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Original article (Orijinal araştırma)

Persistence and insecticidal efficacy of a diatomaceous earth formulation, Inert-PMS, in stored wheat grain against *Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman*, Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst)

Diatomlu bir toprak formülasyonu olan Inert-PMS'in, depolanmış buğday tanelerinde *Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman*, Rhyzopertha dominica* (F.) ve *Tribolium castaneum* (Herbst)'a karşı kalıcılığı ve insektisit etkinliği

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Summary

Experiment was conducted to determine the persistence and insecticidal efficacy of a new enhanced diatomaceous earth, Inert-PMS, in wheat against four stored grain insect pests [*Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman, *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst)] under the laboratory conditions at 50, 75 and 100 mg/kg, for intervals of 24 h, 4 and 7 d at 28ºC and 65% RH. For persistence, Inert-PMS was applied to stored grain for 0, 30, 60, 90 and 120 d. The results demonstrated that adult mortality was directly proportional to the dose and exposure interval. While the efficacy remained constant up to 60 d, it fell after 90 and 120 d of storage. *L. paeta* and *C. ferrugineus* were the most susceptible to Inert-PMS (100% mortality) followed by *R. dominica* (81%) and *T. castaneum* (72%) at 75 mg/kg after 4 d. Inert-PMS also suppressed the reproduction at lower dose rates. Inert-PMS is an eco-friendly formulation that not only interferes with the growth and development of stored grain insects but is also cheap and free from ill effects.

Keywords: *Cryptolestes ferrugineus*, *Liposcelis paeta*, *Rhyzopertha dominica*, *Tribolium castaneum*, Inert-PMS, new enhanced diatomaceous earth

Özet

Bu çalışma, yeni geliştirilmiş diatomlu bir toprak olan Inert-PMS'in dört farklı depolanmış buğday zararlısına [*Cryptolestes ferrugineus* (Stephens), *Liposcelis paeta* Pearman, *Rhyzopertha dominica* (F.) ve *Tribolium castaneum* (Herbst)] karşı kalıcılığı ve insektisit etkilerini belirlemek amacıyla, 24 saat, 4 ve 7 gün aralıklarla 50, 75 ve 100 mg/kg dozları uygulanarak 28ºC sıcaklık ve % 65 orantılı neme sahip laboratuvar koşullarında gerçekleştirilmiştir. Kalıcılığını belirlemek için, inert PMS'in etkisi 0, 30, 60, 90 ve 120 gün süresince depolanan buğdaylarda değerlendirilmiştir. Sonuçlar, ergin ölümünün doz ve maruz kalma aralığı ile doğru orantılı olduğunu göstermiştir. Etki, 60 günlük depolamada sabit devam ederken, 90 ve 120 günlük depolamada ise düşmüştür. 75 mg/kg uygulama dozunda 4 gün sonunda, *L. paeta* ve *C. ferrugineus* Inert-PMS'ye en hassas türler olmuş (%100 ölüm), bunu *R. dominica* (%81) ve *T. castaneum* (%72) izlemiştir Inert-PMS, ayrıca düşük dozlarda da üremeyi baskılamıştır. Çevre dostu bir formülasyon olan Inert-PMS, depolanmış buğday zararlılarında sadece büyüme ve gelişmeye müdahale etmekle kalmayıp, aynı zamanda ucuzdur ve olumsuz etkileri de yoktur.

Anahtar sözcükler: *Cryptolestes ferrugineus*, *Liposcelis paeta*, *Rhyzopertha dominica*, *Tribolium castaneum*, Inert-PMS*,* yeni geliştirilmiş diatomlu toprak

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Introduction

Stored grain insect pests cause 5-15% damage to stored oilseeds, cereals and pulses (Padin et al., 2002). These insects cause both quantitative and qualitative losses to stored commodities (Bello et al., 2001; Michalaki et al., 2007). Among them, the lesser grain borer, *Rhyzopertha dominica* (F.), is most destructive internal feeders of stored grains. Its female lays eggs outside the grain. After hatching, the new larvae bore into the grain and complete their life cycle within the grain (Arbogast, 1991). Likewise, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), feeds directly on the grain germ, increases mold growth and excretes hydroxyquinone compounds that contaminate and damage to the grain (Assie et al., 2007). Also, the red flour beetle, *Tribolium castaneum* (Herbst), regarded as a secondary pest, feeds on whole cereal grains (Aitken, 1975), and the tropical psocid, *Liposcelis paeta* Pearman, commonly (Rajendran, 1994) reaches damaging population densities under favorable conditions (McFarlane, 1982; Turner, 1994).

