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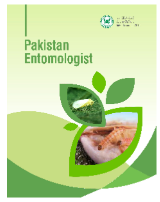

TOXIC AND GROWTH INHIBITORY EFFECT OF DIATOMACEOUS EARTH AND THIAMETHOXAM ALONE AND IN COMBINATION AGAINST *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT

Present study was conducted to investigate the effectiveness of thiamethoxam and three formulations of diatomaceous Earth (DE) (Concern®, Organics® and Food Grade®) against rust red flour beetle, *Tribolium castaneum* (Herbst). Three concentrations of thiamethoxam (0.25, 0.50 and 0.75 ppm) and DE (200, 400 and 600 ppm) were used. Best treatments of thiamethoxam and DEs were selected and their combined effects regarding mortality and progeny inhibition were evaluated. The experiment was performed on sterilized, crushed wheat grains at 30±2ºC and 65±5% R.H. The results showed that maximum mortality (90 %) was achieved in combination of Food Grade® with thiamethoxam and Concern® with thiamethoxam, while combination of organics® with thiamethoxam caused 82.22 % mortality after 21 days. In case of DE tested formulations, Food Grade® showed better results causing 67.77% mortality at the dose rate of 600 ppm after exposure period of 21 days followed by Concern® and Organics® which caused 59.98 % and 51.11 % mortality at dose rates of 600 and 400 ppm, respectively after 21 days exposure time. Similar trend of results were recorded for progeny production of *T. castaneum,* in combined treatments of D.E Food Grade® and D.E Concern® with thiamethoxam that was 0.00%. It can be concluded from these results that the use of diatomaceous Earth (DE) and thiamethoxam would be a better alternative for the protection of stored grains and their products.

Keywords: Diatomaceous earth, Mortality, Progeny inhibition, Thiamethoxam, *Tribolium castaneum*

INTRODUCTION

Stored-product insects are severe pests of stored-grains, durable agricultural commodities and of many value-added food products. Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is severe insect pest of stored grain products in the world (Haines, 1991). Both, adults and larvae are voracious feeder of stored commodities and cause quantitative and qualitative losses to crushed cereal products such as wheat flour, milled rice and peanuts (Rees, 2004). Control of this insect pest is achieved mostly with fumigants and synthetic pesticides (Boyer *et al*., 2012). But the selection of insecticides is limited due to strong needs imposed for the safe use of synthetic insecticides near or on the stored food commodities (Padin *et al*., 2002). Currently, neonicotinoid insecticides are fastest-growing group of insecticides in the market. These insecticides are target specific, neurotoxic to insect and inhibit the nicotinic acetylcholine receptor (nAChRs) of the target (Kim *et al*., 2003). This distinctive approach marks neonicotinoid extremely appropriate against insects which have developed resistance to traditional insecticides like organophosphates and pyrethroids (Maienfisch *et al*., 1999). Thiamethoxam is a contact insecticide belonging to the neonicotinoid. It has low toxicity towards mammals and beneficial insects. Thiamethoxam was assessed first time by Arthur *et al*. (2004) for its grain protection capacity on maize and wheat against *Tribolium castaneum*, *Sitophilus zeamais* and *Oryzaephilus*

