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Entomocidal effect of diatomaceous earth and thiamethoxam alone and in combination against *Tribolium castaneum*

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ABSTRACT

Current investigation was carried out to study the efficacy of thiamethoxam and three formulations (Concern®, Organics® and Food grade®) of Diatomaceous Earth (DE) against *Tribolium castaneum*. Thiamethoxam was applied at 0.25, 0.50 and 0.75 ppm while DE was applied at 200, 400 and 600 ppm concentrations. The experiment was performed on sterilized, crushed wheat grains at 30 ± 2 ºC and 65 ± 5 relative humidity (r.h.) under completely randomized design (CRD) with three replicates for each treatment. The results showed that maximum percent mean mortality (100%) was achieved with combination of DE Food grade® + thiamethoxam and DE Concern® + thiamethoxam after exposure of 21 days while combination of DE Organics® + thiamethoxam caused (82.22%) mean mortality after 21 days. According to results of bioassay in which DE formulations and thiamethoxam were applied alone, higher percent mean mortality (71.66%) was recorded in thiamethoxam @ 0.75 ppm after 21 days. For DE tested formulations, Food grade ® showed better results and caused percent mean mortality (66.77%) @ 600 ppm after exposure period of 21 days. The efficacy was lower for DE Concern ® and Organics ®, which caused 59.98 and 51.11% mean mortality at concentrations of 600 ppm and 400 ppm, respectively, after 21 days exposure time. Efficacy of Diatomaceous Earth can be enhanced with combinations of Diatomaceous Earth (DE) and thiamethoxamat lower rates, potentially improving the management of *Tribolium castaneum*.

Key words: Concentrations, Diatomaceous earth, Exposure time, Mortality, Thiamethoxam, *Tribolium castaneum*

Stored-product insects are serious pests of dried, stored, durable agricultural commodities and of many value-added food products. Red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a 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 of 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). However, the selection of insecticides is limited due to regulatory requirements imposed for the safe use of synthetic insecticides near or on the stored food commodities (Padin et al., 2002).

Resistance of stored-product insect pests,including *T. castaneum,* to chemical pesticides and fumigants is also observed in many countries (Opit et al., 2012). Abundant resistance to some pyrethroid (e.g. resmethrin and bioresmethrin) and organophosphates (e.g. Malathion) was 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 potentially favourable substitute to contact pesticides is the use of Neonicotinoid insecticides and DEs. Neonicotinoid insecticides are popular contemporary group of insecticides. Neonicotinoid insecticides are target-specific and act on insect nervous system by inhibiting the nicotinic acetylcholine receptor (nAChRs) (Kim et al., 2003;

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Tomizawa, 2004). These attributes make neonicotinoids attractive alternatives to traditional organophosphate, carbamate and pyrethroid insecticides to which insects are developing resistance (Maienfisch et al., 1999). Thiamethoxam is a broad spectrum (i.e. effective against large number of insects) and contact insecticide belonging to the neonicotinoid and is used as a seed treatment (Hofer and Brandl, 1999). 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* (red flour beetle), *Sitophilus zeamais* (maize weevil) and *Oryzaephilus surinamensis* (sawtoothed grain beetle).

DE is nearly pure silicon-dioxide, made-up of fossilized-diatoms of algae (Shah and Khan, 2014) which causes insect death through dryness and disruption of the lipid layer in the cuticle of insect. Latest diatomaceous earth formulations were found effective protectants against numerous stored-product pest species, involving *T. castanaeum* (Athanassiou and Steenberg, 2007). Objective of the study was to evaluate the efficacy of various combinations of DE and thiamethoxam against red flour beetle, *Tribolium castaneum.*

MATERIALS AND METHODS

The current study was steered in Grain Research, Training and Storage Management Cell of the Department of Entomology, University of Agriculture, Faisalabad, during the year 2013-14.

Collection and rearing of insects: 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 under optimum conditions inside incubator at 30° ± 2°C and 65° ± 5% r.h. Wheat flour was used as culture media for rearing *T. castaneum*. In each plastic jar, 100 adults of test insects were released on 250 g sterilized flour and covered with muslin cloth. 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. Homogenous population was achieved after a 28-35 days (Islam and Talukder, 2005).

Actara formulation (wettable granule) of thiamethoxam was used, which contains 250 g/kg of active ingredient. Various formulations of DE Concern® (85% SiO_2, 10% other elements oxides and 5% moisture), Organics® (85% SiO_2, 10% other elements oxides and 5% moisture) and Food Grade® (100% pure diatomaceous earth) were used. Infestation free crushed wheat (*Triticum aestivum)* was used as test stored commodity.

Bioassay for percent mortality through thiamethoxam and Diatomaceous Earth against Tribolium castaneum*:* Three concentrations (0.25, 0.50 and 0.75 ppm) of Diatomaceous Earth (DE) and thiamethoxam (200 ppm, 400 ppm and 600 ppm) were applied on the sterilized, crushed wheat of 50 g and were allowed to equilibrate moisture content for a reasonable time period and then placed in the plastic jars. Thirty 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°C and 65 ± 5% r.h. Each treatment was replicated three times using completely randomized design (CRD). Adult mortality was recorded after exposure period of 2, 7, 14 and 21 days.

Bioassay for percent mortality of Tribolium castaneum *through combinations of thiamethoxam and diatomaceous Earths:* Experiments were conducted to evaluate the the optimal concentration of thiamethoxam and each DE formulation applied in combination on sterilized, crushed wheat of 50 g. Initially commodity was treated with thiamethoxam and allowed for reasonable time period to equilibrate moisture. After that, DE formulations were applied to wheat treated with thiamethoxam. Control was maintained by treating the commodity with water only. Thirty 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°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.

