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PUISSANCE OF NATURE'S WISDOM FOOD GRADE DIATOMACEOUS EARTH AGAINST *Tribolium castaneum* (HERBST); A STEP TOWARDS ECOFRIENDLY PEST MANAGEMENT

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Diatomaceous earth (DE) formulations are being used as grain protectants with reduced environmental issues and insecticide resistance. In this study binary response of 2^{nd}, 4^{th} larval instars and adults of *Tribolium castaneum* (Herbst) were recorded (1, 2, 5, 7 and 14 days exposure interval) on DE treated (50, 100, 150, 300, 400 and 500 mg/kg) wheat. Mortality percentage for different life stages and F_1 Progeny reduction at low and high temperature and relative humidity conditions were observed as efficacy determining tools. About 100% mortality was recorded after 7 day interval at 28±2°C; 55±5% RH with low LC_{50} and LC_{90} values in 2^{nd} instar larvae as compared to high LC_{50} and LC_{90} values for 4^{th} instar larvae with a maximum of 81.94% mortality at 28±2°C; 55±5% RH. Mortality rates for 2^{nd} instar (70%) and 4^{th} instar larvae (65%) were much lower at 22±2°C; 40±5% RH. Adults of *T. castanium* appeared to be more tolerant with a maximum of 42.5% response at 28±2°C, 55±5% RH. There was greater than 70% progeny suppression at 400mg/kg dose rate and it was also observed that there was complete progeny suppression (100%) at low temperature and humidity conditions. So, from these results we can conclude that Nature's Wisdom DE can be used as effective gain protectant and its low silica content (<1%) avoids the risk of carcinogenicity as compared with other insecticide based DE formulations.
Keywords: Diatomaceous earth, Nature's Wisdom, Food grade, life stages, *Tribolium castaneum,* wheat.

INTRODUCTION

Fumigation is the most commonly used practice to control stored-product pests. However, in the last few decades, phosphine resistance in insects of stored-product commodities has become a global challenge that may limit its use in future (Benhalima *et al.*, 2004; Nayak *et al.*, 2003; Opit *et al.*, 2012). Currently, there is a clear documented evidence that phosphine resistance is present in stored-product species from all around the world (Campolo *et al.*, 2014; Cato, 2015; Chen *et al.*, 2015; Jittanun and Chongrattanameteekul, 2014; Kocak *et al.*, 2015; Pimentel *et al.*, 2010; Riudavets *et al.*, 2014; Sağlam *et al.*, 2015). Besides, phosphine resistance such chemicals pose threat to environment and human health (Campolo *et al.*, 2014; Campolo *et al.*, 2013; Riudavets *et al.*, 2014). As a consequence of these factors, demand for residue free food and eco-friendly pest control strategies is rising that has paved way to development of non-toxic, potentially effective grain protectants.

Diatomaceous earth (DE) composed of unicellular algae diatoms is an amorphous Silicon dioxide (SiO_2) with trace amounts of some additional minerals (Aluminum, Calcium hydroxide, Iron oxide, Sodium and Magnesium) is considered as one of the most effective grain protectant and alternative to long used residual insecticides (Athanassiou *et al.*, 2014b; Bougherra-Nehaoua *et al.*, 2015; Campolo *et al.*, 2014; Vayias and Stephou 2009).

Inert dusts work by absorbing the epicuticular wax from the insect cuticle and increase water evaporation rate due to which insects die of desiccation (Korunic, 1998; KoruniĆ, 1997; Subramanyam andRoesli, 2000). It has been documented that inert dusts do not affect metabolic pathway and hence reducing the likelihood of insect resistance (Ebeling, 1971). The efficacy of inert dusts is dependent on type of commodity, commercial formulation, stage of insect, geographical origin, relative humidity and many other factors (Athanassiou *et al.*, 2014a; Athanassiou *et al.*, 2011, Baldassari and Martini, 2014; Rigaux *et al.*, 2001; Stathers *et al.*, 2004; Vayias *et al.*, 2006). When insects lost about 60 percent of water or approximately 30 percent weight of their body, they dies (Ebeling, 1971).

