

Evaluation of parasitic potential of *Anisopteromalus calandrae* (Howard) against *Callosobruchus maculatus* (F.), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) in grains treated with diatomaceous earths

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The herein reported study was conducted to evaluate the parasitic potential of *Anisopteromalus calandrae* (Howard) against larvae of *Callosobruchus maculatus* (F.), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) while, adult pests on the food medium treated with two formulations of diatomaceous earth (DE); Marine (Celite) and fresh water (Perma Guard) as grain protectants. The DE was applied at three dose rates i.e., 200, 400 and 600 ppm, at 25°C temperature and 65% relative humidity. Mortality and emergence of adults of tested insects and parasitoid were observed after 14 and 28 days of exposure respectively. The highest mortality of *C. maculatus* was 43.37% against higher dose of Perma Guard (600 ppm) while the maximum mortality of *S. oryzae* was recorded 39.56% on application of Celite (600 ppm). The maximum mortality of *A. calandrae* parasitoid was observed 66.86% in *S. oryzae* infested grains treated with higher dose of Perma Guard (600 ppm), while 63.81% mortality of parasitoid was observed in *S. oryzae* infested grains at higher dose of Celite DE (600 ppm). The Perma Guard effectively controls population of tested insect's mortality than the Celite. The highest emergence of *C. maculatus* observed was 62.44% at lower dose of 200 ppm of Perma Guard DE while the highest emergence recorded on application of Celite was 60.66% from *C. maculatus*. The highest emergence of *A. calandrae* was 65.65% from *S. oryzae* at lower dose of 200 ppm of Perma Guard DE while the highest emergence of parasitoid recorded on application of Celite was found 60.66% at lower dose of 200 ppm from *C. maculatus*. The emergence of tested insects and parasitoid increased with the decrease in dose rate of DE in most of the tested combinations. Higher dose (600 ppm) of both DE (Celite and Perma Guard) used in experiment showed mortality of tested insects and parasitoid activity of *Anisopteromalus calandrae* and furthermore release of *A. calandrae* on host insects would be adversely affected by use of diatomaceous earth product on stored grains. The experiments were carried out in laboratory of Grain Research, Training and Storage Management cell, Department of Entomology, University of Agriculture Faisalabad.

Keywords: *Anisopteromalus calandrae*, *Callosobruchus maculatus*, Diatomaceous earth, Parasitism, *Rhyzopertha dominica* and *Sitophilus oryzae*.

INTRODUCTION

Pulse beetle, *Callosobruchus maculatus* L. (Coleoptera: Bruchidae) also known as dhora beetle is the most destructive cosmopolitan pest of stored gram. It is a pest of stored pulses cause 12-13% loss by feeding the protein contents of grains. *C. maculatus* causes up to 10% damage to stored chick pea and up to 90% loss to stored gram. (Sidra-Tul-Muntaha *et al.*, 2017)

Losses caused by the insect pests may reach up to 30% during storage. About 2-6% food grain production of Pakistan is lost every year during storage by stored grain insect pests including *Rhyzopertha dominica* (Coleoptera: bostruchidae)

(Fabricious 1792), *Sitophilus oryzae* (Coleoptera: Curculionidae) (Linnaeus, 1763) (Wakil, W. and A. Shabbir, 2013)

Diatomaceous earths (DEs) used for insect pest mortality, because of unique mode of action, no or low mammalian toxicity (Subramanyam and Roesli, 2000). *A. calandrae* females avoid strongly against treatment of Protect-It on wheat and a significantly prefers parasitizing weevils in untreated wheat (Perez-mendoza 1999). Its affectivity depends on type of insects, stored commodity and environmental factors (Athanasios *et al.*, 2011). Athanasios *et al.* (2003) found the mortality effect of DE SilicoSec® against *Sitophilus oryzae* (L.) on two commodities at 1000

Nawaz, H., M.A. Ali, R.M. Atif, A. Nawaz and A. Abbas. 2021. Incidence of fusarium wilt in major tomato growing areas of Punjab. Pak. J. Agri. Sci. 58:1161-1167.