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surinamensis. Diatomaceous earth (DE) is nearly pure shapeless silicon-dioxide, made-up of fossilised-diatoms of algae (Shah and Khan, 2014) which is responsible for insect death through dryness and disruption of the lipid layer in the cuticle of insect (Athanassiou and Steenberg, 2007). Recently, diatomaceous earth formulations prooved effective protectants against numerous stored-product pest species, involving *T. castanaeum* (Saez and Mora, 2007). The toxicity of DE is influenced by growth temperature (Athanassiou *et al*., 2011), DE source (Rojht *et al*., 2010), moisture content of grain (Mewis and Ulrichs 2001), nature of stored product (Ziaee *et al*., 2007) and stage of pest (Rigaux *et al*., 2001). Hence, it is of great importance to know the effect of prevailing conditions for effective usage of DEs. Resistance in insect pests of stored-grain products including *T. castaneum* to chemical pesticides and fumigants has also been observed in many countries (Opit *et al*., 2012). A high level of resistance to some pyrethroid (e.g. resmethrin and bioresmethrin) and organophosphates (e.g. Malathion) had been observed in *T. castaneum* (Arthur, 1992). Beside the resistance, consumer demands for pesticide residue-free food and health concerns emphasize on the need for assessing alternative control approaches that can be effective against *T. castaneum* (Arthur, 1996). One of the extreme favorable substitutes to contact pesticides is the use of diatomaceous earths (DEs). Many scientists have assessed the use of diatomaceous earth at low application rates with combination of other control techniques such as heat treatment (Vayias and Athanassiou, 2004).

Keeping in view the above facts, the current study was conducted to probe out the lethal and growth inhibitory potential of thiamethoxam and three formulations of diatomaceous earth, alone and in combination, against *T. castaneum.*

MATERIALS AND METHODS

Insect collection and rearing

Infested wheat flour was collected from flour mills, grain market and godowns located in Faisalabad and were sieved out to collect adults of *Tribolium castaneum*. Collected insects were kept in plastic jars inside incubator maintained at 30±2°C and 65±5% R.H. for rearing. Clean and sterilized crushed wheat grains were used as culture media for rearing *T. castaneum*. In each plastic jar, 100 adults of test insects were released on 250 g sterilized crushed wheat grains and covered with muslin cloth for aeration and to avoid the escapes of beetles. Adults were allowed to mate and lay eggs. After oviposition period of 3 days, beetles were sieved out from the flour. The flour, having the eggs, was again put into jars which were kept in incubators at optimum growth conditions (30±2°C and 65±5% R.H.). Homogenous population was achieved after a time period of 28-35 days as illustrated by Islam and Talukder (2005).

Mortality and growth-inhibitory bioassays of alone DE and thiamethoxam concentrations

In this bioassay, three formulations of diatomaceous Earth (DE) (Concern®, Organics® and Food Grade®) and a nicotinoid (thiamethoxam) were evaluated. Three concentrations of each DE formulation (0.25, 0.50 and 0.75 ppm) as well as of thiamethoxam (200 ppm, 400 ppm and 600 ppm) were applied on the sterilized, crushed wheat grains (50 g) and were allowed to equilibrate moisture content for an hour and then placed in the plastic jars. Thirty mixed sex adults of T. castaneum were released in the plastic jars containing treated commodity. The plastic jars were tightly covered with muslin cloth and were placed in incubator at 30±2^{o}C and 65±5% R.H. Each treatment was replicated three times using completely randomized design (CRD). Adult mortality was observed after exposure period of 2, 7, 14 and 21 days and for progeny development experiment, data was recorded after exposure periods of 35 and 75 days.

Mortality and growth-inhibitory bioassays of combined treatments of best thiamethoxam and DEs concentrations

Best concentration of thiamethoxam (0.75ppm) and of each DE formulation (600ppm) was applied in combinations on sterilized, crushed wheat of 50 g. For this purpose, initially commodity was treated with thiamethoxam and allowed for reasonable time period to equilibrate moisture. After that DEs formulations with their best concentration (600ppm) were applied to wheat treated with thiamethoxam. Control was maintained by treating the commodity with water only. 30 adults of *T. castaneum* were placed in the plastic jars containing treated commodity. Jars were tightly covered with muslin cloth and were placed in incubator at 30±2^{o}C and 65±5% R.H. Experiment was replicated three times using CRD. Adult mortality was recorded after exposure period of 2, 7, 14 and 21 days. Post treatment progeny development data were recorded after 35 and 70 days.