The genus *Cryptolestes* contains the species that have been identified as the most susceptible to DE, while *Oryzaephilus* and *Sitophilus* species are moderately susceptible, and flour beetles of the genus *Tribolium* and *Rhyzopertha* the least one (Arnaud *et al.*, 2005; Golob, 1997; Korunic, 1998; KoruniĆ, 1997). The utmost communal tolerant pest against DE is *T. castaneum* therefore if a formulation of DE is proven capable to control the flour beetles then it should be capable to control mostly insects which occurs in food storages (Arnaud *et al.*, 2005; Fields *et al.*, 1997; KoruniĆ, 1997).

Considering the above mentioned facts, current research was planned to estimate different doses of DE against different life stages and subsequent F_1 progeny of *T. castaneum* exposed to treated wheat grains, at distinctive temperature and relative humidity conditions.

MATERIALS AND METHODS

***Insect Culture*:** Insects of *T. castaneum* were reared in wheat flour containing 10% brewer's yeast in glass jars of 2.5 ml in Stored Grain Laboratory of Entomological Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan. The mouth of jars were enclosed with the muslin cloth and held at 28±2 ° C and 65±5%RH in incubator. To obtain the larvae, 50 unsexed mix aged adults of *T. castaneum* were set free in flour with 10% brewer's yeast in glass jars. The jars were placed at the same rearing conditions as for mass rearing of *T. castaneum* insects. Larvae of 2nd and 4th instar were removed after 7 and 20 days of interval respectively, with the help of 50 mesh sieve.

***Diatomaceous Earth Formulation*:** The formulation of DE used in the study was of fresh water origin made in US. The formulation was Nature's Wisdom 100 percent pure food grade DE that is registered by FDA and contains less than 1 percent crystalline Silica and is considered as ecofriendly and environmentally safe. There are 600 deposits of DE in the US out of which 4 are considered food grade.

***Quantal Bioassays Against larvae*:** Quantal studies involving binary responses were carried out at22±2 °C, 40±5% RH and 28±2 °C, 55±5% RH. About 200 g. un-infested crushed wheat grains of Faisalabad-2008, a commonly grown wheat variety in Pakistan, were added in 1ml glass jars. DE was tested at 6 application rates (50, 100, 150, 300, 400 and 500mg/kg).The measured amount of DE was added in 200 g. wheat grains and shaken well for uniform mixing of dust. Then 20 g. treated and untreated (control) wheat grains were added in 90 mm diameter glass petri plates of each replicate that were repeated four times. Ten2nd and 4th instar larvae of *T. castaneum* were released into each petri plate of each experimental unit. Binary responses of *T. castaneum* larvae were recorded at 1, 2, 3, 5, 7 and14 days of exposure interval.

***Against adults*:** The method employed for larvae was also used for bioassay of adults. Twenty four glass jars of 1 liter volume were prepared having 30 gm crushed wheat grains. Six concentrations of DE i.e., 50, 100, 150, 300, 400 and 500 mg/kg were tested against adults. Ten adults (2-3 weeks old) were introduced into glass jars of each dose rate of each experimental unit. The mouth of jars was covered with muslin cloth for appropriate aeration and to avoid the escape of insects from the jar. All treated and untreated (control) jars were put into incubator at 22±2 °C, 40±5% RH and 28±2 °C, 55±5% RH. Mortality data for adults were taken at 1, 2, 3, 5, 7 and14 days of exposure interval to DE. 14 days later all the adults were taken out and the jars were left for 5 weeks for F_1 progeny emergence.

***Statistical Procedures*:** The larval mortality counts were corrected by applying (Abbott 1925) formula. In case of adults there was no control mortality so, no correction was required. The discrete data for mortality (percent) of 2nd and 4th instar larvae were converted to arcsine square root transformation to make it normalize and continuous in nature to fit the assumption of ANOVA. Mortality percentage data for adults was transformed to square root $\sqrt{(0.5+x)}$ to make it near normally distributed. The progeny data was transformed to $\log(x+1)$. The mortality data were analyzed by repeated measures ANOVA of generalized linear models (GLM) of IBM SPSS 20.0 software (2011). The median lethal dose required to kill 50% and 90% of target insects (LC_{50}, LC_{90}) was determined by (POLO-Plus 2006) Dose-response mortality linear relationship for each life stage at 5, 7 and 14 days interval was calculated by Graph Pad prism 6.02to explain the amount of variation (R^2) explained by different life stages at a particular interval. Means were segregated by; Tukey's Kramer honestly significant difference (HSD) test, at 0.05 significance level (Sokal RR 1995). Percentage of progeny reduction was calculated by (Aldryhim 1990) is given as:

$$\text{Progeny Reduction (\%)} = \frac{\text{No.progeny in control} - \text{No.progeny in treatment}}{\text{No.progeny in control}} \times 100$$