[Received 16-4-2021; Accepted 12-Jul 2021; Published (online) 21 Sep 2021]



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ppm. Efficacy depends upon the Source of DE but basically they are soft rock fossilized algae called diatoms. (Kavallieratos *et al.*, 2005). Mode of action for DE associated with the removal of the insect's cuticular waxes resulted in mortality from desiccation (Athanasios *et al.*, 2005). For protection of seed, it is usually mixed into the grain as a powder. The use of pesticides increases environmental pollution, depletion of ozone, so there is need of time for nonchemical pest control methods. DE is biodegradable and does not show toxic effect (Arthur, 2002).

Regardless of the non-availability of methyl bromide and continuous use of aluminum phosphide as fumigant to control stored product pests (Kavallieratos *et al.*, 2007), out of many available control tactics, only few have been comprehensively assessed. Insect pest can be controlled by different methods like biological (Hoedjes *et al.*, 2011), chemical (Ishii and Shimada 2012) and physical control tactics (Ghimire and Phillips 2008; Ishii 2008). Numerous kind of hymenopteran parasitoids including *Lariophagus distinguendus* (F.), (Hymenoptera: Pteromalidae), *Anisopteromalus calandrae*, have been used to manage stored grain insect pests (Gokhman, 2003; Sasakawa *et al.*, 2012). These species have a highly developed ability to detect prey and can attack numerous tested species. Both *L. distinguendus* and *A. calandrae* are quite effective in managing these storage pests (Konishi *et al.*, 2004). The female of wasps uses certain clues to locate the larvae that are developed mostly in the inner portion of the grains and detect pest movement. It also detects sound of host feeding, before parasitizing. Once the larvae found, the female wasp uses to oviposit by drilling into the grain, kill the host larvae and oviposit an egg on it. The female of *A. calandrae* prefers to oviposit on the later stages of *Sitophilus oryzae* at lab condition $25 \pm 5^\circ\text{C}$ and $65 \pm 5\%$ RH (Islam *et al.*, 2003). The mode of laying egg of female wasp is inserting the ovipositor into seed up to the body of host insect. The larvae of wasp on hatching feed on the fluids of larval body of pest. The wasp possess three larval stages, followed by pupal and finally adult. Emerging adults have tremendous ability to kill the insect pests (Islam, 2006).

Keeping in view the above facts, the herein reported study was conducted to evaluate the application of the Diatomaceous Earths (DE) along with bio-control agent (*Anisopteromalus calandrae*) and the efficacy in term of reducing pest population.

MATERIALS AND METHODS

Insects Pest collections: Three insect pests namely, *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.); *Rhyzopertha dominica* (F.) were collected from farmer field, private and government storage department and reared in laboratory for several generations for the homogenous population that were used in further research. The experiments were carried out in laboratory of Grain Research,

Training and Storage Management cell, Department of Entomology, University of Agriculture Faisalabad.

Parasitoids: Adult females of *Anisopteromalus calandrae* were obtained from stock cultures (initially reared on cow pea grains) maintained in the laboratory at 25°C and 75% RH (Kavallieratos *et al.*, 2005). The *A. calandrae* is a pteromalid wasp, easily reared in the laboratory at $25 \pm 5^\circ\text{C}$ and $65 \pm 5\%$ RH. The populations of parasitoid *A. calandrae* were released in the jar. In the plastic jars, clean cowpea grains was introduced and allowed to infest with larvae of *C. maculatus* and *S. oryzae* in brown rice. Jars placed in incubators. The grains that receive this oviposition were collected and placed in other jar containing grubs population again and again for several generations in order to get the homogeneous parasitoid population.

