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Effect of Grain Type on the Insecticidal Efficacy of Paya® against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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The objective of the current study was to determine the insecticidal effectiveness of diatomaceous earth formulation (DE) Paya®. The effects of grain commodity (wheat, barley, maize and rice), exposure times and dose rates were evaluated. The efficacy of diatomaceous earth (DE) against 1–7 days old adults of *Tribolium castaneum* (Herbst) was evaluated on wheat, rice, maize and barley at 26 ± 1 °C and 60 ± 5% RH in darkness. The mortality of adults was tested at five concentrations: 0.125, 0.25, 0.5, 1 and 1.5 g/kg of grain and different exposure times (24, 48 h, 7 and 14 d). To evaluate the progeny production, dead and alive adults were removed from containers after 14 days and these containers were kept at above-mentioned conditions for 45 days. The results obtained indicated that the highest adult mortality was observed after the longest exposure period on barley and wheat so that the two highest DE dosages caused 100% mortality of *T. castaneum*. The efficacy of Paya® on maize and rice was not satisfactory, given that after a 14 days exposure at the highest dose rate the mortality did not exceed 37% and 66% in the case of maize and rice, respectively. The results demonstrated that the mortality increased with rises in concentration and exposure time and also highly affected by grain type.

Keywords: Red flour beetles, Diatomaceous earth, wheat, barley, rice, maize.

Synthetic insecticides have been used since the 1950s to control stored-products insects (Subramanyam and Hagstrum, 1995). However, the use of these compounds meets with several drawbacks such as (a) they are generally toxic to mammals, (b) they leave residues in the product and (c) many species are resistant to some residual protectants (Arthur, 1996). Some researchers decided to evaluate several new alternative protection methods for stored products (Athanassiou et al., 2005a). As an alternative to chemical control, Diatomaceous Earth (DE) has been increasingly used over the last decade and is recognized as an essential component of Integrated Pest Management (IPM) in stored-products (Korunic, 1999). DE is a light weight, porous sedimentary rock made up of the diatoms (Bacillariophyta), unicellular aquatic plants (Round et al., 1990). The DEs mined currently vary remarkably in their insecticidal activity, depending upon the geological and geographical origin as well as certain characteristics, such as SiO_2 content, pH, tapped density and adherence to kernels (Korunic, 1997). Environmental conditions,

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primarily temperature and air and medium humidity or moisture content, have significant impact on the effectiveness of DE against stored-product insects (Fields and Korunic, 2000; Arthur, 2001, 2002; Athanassiou et al., 2005b; Vardeman et al., 2006).

DE have some incontestable advantages over the residual insecticides, given that (a) they are non-toxic to mammals, (b) they can be easily removed from the product during processing, (c) are very effective for a wide range of species and (d) given that only a physical method is involved (Golob, 1997; Korunic, 1998; Subramanyam and Roesli, 2000; FDA, 1995). Nevertheless, there are two major disadvantages in the use of DE. First, DEs negatively affect the bulk density, which may have a certain impact on the commercial value of the treated grains (Korunic, 1998). Second, DEs should be applied at high dose rates, which often exceed 1000 ppm (Athanassiou et al., 2003, 2005b; Kavallieratos et al., 2005). While most grain insecticides is usually effective at application rates < 10 ppm (Arthur, 1996).

DEs act on the insects' exoskeleton (cuticle) causing rapid desiccation resulting in death through water loss as long as DE particles are trapped and adhered to insects' body (Ebeling, 1971). The red flour beetle, *Tribolium castaneum* (Herbst) is a major pest of stored grains and grain products throughout the world (Sinha and Watters, 1985). *Tribolium castaneum* is one of the least susceptible stored-product pests to DE, so a DE formulation able to control flour beetles should be able to control most insects occurring in stored food (Korunic, 1998; Fields and Korunic, 2000; Arnaud et al., 2005). In the present study, the efficacy of Paya (Iran DE) was assessed against *Tribolium castaneum* on four different types of grain at five dose rate under laboratory conditions of 26 ± 2 ºC, 65 ± 5% RH. Furthermore, the progeny production of the above species in substrates treated with the DEs was also evaluated.

