed that mortality showed by *S. oryzae* was more on rice grains as compared to maize treated with silicosec (DE) [32]. Lufenuron showed better results as compared to tebufenozide so lufenuron was preceded in further studies by making its combinations with DE. Combined concentrations (lufenuron + DE) were tested against Khapra beetle and mortality data were assessed after 7, 14 and 21 days. Maximum mean mortality of 91.63% was recorded at the highest dose rate (0.75 ppm + 750 ppm, respectively) (Table 1). Mortality was increased with the increase in dose rate. These results are in accordance with Arthur [34] who performed experiments to evaluate the combined effect of methoprene and DE against *Rhyzopertha dominica* and stated that survival of the insects was greatly decreased with an increase in dose rate. The combined effect of spinosad and DE was studied by Vayias et al. [35] and they verified that growth, development and survival of insects decreased when the dose of spinosad + DE was increased.

The data regarding the pupal emergence of khapra beetle was recorded after the exposure time of 14 days. Results revealed that with the increase in dose rate of DE and IGRs, a significant reduction in pupal emergence was determined in all treatment compared to control. The minimum pupation of 1.55 was seen with lufenuron at the dose rate of 0.75 ppm over control. Tebufenozide gave minimum mean pupation of 2.66 at the dose rate of 0.75 ppm and DE gave minimum pupation of 3.88 at the dose rate of 750 ppm over control. In case of the combined application of lufenuron and DE, a further decrease in pupation (1.22) was found compared to control (Table 2). In another study, lufenuron drastically affected the nymph of the desert locust by reducing its growth and development and prolonged the pupation [36]. Smagghe et al. [37] stated that tebufenozide affected the pupation of codling moth by reducing the larval feeding and stopping the growth, and showed the symptoms of double head capsule (Table 2). Adult emergence of the khapra beetle was noted after the exposure time of 30 days. At the maximum dose rate of DE, lufenuron and tebufenozide, minimum mean adult emergence of 2.77, 1.11 and 2.11, respectively, was determined. The combined use of lufenuron and DE revealed that at their highest dose (0.75 + 750 ppm) minimum mean adult emergence was 0.22. These results vary, when compared to the alone treatments of DE and IGRs (Table 2). The combined treatments of lufenuron and DE not only further delayed the pupal and adult emergence of Khapra beetle but also revealed the importance of their combined use as compared to alone application. These results are supported by previous observations of Kostyukovsky et al. [38], where 0.01 ppm of pyriproxyfen (IGR) completely inhabited the F_1 adult emergence of both S and R strains of red flour beetle.

Table 3 Effect of different concentrations of diatomaceous earth, lufenuron, tebufenozide and combined application of lufenuron + diatomaceous earth on progeny production of Khapra beetle after 60 days.

Treatment	Concentration (ppm)	Progeny production
Diatomaceous earth	250	181.00 ± 0.57b
	500	171.00 ± 0.57 c
	750	161.33 ± 0.88 d
	control	292.00 ± 1.00 a
Lufenuron	0.25	99.00 ± 0.58 b
	0.50	90.00 ± 0.57 c
	0.75	71.33 ± 0.88 d
	control	181.00 ± 0.59 a
Tebufenozide	0.25	131.00 ± 0.57b
	0.50	110.00 ± 0.56c
	0.75	100.66 ± 0.88 d
	control	293.66 ± 2.02 a
Lufenuron + diatomaceous earth	0.25 + 250	90.00 ± 0.58 b
	0.50 + 500	71.00 ± 0.55 c
	0.75 + 750	64.00 ± 0.57 d
	control	298.00 ± 2.07 a

Value ± standard deviation; Means sharing similar letters are not significantly different at $P<0.05$.

The data regarding progeny production was observed after 60 days. Progeny production of lufenuron and tebufenozide was 71.3 and 100.7 over control, respectively, while in case of DE, 161 over control and in combination (lufenuron + DE) 64 over control was recorded (Table 3). In another study, tebufenozide induced sterility in the male and female codling moths that produced less F1 progeny [37]. Bakr et al. [39] also stated that the lufenuron inhabited the adult emergence when the last instar nymph of desert locust was treated with lufenuron. While in another experiment when methoprene and DE were used in combination, they showed 100% suppression of the progeny [34]. The present study also revealed that a combination of lufenuron + DE has great potential against stored grain insect pests. Similar findings were reported by Awais et al. [40] in a laboratory bioassay with DE and insect growth regulators (lufenuron and tebufenozide) against *Tribolium castaneum*.

Conclusion

The present study revealed that though the application of diatomaceous earth and insect growth regulators (lufenuron and tebufenozide) alone was effective in reducing the deleterious effects of khapra beetle on stored grains, the combined application of both was most effective. So, it is suggested that the diatomaceous earth and insect growth regulators should be used in combination for the better management of stored grain insect pests.

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Conflict of Interest

The authors declare no conflict of interest.

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