Diatomaceous Earth: Different formulations of Marine (Celite, 80–90% silica, with 2–4% alumina and 0.5–2% iron oxide) (Sigma-Aldrich) and fresh water (Perma Guard 80% silica) (PubChem CID) DE were imported from USA. DE was used at the rate of 200, 400 and 600 ppm

Effect of diatomaceous earth on parasitic potential: Grains (wheat, rice and cowpea) were treated with DE at different concentration (200, 400 and 600 ppm) 24 hr before releasing the insect pests and *A. calandrae*. All the tests to examine parasitism of *A. calandrae* were conducted at temperature 30°C and replicated three times. Thirty (30) samples of adults of insect pests were introduced in a plastic box separately. Five pairs of *A. calandrae* were introduced into treatment, after 14 days alive or dead adults of pest data were noted and lateral larval stages of F1 progeny allowed to parasitize for 24 hours on the later stage of larvae of insect pest. Jars were placed in incubator under controlled conditions. The larvae of pests that receive this oviposition were observed till the parasitoid emergence. Mortality data for pest and parasitoid was collected after 14 days and Emergence data for pest and parasitoid was collected after 28 days.

Former to setting up of experiment the grains (wheat, rice and cowpea) were cleaned and disinfested by exposing to 60°C for three hours in an air-circulated oven. The treatments consisted of two DE formulations and three DE dose rates and an untreated control, each replicated three times. Lots of 150 g of wheat were treated with four dose rates of 0 (control), 200, 400 and 600 mg/kg of each DE formulation. The lots were placed in 500 ml capacity bottles and the above mentioned dose of DE was added in each lot. The bottles were shaken manually for 2 minutes to achieve an even distribution of the DE on the grains. Subsequently, three samples of 50 g of treated or control grains were taken from each lot, and placed in 250 ml capacity glass jars. In each jar, thirty (30) adults of insect pests were introduced and then the jars were covered with perforated plastic lids fitted with filter papers to allow gaseous exchange. Five pairs of *A. calandrae* were introduced into treatment, allowed to parasitoid the later stage of larvae for 24 hr on removal of alive or dead adults after 14

days. The jars were kept under laboratory conditions at 30°C and 65% RH. Adult mortality of insect pest was assessed after 14 days and emergence of parasitoids after 28 days of exposure.

Statistical Analysis: Collected data of parasitoid and tested insects were corrected by using Abbott's formula (Abbott, 1925). After that the treatments were compared by using analysis of variance (ANOVA) through Statistic 8.0. Treatment means were separated using Tukey Kramer HSD test at $\alpha = 0.05$ level.

RESULTS

The results reveal that for fresh water DE the highest mortalities 43.37 %, 30.81 and 32.56 % were recorded in 600 ppm concentration for *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Lowest mortalities of 14.44, 11.05 and 16.86% were observed with 200 ppm concentration for *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Mortalities of tested insects increased with an increase in DE concentration. *C. maculatus* showed higher mortality 43.37% at maximum dose as compared to other tested insects in bioassay (Fig. 1).

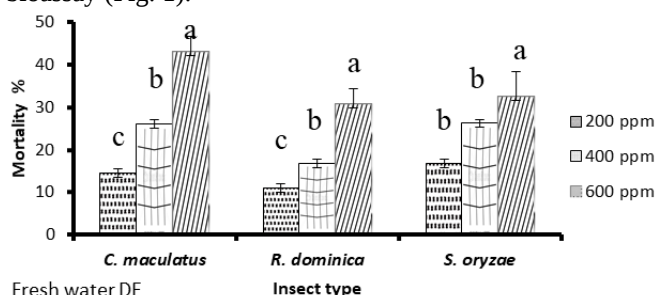


Figure 1. Comparison of means of percent mortality of tested insects, *C. maculatus* ($F=25.39$; $P<0.001$), *R. dominica* ($F=9.54$; $P<0.001$), *S. oryzae* ($F=4.28$; $P<0.001$) at different dose (ppm) of Perma Guard diatomaceous earth after 14 days' time interval