RESULTS

All the main effects and their pertinent interactions were found significant (P<0.05) with the exception of life stage*temperature/RH (P = 0.240) and exposure interval*life stage*temperature/RH*dose (P = 0.072). In addition, assumption of Mauchly's test of sphericity for within exposure intervals was met χ^2 (9) = 113.397, P = <0.05 so, we reject the null hypothesis that variances across all exposure interval pairs are equal (sphericity has been violated). Repeated measures ANOVA for main effects and associated interactions is presented in Table 1.

At 22±2 °C and40±5% RH, 2nd instar larvae did not show any response to low dose rates of 50, 100 and 150 mg/kg for the

1st 24 hours and even after 2 days exposure interval mortality was not recorded when treated with dose rates 50 and 100 mg/kg. After 5 days interval at the same temperature and humidity2nd instar responded to all dose rates and the highest mean mortality (47.5%) was recorded at 500 mg/kg. Similarly, after 7 and 14 days interval at maximum dose rate (500 mg/kg) mortality response reached to 60 and 70%, respectively. larvae of 2nd instar were more prone to high temperature and humidity (28±2 °C, 55±5% RH) and mortality peaked up to 100% even after 7 day interval.

In case of 4th instar larvae, no statistically significant differences were detected in mortalities after 1, 2 and 5 days exposure intervals at 22±2 °C, 40±5% RH. At 500 mg/kg the highest mortality level (65%) was recorded after 14 days interval whereas, at 28±2 °C, 55±5% RH, all the exposure intervals depicted significant differences between mortalities at different dose rates and mortality reached up to 81.94% at the highest application rate (500 mg/kg).

In both bioassays, all the dose application rates and exposure intervals were not sufficient to achieve the end point mortality for adults. A maximum of 42.5% mortality was recorded at elevated temperature and humidity conditions in comparison with low temperature and humidity conditions where only 20% adult mortality was observed at 14 day interval.

Table 1. Repeated measures (ANOVA) for main effects and associated interactions of *T. castaneum*.

Between exposure intervals	df	F	P
Intercept	1	1109.99	<0.05
Life stage*temperature/RH*dose	10	2.47	<0.05
Life stage*temperature/RH	2	1.37	0.240
Temperature/RH*dose	5	61.44	<0.05
Life stage*dose	10	14.41	<0.05
Life stage	2	16.09	<0.05
Temperature/RH	1	39.46	<0.05
Dose	5	271.82	<0.05
Error	108		
Within exposure intervals			
Exposure interval	4	143.98	<0.05
Exposure interval*life stage*temperature/RH*dose	40	1.37	0.072
Exposure interval*life stage*temperature/RH	8	15.82	0.086
Exposure interval*temp./RH*dose	20	1.47	0.164
Exposure interval*life stage*dose	40	3.19	<0.05
Exposure interval*life stage	8	21.37	<0.05
Exposure interval*temp./RH	4	7.43	<0.05
Exposure interval*dose	20	5.15	<0.05
Error	432		