Materials and Methods

Insect cultures

T. castaneum was reared in plastic containers (20 cm length × 14 cm width × 8 cm height) containing wheat flour mixed with brewer's yeast (10:1, w/w) which were covered by a fine mesh cloth for ventilation. The cultures were maintained in darkness in a growth chamber set at 26 ± 1 °C and 60 ± 5% RH. All adults used in the experiment were 1–7 days old of mixed sex.

DE formulations

Paya® (kimia-sabzavar, Iran) is a white-coloured DE containing approximately 81.5% SiO_2, 5.9% Al_2O_3, 3.1% Fe_2O_3, 1.1% CaO, 0.6% MgO, and 2.5% other oxides (e.g. TiO_3, P_2O_3), and 3–5% moisture content (m.c.). The median particle size is 50 μm.

Bioassay

For each grain, five concentrations were tested: 0.125, 0.25, 0.5, 1 and 1.5 g/kg of grain. For every concentration (grain-dose combination) four replications containing 60 g of treated grain were taken. The samples were treated individually with the required DE quantity to achieve the aforementioned dose rates, as recommended by Subramanyam and Roesli (2000). Four additional samples of untreated grain, for each grain-dose combination, were used as control. Each sample was placed in a small glass vial, which was closed, and borne a hole (1.5 cm in diameter) covered with organtin to allow sufficient aeration. Subsequently, fifty, 1–7 days old unsexed adults were introduced into each vial. Experiments were carried out in the dark at 26 °C and 65 ± 5% RH. Adults were sieved off the grain after 24, 48 h, 7 and 14 days and the numbers of live and dead insects were counted. After the 14 days count, all adults (dead and alive) were removed from the vials. The vials were left in the incubator for 45 d more. After this period, the emerged *T. castaneum* adults were counted, classified as dead or alive and removed from the vials.

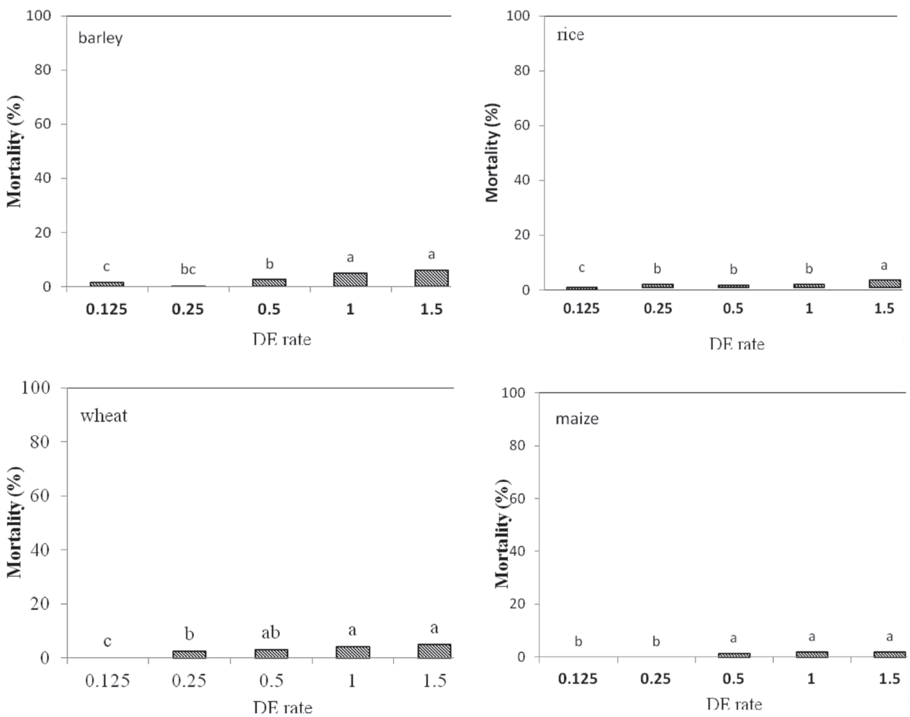

Fig. 1. Mortality (%) (mean ± SE) of *T. castaneum* adults on each grain type treated with five different dose rates of Paya®, after 24 h of exposure (means within the same grain type followed by the same letter are not significantly different; Tukey–Kramer HSD test, at P = 0:05)