Statistical analysis

The mortality data were transformed into percent corrected mortality using Abbotts formula (Abbott, 1925). Analysis of variance (ANOVA) of the data regarding percent corrected mortality and progeny development were computed using statistica 8.1 software. Means of significant treatments were compared using Tuckey HSD test at 5% level of significant.

RESULTS

Thiamethoxam demonstrated the highest mortality (71.64%) at 0.75ppm followed by 53.29% at 0.5ppm and 38.28% at 0.25ppm after 21days. After 14 days of exposure, thiamethoxam showed 58.30, 39.96 and13.33% mortality at 0.75ppm, 0.5ppm and 0.25ppm, respectively. Thiamethoxam demonstrated lowest mortality at 0.25ppm (8.33%) and 0.5ppm (13.33%) after 2 days exposure interval. However, thiamethoxam induced a concentration and exposure-time dependent mortality in *T. castaneum* adults. An increasing mortality in *T. castaneum* adults was observed with an increase in exposure interval and concentration of thiamethoxam (Table 1).

The Table 2 showed that highest mortality (67.76%) was given by Food Grade® at 600 ppm concentration followed by D.E Concern® (59.98%) after 21 days of treatment application. The least mortality 3.33 and 5.55% was observed in case of Concern® and Food Grade® at 200 ppm, after an exposure time of 2 days. Food Grade® and D.E Concern® proved effective against the target insect pest of stored grains. The Table 3 showed that combined effect was very effective and mamximum mortality (100%) was achieved by combination of D.E Food Grade® (600ppm) + thiamethoxam (0.75ppm). The combinations of D.E Organics® (400) + thia.(0.75), D.E Concern® (600)+ thia.(0.75) and D.E Food Grade® (600) + thia.(0.75) proved comparatively least effective and gave 26.66 and 33.33% mortality after 21 days.

The data for thiamethoxam given in the Table 4 shows that maximum progeny 64.33% was obtained in control jar after 75 days, followed by 9.66% at 0.25ppm concentration, 5.66% at 0.50ppm concentration and 2.66% at 0.75ppm concentration. In case of D.E formulations the highest progeny 67.00% was obtained in controls jars of Food Grade®, followed Concern® 65.66% and Organic® 60.66% after 75 days of treatment application (Table 5).

Similarly after 35 days of DE formulations application (Table 5), the highest progeny was10.33, 9.00 and 6.66% at 200ppm as a result of D.E Concern®, Food Grade® and Organic®, respectively. The progeny production was lowest 3.66, 4.33 and 7.66% due to the application of D.E Concern®, Food Grade® and Organic®, respectively at 600ppm concentration of DE formulations. At 400ppm concentrations the progeny production values were 5.66, 6.33 and 5.66% due to the application of D.E Organic®, Food Grade® and Concern®, respectively.

The data given in the Table 6 showed that highest progeny emergency 63.00% and 25.33% was obtained after 35 and 75 days of application in controls jar, respectively. The least progeny emergence (5.66%) was observed in D.E Organics® (400) + thia.(0.75), followed by D.E Organics® (400) + Thia. (0.75) (2.33 %) after 35 days of application of treatment combinations. The Concern® (600)+ thia.(0.75) 0.00 and D.E Food Grade® (600)+thia.(0.75) 0.00 were statistically at par at mean value (0.00%).

In the present experiment three formulations of Diatomaceous earth (Concern®, Organics® and Food Grade®) and a neonicotinoid insecticide, thiamethoxam were evaluated for their effect on the mortality and progeny inhibition of *Tribolium castaneum*. The combination of DE Concern® (600ppm) + thiamethoxam (0.75ppm) and DE Food Grade® (600ppm) + Thiamethoxam (0.75ppm) have been resulted into 100% mortality after an exposure period of 21 days. These results are similar to those of Wakil *et al.* (2013) who reported that combined effect of thiamethoxam and DE SilicoSec resulted in higher mortality in *Rhyzopertha*

Table 1

Percent mortality of *Tribolium castaneum* caused by thiamethoxam at different concentrations and exposure intervals.