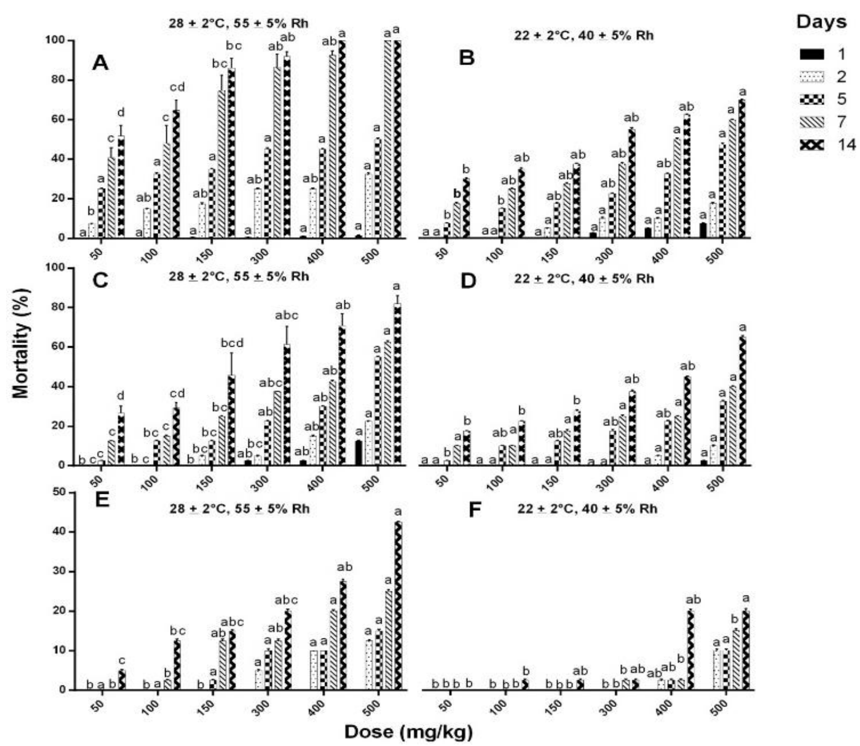

Figure 1. Means ± SE of *T. castaneum* different life stages at varying temperature and humidity conditions exposed to crushed wheat grains treated with 50, 100, 150, 300, 400 and 500 mg/kg dose rates for 1, 2, 5, 7 and 14 days interval. Means and SE are on untransformed data. Means followed by same letter are not significantly different at P = 0.05. Where, A = 2nd instar larvae; B = 2nd instar larva; C = 4th instar larvae; D = 4th instar larvae; E = adults and F = adults.

Table 2. Linear regression equations describing mortality of *T. castaneum* different life stages at 5 day exposure interval

Life stage	Temperature (°C)	RH (%)	Regression Coefficients ± SE			
			a	b	R^2	P
2nd instar larvae	22 ± 2	40 ± 5	4.414 ± 3.003 [a]	0.07734 ± 0.010060 [a]	0.9462	<0.05
4th instar larvae	22 ± 2	40 ± 5	1.992 ± 1.920 [b]	0.05703 ± 0.006430 [b]	0.9099	<0.05
Adults	22 ± 2	40 ± 5	-2.409 ± 1.994 [c]	0.01797 ± 0.006679 [c]	0.7226	<0.05
2nd instar larvae	28 ± 2	55 ± 5	26.050 ± 2.185 [d]	0.05078 ± 0.007318 [d]	0.9148	<0.05
4th instar larvae	28 ± 2	55 ± 5	-2.109 ± 4.752 [e]	0.09844 ± 0.015920 [e]	0.9234	<0.05
Adults	28 ± 2	55 ± 5	-2.344 ± 1.043 [f]	0.03438 ± 0.003494 [f]	0.9328	<0.05

Regression coefficient R^2 is on transformed data. For all regression equations y = mortality (%) and x = dose (mg/kg). Where, a, Y = 0.07734X + 4.414; b, Y = 0.05703X + 1.992; c, Y = 0.01797X - 2.409; d, Y = 0.05078X + 26.05; e, Y = 0.09844X - 2.109 and f, Y = 0.03438X - 2.344

Table 3. Linear regression equations describing mortality of *T. castaneum* different life stages at 7 day exposure interval

Life stage	Temperature (°C)	RH (%)	Regression Coefficients ± SE			
			a	b	R^2	P
2nd instar larvae	22 ± 2	40 ± 5	13.790 ± 1.578 [a]	0.08984 ± 0.005284 [a]	0.9867	<0.05
4th instar larvae	22 ± 2	40 ± 5	6.016 ± 2.711 [b]	0.06094 ± 0.009077 [b]	0.9274	<0.05
Adults	22 ± 2	40 ± 5	-3.307 ± 2.841 [c]	0.02656 ± 0.009515 [c]	0.8123	<0.05
2nd instar larvae	28 ± 2	55 ± 5	41.980 ± 7.092 [d]	0.12730 ± 0.023750 [d]	0.9493	<0.05
4th instar larvae	28 ± 2	55 ± 5	6.523 ± 2.893 [e]	0.10390 ± 0.009687 [e]	0.9690	<0.05
Adults	28 ± 2	55 ± 5	-0.807± 2.428 [f]	0.05156 ± 0.008132 [f]	0.8328	<0.05

Equation parameters a and b are on back transformed data and R^2 is on transformed data. y = mortality% and x = dose (mg/kg). Where, a,Y = 0.08984X + 13.79; b, Y = 0.06094X + 6.016;c, Y = 0.02656X - 3.307; d, Y = 0.1273X + 41.98; e, Y = 0.1039X + 6.523; f, Y = 0.05156X - 0.8073.