Data analysis

Mortality was calculated by pooling the number of dead and alive beetles across every replication, and the proportion of dead insects was calculated. The mortality observed in the treatment was corrected with the mortality in the control (Abbott, 1925). The data were analyzed by using the GLM Procedure of SAS (SAS Institute, 1995), with red flour beetle mortality as the response variable and exposure interval, grain type and dose rate as the main effects. Means were separated by using the Tukey–Kramer (HSD) test, at P = 0.05 (Sokal and Rohlf, 1995).

Results

The effect of the exposure interval on adult mortality of *T. castaneum* was significant at the $P < 0.01$ level. All main effects and associated interactions for mortality levels of *T. castaneum* were significant either between or within exposure intervals at P = 0.01. All main effects and associated interactions for mortality levels of *T. castaneum*

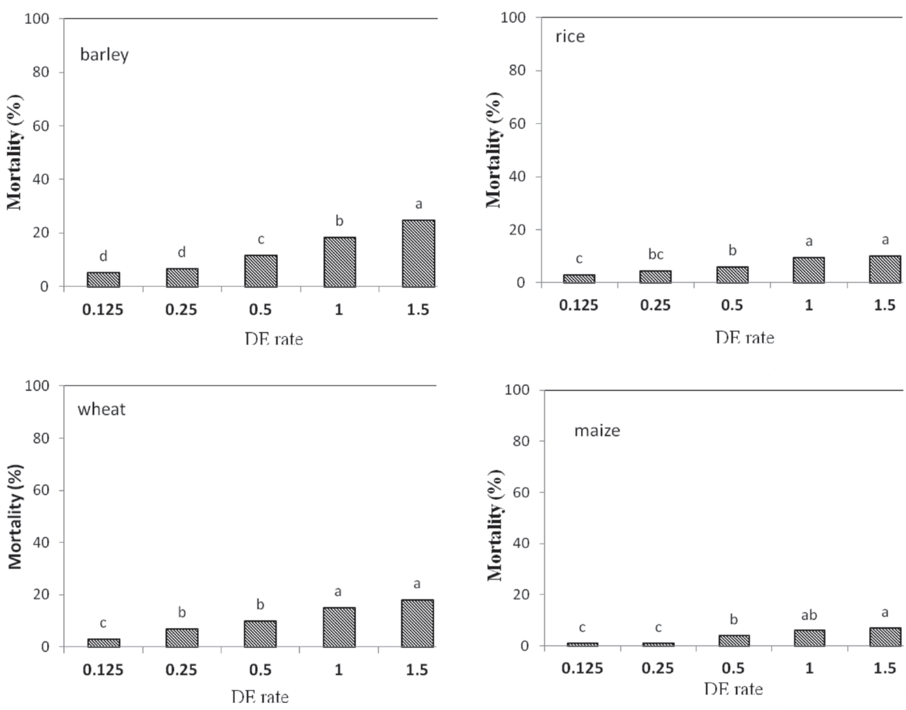

Fig. 2. Mortality (%) (mean ± SE) of *T. castaneum* adults on each grain type treated with five different dose rates of Paya®, after 48 h of exposure (means within the same grain type followed by the same letter are not significantly different; Tukey–Kramer HSD test, at P = 0:05)

were significant within exposure intervals with the exception of exposure × dose and exposure × dose × commodity, which were not significant, all the remainder interactions were significant at P = 0.01. As expected, mortality was observed to increase with the DE concentration. In most of the concentration tested, more adults were dead in treated barley than in treated wheat, maize or rice and the increase of DE rate increased mortality.