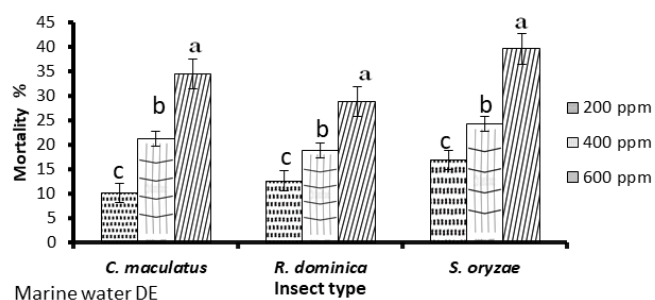


Figure 2. Comparison of means of percent mortality of tested insects, *C. maculatus* ($F=25.39$; $P<0.001$), *R. dominica* ($F=9.54$; $P<0.001$), *S. oryzae* ($F=4.28$; $P<0.001$) at different dose (ppm) of Celite diatomaceous earth after 14 days time interval

The results reveal that for marine water DE the highest mortalities 34.44, 28.81% and 39.56 % were recorded in 600 ppm concentration for *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Lowest mortalities 10.14 %, 12.65 % and 16.86% was observed with 200 ppm concentration for *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Mortality of tested insects increases with the increase in DE concentration but significantly less than fresh water DE. *S. oryzae* showed higher mortality 39.56% at maximum dose as compared to other tested insects in bioassay (Fig. 2).

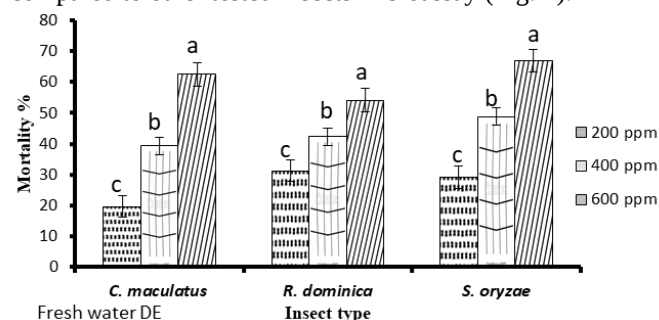


Figure 3. Comparison of means of percent mortality of *Anisopteromalus calandreae* on tested insects, *C. maculatus* ($F=51.0$; $P<0.001$), *R. dominica* ($F=11.6$; $P<0.001$), *S. oryzae* ($F=51.4$; $P<0.001$) at different dose (ppm) of Perma Guard diatomaceous earth after 14 days time interval

The results reveal that for fresh water DE the highest mortalities 62.44, 54.11 and 66.86 % were recorded in 600 ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Lowest mortalities 19.66, 31.17 and 29.13 was observed with 200ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Mortality of *A. calandreae* increases with the increase in DE concentration. *S. oryzae* shows higher mortality 66.86 % at maximum dose in bioassay (Fig. 3).

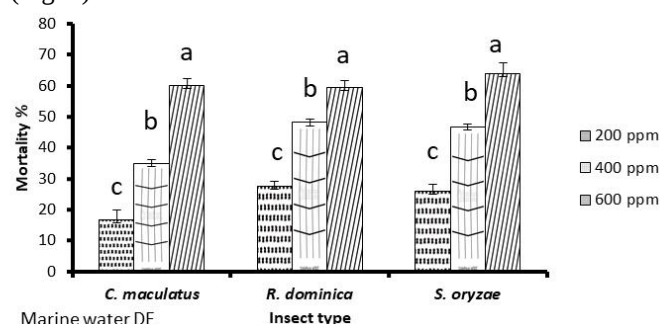


Figure 4. Comparison of means of percent mortality of *Anisopteromalus calandreae* on tested insects, *C. maculatus* ($F=51.0$; $P<0.001$), *R. dominica* ($F=11.6$; $P<0.001$), *S. oryzae* ($F=51.4$; $P<0.001$) at different dose (ppm) of Celite diatomaceous earth after 14 days time interval