Table 4. Linear regression equations describing mortality of *T. castaneum* different life stages at 14 day exposure interval

Life stage	Temperature (°C)	RH (%)	Regression Coefficients ± SE			
			a	b	R^2	P
2nd instar larvae	22 ± 2	40 ± 5	25.48 ± 1.121 [a]	0.09141 ± 0.003754 [a]	0.9942	<0.05
4th instar larvae	22 ± 2	40 ± 5	12.01 ± 3.062 [b]	0.09531 ± 0.01026 [b]	0.9631	<0.05
Adults	22 ± 2	40 ± 5	-3.802 ± 3.564 [c]	0.04688 ± 0.01193 [c]	0.8287	<0.05
2nd instar larvae	28 ± 2	55 ± 5	57.49 ± 7.395 [d]	0.09965 ± 0.02477 [d]	0.9186	<0.05
4th instar larvae	28 ± 2	55 ± 5	21.56 ± 3.031 [e]	0.1243 ± 0.01015 [e]	0.9765	<0.05
Adults	28 ± 2	55 ± 5	2.643 ± 2.867 [f]	0.07109 ± 0.009603 [f]	0.9362	<0.05

Regression for different life stages exposed to 22 ± 2 °C, 40 ± 5 % RH and 28± 2 °C, 55± 5 % RHy = mortality% and x = dose (mg/kg). where; a, Y = 0.09141X + 25.48; b, Y = 0.09531X + 12.01; c, Y = 0.04688X - 3.802; d, Y = 0.09965X + 57.49; e, Y = 0.1243X + 21.56; f, Y = 0.07109X + 2.

Percentage mortalities for 2nd, 4th instar larvae and adults of *T. castaneum* at varying temperature and humidity conditions for 1, 2, 5, 7 and day exposure interval at 6 dose rates 50, 100, 150, 300, 400 and 500 mg/kg were presented in Fig. 1.

***Dose response mortality relationship*:** Dose mortality regression lines drawn for 2nd, 4th instar larvae and adults at 5, 7 and 14 days interval presented in Fig. 2 explained the amount of variation (R^2) contributed by each life at a particular interval by the linear model. Linear regression equations for different life stages are presented in Table 2, 3 and 4, respectively.

Data presented in Table 5 and 6 cleared that LC_{50} and LC_{90} values decreased with increase in time. LC_{50} and LC_{90} for 2nd instar larvae were lower as compared to 4th instar larvae at all the exposure intervals. Chi-square test indicated that the observed and expected mortalities of the 2nd instar and 4th instar larvae were same at 5, 7 and 14 days interval with the exception of 5 day exposure interval for 2nd instar held at 22±2 °C and 40±5% RH. At high temperature and humidity the average number of progeny (Mean ± SE) in the control was 28.25 ±3.24 as compared to 13.25 ±2.65 at low temperature and humidity conditions. Progeny data analysis and progeny

Table 5. The LC_{50} and LC_{90} values (mg/kg) 0f 2nd and 4th instar larvae of *T. castaneum* exposed to wheat grains treated with DE at 22 ±2 °C and 40 ± 5 % RH.

Exposure time	LC_{50}	Confidence limits 95 %	LC_{90}	Confidence limits 95 %	Chi-square (df= 4)
2nd instar larvae					
5	890.3	446.100 – 18184	13241.0	2436.2 – 0.69479E+08	9.20*
7	409.7	146.339 – 289.18	5646.4	2075.5 – 59159	4.38*
14	201.1	139.928 – 266.43	3722.0	1471.5 – 34481	4.17*
4th instar larvae					
5	1519.8	766.200 – 10390	20814.0	4557.9 – 0.18942E+07	4.16*
7	1313.2	649.700 – 11408	24942.0	4626.0 – 0.66115E+07	4.73*
14	404.0	258.717 – 1129.6	5071.5	1545.9 – 0.27575E+06	8.41*

Table 6. The LC_{50} and LC_{90} values (mg/kg) of 2nd and 4th instar larvae of *T. castaneum* exposed to wheat grains treated with DE at 28 ± 2 °C and 55 ± 5 % RH.