We knew from previous works that DE concentration affects mortality and that DEs differ in their efficacies (Fields and Korunic, 2000). It would be interesting to know if one population, which is not satisfactorily controlled with one DE formulation, could be better controlled with another DE.

The results presented in Fig. 1 show the mortality percentages were low 24 h after the introduction of *T. castaneum* adult and did not exceed 6% for *T. castaneum* treated with Paya®. In general, numerically, the highest adult mortality was obtained at the rates 1 and 1.5 g, that the mortality was 5%, 6%, 2% and 3/5% in the case of treated wheat, barley, maize and rice, respectively. However, the mortality went up when the exposure time was increased to 48 h after this period, the highest mortality levels were recorded on barley (24.8%) and wheat (18.1%). In these two grain types treated with the 1 g dose rate the mortality was 18/27% and 15%, respectively. The mortality of rice was 10.04% and the Figure was 7% for maize even at the highest dose rate (Fig. 2).

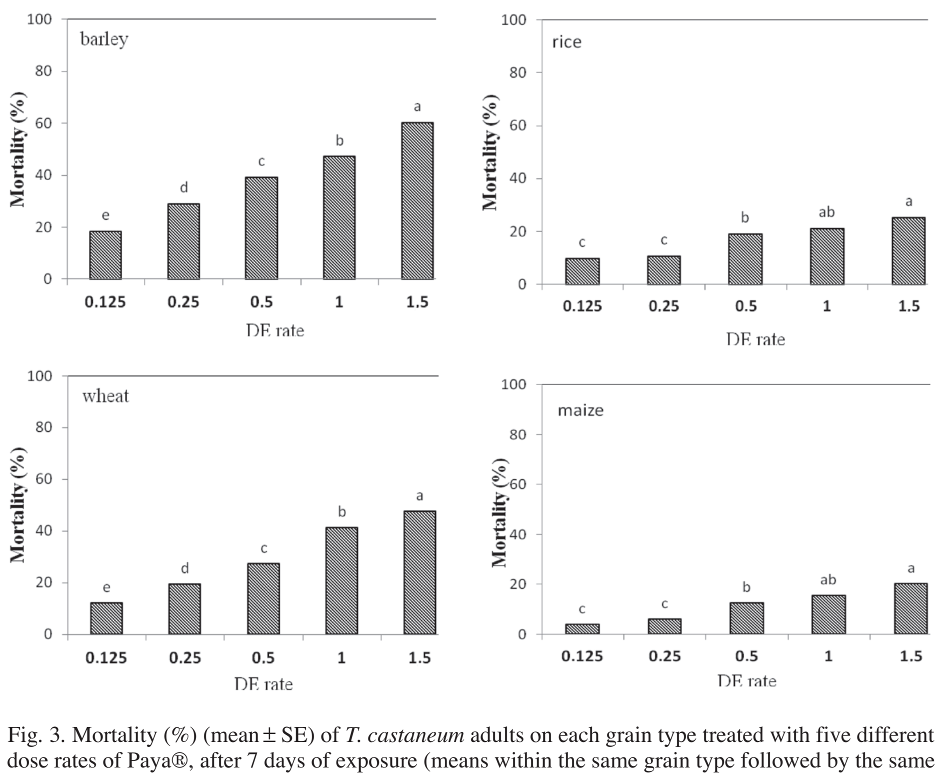

Fig. 3. Mortality (%) (mean ± SE) of *T. castaneum* adults on each grain type treated with five different dose rates of Paya®, after 7 days of exposure (means within the same grain type followed by the same letter are not significantly different; Tukey–Kramer HSD test, at P = 0:05)

Paya® significantly reduced the survival of adult *T. castaneum* after 7 days exposure on barley and wheat. The adult mortality was 60.2%, 47.4%, 21.15% and 20.3% in the case of treated barley, wheat, rice and maize, respectively (Fig. 3). All adults died by day 14 at Paya® dosages of 1 and 1.5 g/kg of cereals on barley and wheat, while at rate 0.5 g the mortality of treated barley was 68.96% and the Figure was 47.03% for wheat. In contrast, the highest mortality on rice and maize was 66.82% and 37.38%, respectively (Fig. 4).