The results reveal that for marine water DE the highest mortalities 60.0, 59.34 and 63.81 % were recorded in 600ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Lowest mortalities 16.66, 27.61, and 25.83 % were observed with 200ppm for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Mortality of *A. calandreae* increases with an increase in DE concentration. *S. oryzae* showed higher mortality (63.81%) for *A. calandreae* at maximum dose in bioassay (Fig. 4).

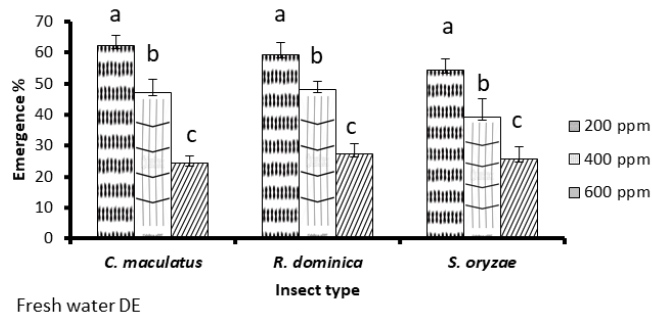


Figure 5. Comparison of means of percent emergence of tested insects, *C. maculatus* ($F=6.07$; $P<0.001$), *R. dominica* ($F=11.6$; $P<0.001$), *S. oryzae* ($F=21.18$; $P<0.001$) at different dose (ppm) of Perma Guard diatomaceous earth after 14 days time interval.

The results reveal that for fresh water DE the highest emergence 62.26, 59.34 and 54.81% were recorded in 200ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Lowest emergence 24.41, 27.21 and 25.70% was observed with 600ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Emergence of tested insects decreases with an increase in DE concentration. *C. maculatus* shows higher emergence 62.44% at minimum dose in bioassay (Fig. 5).

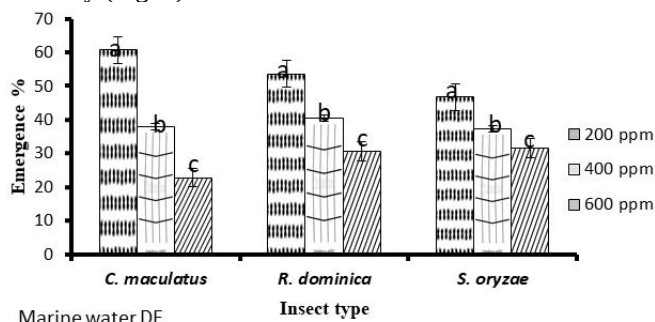


Figure 6. Comparison of means of percent emergence of tested insects, *C. maculatus* ($F=6.07$; $P<0.001$), *R. dominica* ($F=11.6$; $P<0.001$), *S. oryzae* ($F=21.18$; $P<0.001$) at different dose (ppm) of Celite diatomaceous earth after 14 days time interval

The results reveal that for marine water DE the highest emergence 60.66, 53.64 and 46.71 % were recorded in 200 ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Lowest emergence 22.81, 30.60 and 31.63% were observed with 600 ppm concentration of diatomaceous for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Tested insects showed higher emergence at lower concentration as compared to higher concentration applied. *C. maculatus* shows higher emergence (60.661%) at minimum dose in bioassay (Fig. 6).