Exposure time	LC_{50}	Confidence limits 95 %	LC_{90}	Confidence limits 95 %	Chi-square (df= 4)
2nd instar larvae					
5	559.0	318.734 - 3034.862	61069.0	7082.100 - 0.11720E+09	0.306 NS
7	77.5	39.152 - 110.897	370.5	235.799 - 1113.463	6.1179*
14	53.7	14.300 - 83.895	209.9	136.144 - 710.352	9.1325*
4th instar larvae					
5	628.7	388.859 - 2740.362	3603.5	1271.800 - 0.18527E+06	12.608*
7	427.4	295.855 - 871.179	3423.5	1387.500 – 35126	7.4812*
14	173.3	125.645 - 232.753	1179.2	682.510 - 3555.0	6.477*

reduction (%) are presented in Table 7 and Fig. 3, respectively.

Table 7. Univariate (ANOVA) of progeny in relation to temperature/RH and dose.

Between exposure intervals	df	F	P
Intercept	1	13.914	<0.05
Temperature/RH*dose	1	0.394	0.533
Temperature/RH	1	22.712	<0.05
Dose	1	7.803	<0.05
Error	52		

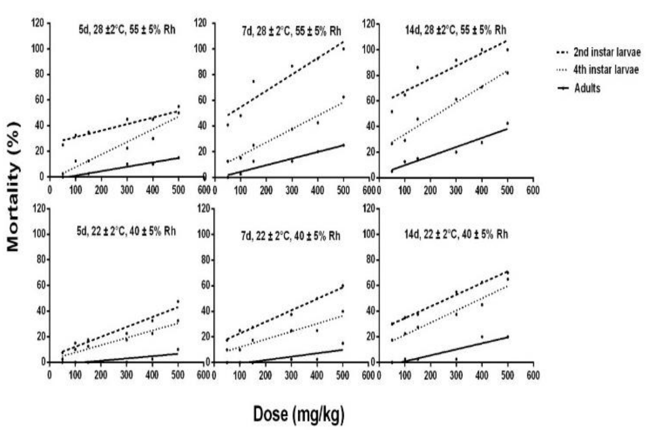

Figure 2. Back transformed mortality lines of *T. castaneum* 2nd, 4th instar larvae and adults exposed for 5, 7 and 14 day exposure interval.

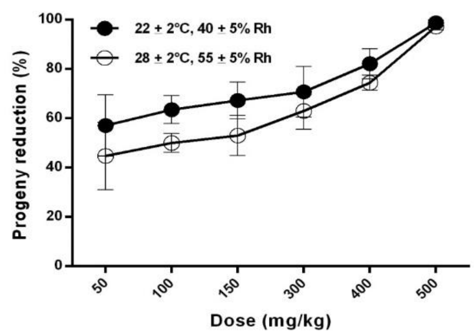

Figure 3. Means ± SE of percentage reduction at 50, 100, 150, 300, 400 and 500 mg/kg dose rate after 5 weeks interval.