Statistical analysis

There corded data were analyzed using statistical software and the corrected mortality was computed following measured Abbotts formula (Abbott, 1925). Analysis of variance (ANOVA) of the data was computed using statistica 8.1 software. Means of significant treatments were separated using Tuckey HSD test at $\alpha = 5\%$.

RESULTS AND DISCUSSION

The data (Table 1) showed that maximum mortality was obtained after 21 days at concentration 0.75 ppm followed by 0.5 ppm and 0.25 ppm. Data after 14 days showed that maximum per cent mean mortality was 58.30% followed by 0.5 ppm (39.96%) and 0.25 ppm (26.61 %). Least percent mean mortality (8.33%)

Table 1 Percent mean mortality of *Tribolium castaneum* using thiamethoxam at different concentrations and time exposures

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

mortality was observed at 0.25 and 0.5 (13.33%) after 2 days exposure time. Time and concentration relationship was found synergistic.

Maximum mortality (59.98%) was given by Food Grade® and DE Concern® (Table 2) and were statistically at par at concentration 600 ppm after 21 days of treatment application, followed by 52.20% at 400 ppm. The least mortalities 6.66 % and 7.77% were observed in DEOrganics®and Concern® at 200 ppm and 400 ppm, after exposure of 2 days. Food Grade® and DE Concern® proved effective against the target insect pest of stored grains.

Combined effect (Table 3) was very effective and maximum mortality (100%) was achieved by combination of DE Food Grade® (600 ppm) + Thiamethoxam (0.75 ppm). The combinations of DE Organics® (400) + thia.(0.75), DE Concern® (600)+ thia.(0.75) and DE Food Grade®(600) + thia.(0.75) proved comparatively least effective and gave 26.66% and 33.33% after 2 days.

In the present experiment three formulations of DE (Concern®, Organics® and Food Grade®) and a new inorganic insecticide thiamethoxam against *T.castaneum* adults to check the effect on mortality. The results showed that combination of DE and thiamethoxam were more effective against this stored grains pest .The maximum mean mortality in combination of these tested insecticides was 100% after an exposure period of 21 days. These results are similar to Wakil et al. (2013). They observed the combined effect of thiamethoxam and diatomaceous earth (DE), SilicoSec on wheat, maize and rice against of *Rhyzoperthadominica*. After 14 d exposure the greater mortality was observed with combination of DE and low doses of thiamethoxam as compared to thiamethoxam alone. The DE showed synergism effect with other integrated pests management techniques as reported by (Korunic and Rozman, 2010). They investigated the combined efficiency of deltamethrin and diatomaceous earth against *R.dominica, T. castaneum* and *S.zeamais.* Results showed higher synergism effect between deltamethrin and DE.

Among alone application of tested insecticides, thiamethoxam caused mortality more than 70%, which also reported by Arthur et al. (2004). Their study reveal that that thiamethoxam is very efficient against *T. castaneum*, *R. dominica, S. zeamais,* and *S.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

Table 2 Percent mean mortality of *Tribolium castaneum* using Diatmaceous Earths at different concentrations and time exposures

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

Table 3 Combined effects of diatomaceous earths and thiamethoxamn *Tribolium castaneum* using different concentrations and time exposures

Time (Days)	Concentrations (ppm)	Mortality(%)± SE
2	D.E Organics® (400) + Thia. (0.75)	26.66 ± 1.92e
	D.E Concern® (600)+ Thia. (0.75)	33.33 ± 2.33de
	D.E Food Grade® (600)+ Thia. (0.75)	36.66 ± 2.10cde
7	D.E Organics® (400) + Thia. (0.75)	45.55 ±2.93a
	D.E Concern® (600) + Thia. (0.75)	54.44 ± 2.00a
	D.E Food Grade® (600) + Thia. (0.75)	60.00 ± 2.93a
14	D.E Organics® (400) + Thia. (0.75)	66.63 ± 2.84b
	D.E Concern® (600) + Thia. (0.75)	81.08 ± 2.93ab
	D.E Food Grade® (600) + Thia. (0.75)	89.97 ± 2.33a
21	D.E Organics® (400) + Thia. (0.75)	82.21 ± 2.84a
	D.E Concern® (600) + Thia. (0.75)	100.00 ± 0.00b
	D.E Food Grade® (600) + Thia. (0.75)	100.00 ± 0.00b

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 DE proved significant results against *T. castaneum* adults. In these formulations Food Grade proved better results, which cause mean mortality more than 65% after exposure period of 21 days @ 600 ppm followed by DE Concern, which is responsible for about 60% mean mortality of tested beetle. However the DE formulation Organics showed lower level of mean mortality 52% among all tested formulations after exposure of 21 days @ 400 ppm. It was observed that effectiveness of DE increased with increase in dose rates and exposure period. These results matched with the findings of Shayesteh and Ziaee (2007). They evaluated the toxicity of diatomaceous earth formulation SilicoSec® against adults and larvae of *T. castaneum* on wheat. 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.

CONCLUSION

From this experiment it is concluded that these insecticides can efficiently be used in integrated pest management strategies of stored grain pests. Diatomaceous earth is a natural compound having more than 80% of SiO_2 which is safer for human health and environmental friendly. Similarly, thiamethoxam is very less persistent in the environment and have very low mammalian toxicity. These insecticides have great synergetic effect on *T. castaneum* in prevailing population and also on the progeny.

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