Concentrations (ppm)	Percent mortality ± SE			
	2 days	7 days	14 days	21 days
0.25	8.33 ± 1.66 b	16.65 ± 2.41 b	26.61 ± 2.26 a	38.28 ± 1.27 a
0.5	13.33 ± 1.66 ab	23.32 ± 2.41ab	39.96 ± 2.64 a	53.29 ± 2.65 a
0.75	20.00 ± 2.88 a	36.65 ± 2.41 a	58.30 ± 2.82 a	71.64 ± 2.67 a

Table 2

Percent mortality of *Tribolium castaneum* caused by different formulations of diatomaceous earths at different concentrations and exposure intervals.

Exposure intervals (days)	Concentrations (ppm)	Percent mortality ± SE		
		Organics®	Concern®	Food Grade®
2	200	6.66 ± 1.92 a	3.33 ± 1.92 a	5.55 ± 2.93 a
	400	8.88 ± 1.11 a	6.66 ± 1.92 a	8.88 ± 1.11 a
	600	7.77 ± 1.11 a	12.22 ± 2.93 a	13.33 ± 3.84 a
7	200	25.55 ± 2.93 a	14.42 ± 2.94 b	16.66 ± 1.92 a
	400	27.77 ± 2.93 a	24.42 ± 2.94 ab	27.77 ± 2.93 b
	600	26.66 ± 1.92 a	34.43 ± 1.44 a	36.66 ± 1.92 b
14	200	37.76 ± 2.00 a	29.98 ± 2.09 b	32.20 ± 2.00 a
	400	39.98 ± 2.33 a	41.09 ± 2.94 ab	46.65 ± 1.92 b
	600	38.87 ± 2.18 a	48.87 ± 1.94 a	52.21 ± 2.94 b
21	200	51.08 ± 2.28 a	43.31 ± 2.09 a	45.53 ± 2.88 a
	400	52.20 ± 2.22 a	52.20 ± 2.84 a	57.75 ± 2.94 a
	600	51.08 ± 2.94 a	59.98 ± 2.82 a	67.76 ± 1.78 a

Table 3

Combined effects of the best concentration of each formulation of diatomaceous earths and thiamethoxam against the mortality of *Tribolium castaneum* at different exposure intervals.

Treatments (ppm)	Percent mortality ± SE			
	2 days	7 days	14 days	21 days
DE Organics® (400) + Thiamethoxam (0.75)	26.7±1.9e	45.5±2.9a	66.6±2.8b	82.2±2.84a
DE Concern® (600)+ Thiamethoxam (0.75)	33.3±2.3de	54.4±2.0a	81.1±2.9ab	100.0± 0.0b
DE Food Grade® (600)+ Thiamethoxam (0.75)	36.7±2.1cde	60.0±2.9a	89.9±2.3a	100.0±0.0b

Table 4

Percent progeny emergence of *Tribolium castaneum* at different concentrations of thiamethoxam after 35 and 75 days exposure intervals.

Concentrations (ppm)	Progeny Emergence (%) ± SE	
	35	75
0.25	9.66 ± 1.20 c	18.33 ± 1.45 c
0.5	5.66 ± 0.88 bc	18.33 ± 2.40 c
0.75	2.66 ± 0.33 b	6.33 ± 0.88 b
Control	24.66 ± 1.20 a	64.33 ± 1.45 a

Table 5

Percent progeny emergence of *Tribolium castaneum* at different formulations of diatomaceous earths after 35 and 75 days exposure intervals.