Results of the present study indicated that mortality of the *T. castaneum* exposed to Diatomaceous earth (DE) varied considerably with respect to its life stages, exposure interval, dose rate, relative humidity conditions and temperature. Our results revealed that high relative humidity conditions and temperature were more conducive in achieving the higher mortality rates. The results of the study are in accordance with work of Chanbang *et al.* (2007), Collins *et al.* (2001), Vayias & Stephou (2009), that DE is more effective at high temperature. This is probably due to the fact that insects become agitated when they come in contact with the DE particles and they remained in contact with diatomaceous earth particles for longer time period. Besides this, at high temperature respiration rate is increased and consequently there is a more water loss via spiracles accelerating desiccation (Cotton 1932, ZACHARIASSEN 1991). It is thus clear from the findings of our study and many other previous investigations that high temperature has a synergistic effect with inert dusts formulations and can be a useful tool on commercial level to control stored grain pests in food processing plants (Fields *et al.*, 1997). There is a general observation that insect mortality is high at reduced humidity levels but this is not supported by the results of our experiment where a combination of high relative humidity and high temperature achieved high mortality rates at all exposure intervals and dose application rates. These results are not supported by the work of Athanassiou *et al.* (2016), Athanassiou *et al.* (2014a) that *T. castaneum, S. oryzae, R. dominica, and T. confusum* adults mortalities were more at 55% RH than at 65 and 75% RH. The difference might be due to the fact that humidity levels we have studied during our experiment were not incorporated by the above said scientists in their studies. There is also a possibility that high temperature might have dominated the effect of humidity as there was no further categorical division of RH levels at that particular temperature. Our findings depicted that at 55±5% RH, mortality of the 2nd, 4th instar larvae and adults was 100, 81.94 and 42.5% compared to 70, 65 and 20% mortalities at 40±5% RH for 2nd, 4th instar larvae and adults, respectively. It has however been documented that insects vary in responses to DE particles (Arnaud *et al.*, 2005; Golob 1997; Korunic 1998; Vayias and Stephou 2009).

Mortality of the *T. casatneum* increased as the exposure interval increased. Lengthier exposure interval was needed to achieve 100 % mortality of adults and 4th instar larvae as compared to 2nd instar larvae that showed 100% mortality due to their fragile cuticle after 14 day exposure period held at high temperature and humidity conditions, because more dust particles are adhered to the insect cuticle when they are in contact with DE treated substrate for longer time period that results in more water loss and desiccation ultimately leading to the death of the insect(Arthur, 2000). Larvae of 2nd instar were more sensitive to DE than 4th instar and adults were least susceptible. This is in accordance with the findings of Vayias and Athanassiou (2004), who exposed younger larvae (1-3rd instar) and older larvae (4-7th instar) of *T. confusum* to Silocosec**®** and reported that all the young larvae were killed after 24 hours exposure interval due to their prolonged contact with the DE prior to pupation whereas, for old larvae mortality was just 26%. A similar trend in mortality of *T. casatneum* different life stages was observed by Shayesteh and Ziaee (2007) when they were studying the effect of Silocosec**®** against different life stages of *T. castaneum* and observed that larval stages especially young larvae were more sensitive and adults were more tolerant to DE. The reason for less susceptibility of adults to DE is that they have hard cuticle which is less abraded by the DE particles. Another important factor that aids adults of Coleopteran beetles feeding in dried cereals is a well-developed Cryptonephridial system that is involved in reabsorbing extra water from the excrement through the rectal area (Chapman, 1998).

Another key factor in the effectiveness of DE is dose. Usually, high dose rates are required for satisfactory level of protection against stored grain insects. High dose rate adversely alter the physical properties of the grain principally grain flow ability and bulk density (Fields, 1998). Our study indicated that 500 mg/kg dose caused 100% mortality in 2nd instar larvae and >80% mortality for 4th instar larvae with complete suppression of progeny combined with high temperature and high humidity conditions. To achieve the same mortality level for adults the dose rate and exposure interval might have to be increased. The results of this study are not in agreement with findings of Arnaud *et al.* (2005) that revealed food grade DE Perma-Guard^{TM} was effective aginst the adults of *T. castaneum* strains from Pakistan origin with 97% mortality at 400 mg/kg dose rate. However, in a separate study by Ziaee and Khashaveh (2007), the same DE formulation was found least effective againstthe adults of stored grain insects when applied at a dose rate of 0.5g/cm^2. The differences might be due to the method of application and type of food grade DE they have used in their experiment because in our study we have used Nature's wisdom food grade DE.

It has been reported earlier that DEs are highly effective when used on the wheat grains rather than applied on other storage commodities e.g., corn, oat, barley, milled rice (Z. Korunic, personal communication). As this research was executed on wheat gains, it is more likely to use high dose rates in other type of stored grains to achieve the same results.

***Conclusion*:** Immature stages are more sensitive to DE with a synergistic effect of relative humidity and temperature. A dose rate of >500 mg per kg would be required to kill all of *T. casatneum*. Nature's wisdom food grade DE can be used without any fear in stored commodities as it contains >1% crystalline silica and is safe for humans, pets and animals.

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