All these insects are associated with stored grain, including household grain storage through to large commercial facilities. The economic threshold for these insects under storage conditions is zero (Flinn et al., 2007). So measures for control of stored grain insects must be applied at the time storage. Since 1950, insecticide application has been the most common control practice in Pakistan (Subramanyam & Hagstrum, 1995). However, the efficacy and effectiveness has declined due to resistant insect populations, increased costs, pesticide residues in animal and human food, and environmental pollution. These problems compelled pest management specialists to search alternatives, such as diatomaceous earth (DE), that are more specific and free from all ill effects (Lorini & Galley, 1999, 2000). DE has become more important in chemical control over the last decade and is now regarded as key component in integrated pest management (IPM) for stored grains (Korunic, 1999). DE is a soft rock composed of fossilized residues of unicellular algae called diatoms. According to geological sources; it is pure amorphous silicon dioxide and nontoxic to mammals (IARC, 1997). DE absorbs cuticular waxes of the insects, causing death by to desiccation (Rigaux et al., 2001) and it also abrades the cuticles of insects (Ebeling, 1971).

The efficacy of DE against stored grain pests is dependent on the commodity, the insect species, temperature and grain moisture content (Fields & Muir, 1995; Fields & Korunic, 2000). The researchers working in different parts of the world have found that variation in DE formulation greatly effects its efficacy, such as for Insecto and SilicoSec (Vayias & Athanassiou, 2004; Kavallieratos et al., 2005). Optimizing the DE application rate is as important factor in this context (Jackson & Webley, 1994; Korunic et al., 1996; Korunic & Ormesher, 1996; Korunic, 1997). Also, promising and persuasive results have been obtained when plant-based mixtures in conjunction with DE were applied to stored grain insects (Athanassiou et al*.,* 2008).

The present study was designed to assess the persistence and efficacy of a new enhanced formulation of DE, Inert-PMS, as surface treatment in stored wheat grain, by measuring its effect on reproduction of *C. ferrugineus*, *L. paeta, R. dominica* and *T. castaneum*.

Materials and Methods

Grains

Untreated, clean, pest-free soft wheat was used. The grain moisture contents was 10.5-11.5% measured by mini Gac Plus (Dickey-John Crop., Auburn, IL, USA) grain moisture meter (Athanassiou & Kavallieratos, 2005).

Rearing of insect pests

The four most important insect pests of stored grain, *C. ferrugineus*, *L. paeta, R. dominica* and *T. castaneum,* were obtained from infested wheat samples and populations were established and maintained at the stored grain laboratory, University of Agriculture, Faisalabad, Pakistan. The insect populations were reared on clean, pest-free wheat in plastic jars. Beetle cultures were maintained at 25-28ºC and 65±5% relative humidity (Kavallieratos et al., 2012), and the psocid population at 30ºC and 75±5% relative humidity (Opit & Throne, 2008).

Diatomaceous earth formulation and bioassay

The DE formulation used, Inert-PMS (Biofa GmbH Münsingen, Germany), is a new enhanced formulation of DE containing of the insecticides, spinosad and pirimiphos-methyl. Test was conducted with dosage rates of 0, 50, 75 and 100 mg/kg. One kg of wheat was prepared for each treatment and placed in cylindrical jars. Inert-PMS was mixed with the grain and 50 g of grain transferred to vials. Thirty mixed-sex adults of *C. ferrugineus*, *L. paeta, R. dominica* and *T. castaneum* were introduced into separate vials. Each treatment was replicated nine times in a completely randomized design, with untreated vials as a control. The jars were incubated at 28ºC and 65% RH. The humidity was maintained in the incubators by using saturated solutions of sodium chloride (Greenspan, 1977). The number of dead and live adults was counted after 24 h, 4 d and 7 d, and progeny data was also assessed. The residual bioassays of Inert-PMS were conducted after 0, 30, 60, 90 and 120 d exposure by the procedure described above. After 7 d, all adults (dead and alive) were removed from the vials and the vials retained for an additional period of 63 d for the beetles and 30 d for psocid under the same conditions. Finally, the vials were opened and the number of adult progeny counted.