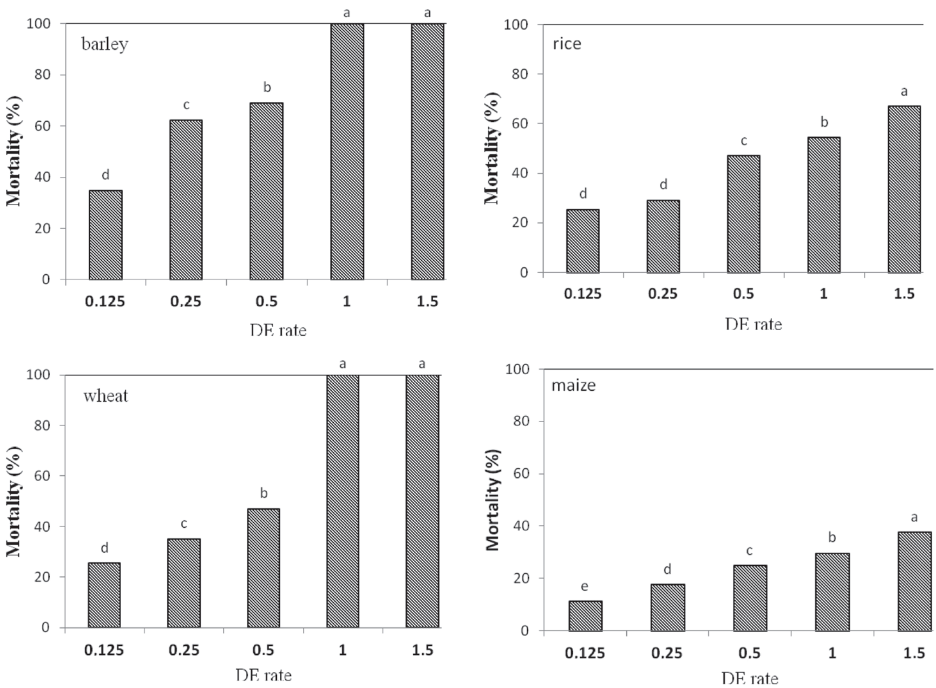

Fig. 4. Mortality (%) (mean ± SE) of *T. castaneum* adults on each grain type treated with five different dose rates of Paya®, after 14 days of exposure (means within the same grain type followed by the same letter are not significantly different; Tukey–Kramer HSD test, at P = 0:05)

The data on progeny production of *T. castaneum*, presented in Table 1, show the progeny production of *T. castaneum* on grain treated with DE after 14 days of parental exposure. Forty five days after remove adult calculated number of adults emerged. The highest rate of the Paya® formulation inhibited progeny production almost as effectively as other dose rate. Considerable variation among different grains was also noted, the progeny production was significantly higher on maize, compared to other grains and lowest progeny production related to barley.

Discussion

The results of the current experiment indicate that DE concentration, insect species (external or internal feeder), developmental stage and exposure time to the treated commodity influenced the efficacy of tested DE. Stored-product insects show a wide range of susceptibility to DE (Aldryhim, 1990; 1993). In general, the most sensitive species are in the genus *Cryptolestes*, while *Sitophilus* spp. are less susceptible, followed by *Oryzaephilus, Rhizopertha*, and *Tribolium* spp. which appear to be the most resistant (Korunic and Fields, 1995; Fields and Muir, 1995). Among the stored-products pests, *Tribolium* flour beetles are one of the most resistant species to DE (Korunic, 1994, 1998; Fields and Muir, 1995). The control of the red flour beetle with DE may require the use of higher doses than those normally recommended. Our results show that good control of red flour beetle in stored grain is not currently possible with the use of low DE dose rates.