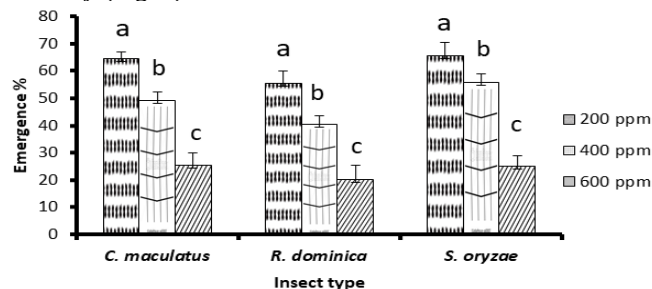


Figure 7. Comparison of means of percent emergence of *A. calandreae* on tested insects, *C. maculatus* ($F=29.13$; $P<0.001$), *R. dominica* ($F=8.62$; $P<0.001$), *S. oryzae* ($F=2.59$; $P<0.001$) at different dose (ppm) of Perma Guard diatomaceous earth after 14 days time interval

The results reveal that for fresh water DE, the highest emergence of 64.51, 55.25 and 69.65 % were recorded in 200 ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae*, respectively. Lowest emergence 25.22, 20.06 and 24.96% were observed with 600 ppm concentration for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae*, respectively. Emergence of *A. calandreae* decreased with an increase in DE concentration. *A. calandreae* showed higher emergence at lower dose applied. *S. oryzae* shows higher emergence (69.65%) at minimum dose of 200ppm in bioassay (Fig. 7).

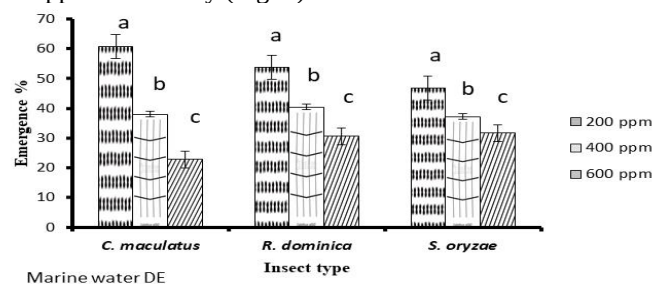


Figure 8. Comparison of means of percent emergence of *A. calandreae* on tested insects, *C. maculatus* ($F=29.13$; $P<0.001$), *R. dominica* ($F=8.62$; $P<0.001$), *S. oryzae* ($F=2.59$; $P<0.001$) at different dose (ppm) of Celite diatomaceous earth after 14 days time interval

The results reveal that for marine water DE the highest emergence were recorded in 200 ppm treatment 60.66, 53.64 and 46.71 % for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. Lowest emergences were observed with 600 ppm treatment, 22.81 30.60 and 31.63 % for *A. calandreae* on *C. maculatus*, *R. dominica* and *S. oryzae* respectively. *A. calandreae* showed higher emergence at lower concentration applied. *C. maculatus* shows higher emergence (60.66 %) at minimum dose of 200ppm in bioassay (Fig. 8).

DISCUSSION

The results in our experiments revealed that the highest mortality of *C. maculatus* was recorded at 600 ppm concentration of fresh water and marine water formulation of DE against *S. oryzae*. This showed the adverse effect of DE on survival of pests. Lowest mortality 11.05% was observed with 200 ppm concentration for *R. dominica* and 10.14% for *C. maculatus*. This showed that mortality at low dose caused to DE treatment and pest showed susceptibility towards DE. Similarly Moras *et al.* (2006) found results that Keepdry® was effective for *S. oryzae* species at higher doses and kills the tested beetle maximum but survival for the *S. oryzae* was high at lower doses, the same DE was effective at higher doses against *S. zeamais* on maize and *A. obtectus* on beans after 3 days of exposure. This result shows that the mortality depends on dose rate; higher dose shows more mortality as compared to lower dose rate. Our result was also satisfied by Ceruti *et al.* (2006) showed that Keepdry® killed completely all population of *S. oryzae* adults on pearl millet seeds treated with 1000 ppm DE dose after 14 d of exposure at 25°C.