Time (Days)	Concentrations (ppm)	Progeny Emergence (%) ± SE		
		Organics®	Concern®	Food Grade®
35	200	6.66 ± 0.88 b	10.33 ± 1.15 c	9.00 ± 1.15 b
	400	5.66 ± 1.45 b	6.33 ± 0.33 bc	5.66 ± 0.88 b
	600	7.66 ± 0.88 b	4.33 ± 0.33 b	3.66 ± 0.66 b
	Control	26.00 ± 1.05 a	25.00 ± 1.15 a	24.33 ± 2.40 a
75	0.25	25.33 ± 1.45 c	23.66 ± 1.85 c	21.66 ± 1.76 c
	0.5	14.00 ± 1.73 b	15.33 ± 1.45 c	13.33 ± 0.88 c
	0.75	22.33 ± 1.33 c	10.66 ± 1.45 b	8.66 ± 1.20 b
	Control	60.66 ± 1.76 a	65.66 ± 1.33 a	67.00 ± 1.15 a

Table 6

Percent progeny emergence of *Tribolium castaneum* at different combinations of thiamethoxam and DE formulation after 35 and 75 days exposure intervals.

Treatments (ppm)	Progeny Emergence (%) ± SE	
	35 days	75 days
DE Organics® (400) + Thiamethoxam (0.75)	2.33 ± 0.88 b	5.66 ± 1.20 b
DE Concern® (600)+ Thiamethoxam (0.75)	0.00 ± 0 b	0.00 ± 0 c
DE Food Grade® (600)+ Thiamethoxam (0.75)	0.00 ± 0 b	0.00 ± 0 c
Control	25.33 ± 1.02 a	63.00 ± 1.73 a

dominica on wheat, maize and rice. After 14 d exposure the greater mortality was observed with combination of DE and low doses of thiamethoxam as compared to thiamethoxam alone. Similar results were documented by Korunic and Rozman, (2010). Among alone application of tested insecticides, Thiamethoxam caused mortality more than 70% which is supported by the findings of Arthur *et al.* (2004). Their study reveals that that thiamethoxam is very efficient against *T. castaneum*, *R. dominica, S. zeamais,* and *Sitophilus oryzae*. The mortality of *R. dominica* and *S. oryzae* was below than 60% at the exposure period of 1 and 2 days on treated wheat, but after exposure period of 6 days it gave 100% mortality. However, in that experiment they used 1 to 4 ppm dose rate of thiamethoxam which are significantly higher than the application rates of 0.25 to 0.75 ppm. This dissimilarity in the dose rates may be responsible for higher rate of mortality at short exposure period. The tested formulations of diatomaceous earth proved significantly effective against *T. castaneum* adults. In the tested formulations, Food Grade® demonstrated better results causing more than 65% mortality after exposure period of 21 days and at the dose rate of 600 ppm followed by Concern® which is responsible for about 60% mortality of tested beetle. However the DE formulation Organics® showed lower level of mortality (52%) among all tested formulations after exposure of 21 days and at the dose rate of 400 ppm. These results are consistent with the findings of Shayesteh and Ziaee, (2007). They evaluated the toxicity of diatomaceous earth formulation SilicoSec® against adults and larvae of *Tribolium castaneum* on wheat. Their results revealed that mortality influenced by dose rates and exposure period. Similarly, Mohale *et al.* (2010) applied diatomaceous earth against *T. castaneum* and reported higher mortality at higher dose rates. These results are also in agreement with our findings. In progeny reduction, thiamethoxam in combination with DE formulations greatly inhibited the adult progeny emergence of *T. castaneum.* In some treatments total inhibition of progeny production of *T. castaneum* on treated wheat was probably due to higher mortality of adult. These results are also related with the results illustrated by Wakil *et al.* (2013) who documented total progeny inhibition in combination of thiamethoxam with diatomaceous earth at higher dose rates. The similar types of results were obtained by Chintzoglou *et al.* (2008) in combination of Diatomaceous earth and spinosad. From this experiment it is concluded that thiamethoxam in combination with DE can efficiently be used in integrated pest management strategies of stored grain pests.

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