Important factor which affect the *T. castaneum* mortality is also the exposure interval. Mortality was confirmed to increase with the duration of exposure to DE-treated grain (Fields et al., 2003; Athanassiou et al., 2005b). DE has physical action, since the DE particles grasp insect cuticle mean while red flour beetle are moving on treated grain (Athanassiou et al., 2005a). Further exposure means active contact with DE particle,

Table 1

Progeny production (mean number of adults ± SE) and percentage (% ± SE) of dead progenies on each grain type treated with Paya®, 45 d after the removal of the exposed *T. castaneum* adults

DE rate (g/kg of grain)	Grain type	No. progeny a	% dead adults b
0.125 g ($F_{3,12}$=9.78; P = 0.0015)	Barley	8.5 ± 0.86b	44.11 ± 10.04
	Wheat	13.75 ± 1.49b	34.54 ± 6.03
	Rice	11.25 ± 1.54b	28.88 ± 9.46
	Maize	21.25 ± 2.62a	10.58 ± 2.56
0.25 g ($F_{3,12}$ = 20; P = 0.0001)	Barley	6.5 ± 10.04c	57.69 ± 10.48
	Wheat	12.25 ± 1.2b	32.60 ± 6.69
	Rice	9.5 ± 0.86bc	42.1 ± 13.05
	Maize	18.75 ± 1.43a	18.66 ± 4.49
0.5 g ($F_{3,12}$ = 13.7; P = 0.0004)	Barley	4.75 ± 1.03c	73.68 ± 18.97
	Wheat	9.5 ± 0.86b	47.38 ± 14.39
	Rice	7 ± 0.91bc	60.71 ± 14.93
	Maize	13.25 ± 1.1a	30.18 ± 8.48
1 g ($F_{3,12}$ = 11.99; P = 0.0006)	Barley	3 ± 0.91b	75 ± 23.62
	Wheat	4.5 ± 0.64a	61.11 ± 15.11
	Rice	4.25 ± 0.47b	58.82 ± 11.9
	Maize	8.5 ± 0.64b	47.05 ± 5.11
1.5 ($F_{3,12}$ = 3.18; P = 0.0631)	Barley	3 ± 0.7b	91.66 ± 5
	Wheat	4.25 ± 0.62ab	88.23 ± 6.25
	Rice	3 ± 0.91b	66.66 ± 67.12
	Maize	6.25 ± 1.1a	44 ± 22.78

which damages weevil wax layer and insect dies through desiccation (Korunic, 1998). So that 7 days of exposure treated barley at the highest dose rate only 60.2% of adult red flour beetle died, while after 14 days the survival was nil.

The efficacy of DE is determined by the type of storage product the dust is applied to. Our results indicated that the same dose rate does not provide the same level of protection when applied on different grains. It is known that the physical and chemical properties of the grain determine the amount of dust that is retained on kernels (Korunic and Ormesher, 2000). DE effectiveness was always lower in maize than in wheat (Subramanyam and Roesli, 2000; Athanassiou et al., 2003). High lipid content of maize kernels may cause an increased oil absorption by DE particle and thus the DE becomes inactivated (Vayias et al., 2006).

Athanassiou et al. (2003), using "Silicosec" in dose-response tests on *S. oryzae* adults in peeled rice, paddy rice, barley and maize, found that notably varied in different types of grain. Our results stated that after 14 days of exposure treated barley and wheat at the highest dose rate all adults died whilst on treated maize at the highest rate killed only 37.38% of the exposed adults.

Subramanyam and Roesli (2000) have noted that it is often more important in practical conditions of cereal storage to prevent progeny formation than to concentrate on obtaining direct lethal effects of DE against parent insects. In grain type, adult emergence, due to oviposition of the exposed females, could not be avoided.

This investigation illustrate that good control of the red flour beetle in stored grain is not currently possible with the use of low DE concentrations, especially on maize and rice.

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