Wakil *et al.*, 2014 concluded in experiment by using (Protect-It) DE against adults of *R. dominica* (F.). The 500 and 600 ppm dose rates were highly effective resulting in about 100% mortality in three weeks of exposure interval to treated wheat. Mortality of insects depends on the time intervals of exposed treatment as well as concentration applied.

Different insects have different susceptibility to DE some populations of insects, which are more tolerant can be significantly controlled by DE formulations (Rigaux *et al.*, 2001). In present study, almost all the bruchids in control treatment infesting grains developed and emerged to a new generation of *C. maculatus*, *R. dominica* and *S. oryzae*. However, treatment with lower dose mortality was lower and higher emergence found for all the tested insects.

The highest mortality 66.86 ± 1.95 % was recorded in 600 ppm concentration for *A. calandreae* on *S. oryzae* in fresh water DE treatment and 63.81 ± 2.28 % was recorded for *A. calandreae* on *S. oryzae* in Marine water DE treatment. Lowest mortality 19.66 ± 2.09 % was observed with 200 ppm concentration for *A. calandreae* on *C. maculatus* and 16.66 ± 2.11 % for *A. calandreae* on *C. maculatus*. Alan and Fields (2012) found the same result on treating the *A. calandreae* with DE and conclude that the DE mode of action is the same for all the

insects regardless of predator and prey. Its capability of killing equally affects all the insects.

Cheaseng *et al.*, 2017 successfully control the specie *S. zeamais*, by treatment of Keepdry®. 100 % of insect mortality was observed after 20 days of application.

The highest emergence 62.44% and 60.66% were recorded in 600 ppm concentration for *A. calandreae* on *C. maculatus* for fresh water and marine water DE treatment respectively. Lowest emergence 24.41% and 22.81% was observed with 200 ppm concentration for *A. calandreae* on *C. maculatus* for fresh water and marine water DE treatment respectively.

Fieldsa, and Korunic in (2010) found that lower dose treatments of DE emergence of tested insects found greater than the higher dose treatment. Arthur in (2010) found that *A. calandreae* reproduce in higher rate when abundant amount of host insects found in favorable environment conditions.

For fresh water DE the highest emergence 65.65% were recorded in 600 ppm concentration for *A. calandreae* on *S. oryzae*. For marine water DE the highest emergence were recorded in 600 ppm treatment 60.66% for *A. calandreae* on *C. maculatus*. Lowest emergence 20.06 % was observed with 200 ppm concentration for *A. calandreae* on *R. dominica*. Lowest emergence was observed with 200 ppm treatment, 22.81% for *A. calandreae* on *C. maculatus*.

Wakil and Shabbir 2013 evaluate the mortality and emergence of tested insects by application of SilicoSec® on treated grains. Grains were allowed to infest by *S. oryzae* adults and all the exposed adults were removed and progeny production was estimated. Data collected after 3 weeks showed complete suppression of insects achieved and no progeny found time interval and dose rate played a significant role for the suppression of tested insects and their progeny. In present study the DE treatment equally affects the mortality and progeny production of the tested insects, parasitoid and its efficacy. Effect of DE on parasitoid and tested insects reduce the production of tested insect and also affect the parasitism of *A. calandreae* toward the tested insects. Mortality is directly proportional to the exposure time and dose rates applied.

Conclusion: Higher dose of DE treatment showed mortality effect both on the parasitoid and pests however use of proper dose of DE along with biological control can be very effective for the control of insect pests. In conclusion, the findings of this study indicate that, both DEs formulation i.e., Fresh water (Perma Guard) and Marine water (Celite) could provide excellent control of insect pests (internal feeders) used, when treated at 600 ppm causing mortality as compared with those at lower dose rates. Higher dose used caused the mortality of population of both the insect pests and the parasitoid and reduce emergence ultimately. In the dosages of DE varying from 200 to 600 ppm can be used in stored cowpea, wheat and rice, as grain protectants to reduce the population of insect

pests, however concentrations of DE applied also showed adverse effect on the parasitoids population.

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