Data analysis

Control mortality was corrected by using the Abbott's formula (Abbot, 1925). The data were analyzed by two-way ANOVA, using the GLM procedure of MINITAB 13.2 (Minitab 2002 Software Inc., Northampton, MA, USA). The means were separated by Tukey-Kramer HSD test at 5% significant level (Sokal & Rohlf, 1995).

Results

Cryptolestes ferrugineus

Similar mortality trend was obtained when *C. ferrugineus* were exposed to different dose rates and exposure intervals. However, higher mean mortality (94.1%) was obtained at 120 d at 100 mg/kg (Figure 1). Likewise, mortality decreased after 60 d, resulting in negative impact on progeny production of *C. ferrugineus*. The mean number of progeny from treated grain was significantly lower (0.33) at 100 mg Inert-PMS/kg (Figure 5) in the initial bioassay.

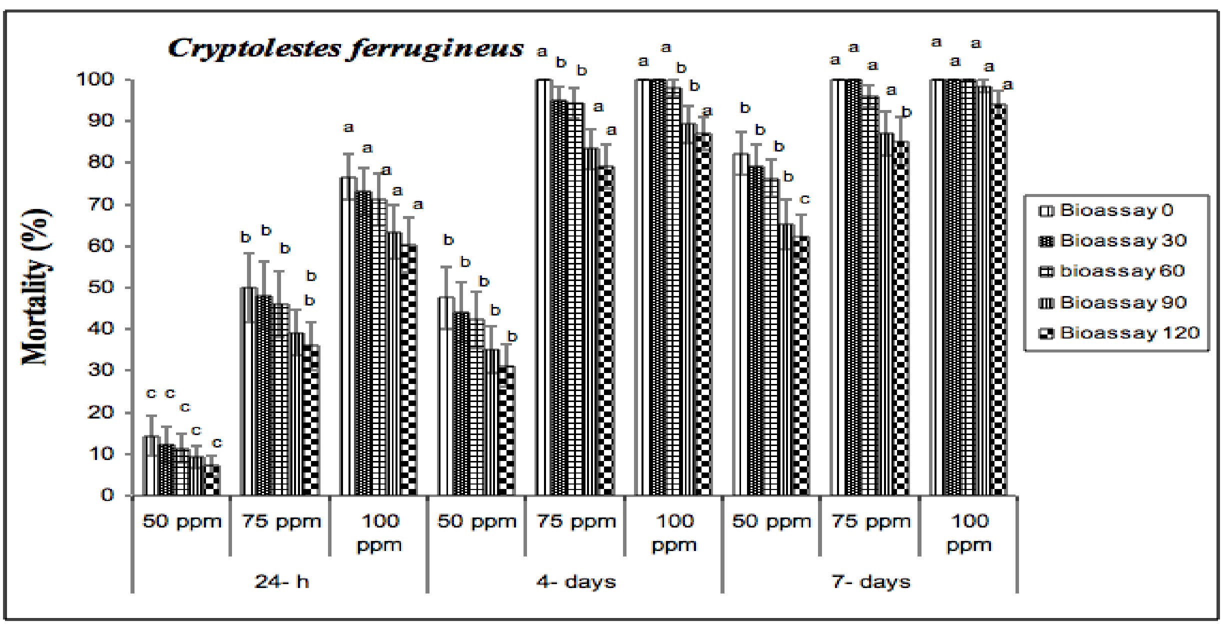

Figure 1. Mean mortality (% ± SE) of *Cryptolestes ferrugineus,* exposed for 24 h in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by the same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

Liposcelis paeta

Mortality of *L. paeta* increased with increase of exposure interval and dose rate. The lowest mortality rate (10.2%) occurred at 50 mg Inert-PMS/kg after 24 h following 120 d of storage. Increasing the exposure interval from 24 h to 4 d and 7 d, increased adult mortality to 44.0 and 71.4% (Figure 2). A similar mortality occurred until 60 d, but mortality decreased rapidly after 60 d. Moreover, progeny production after the exposure to Inert-PMS was generally low and decreased with the increasing dose rate. For the initial bioassay, mean number of progeny was 0.41 at 50 mg/kg and 0.25 at 100 mg/kg. However, mean number of progeny from the untreated stored grains were always higher than from treated ones (Figure 5).

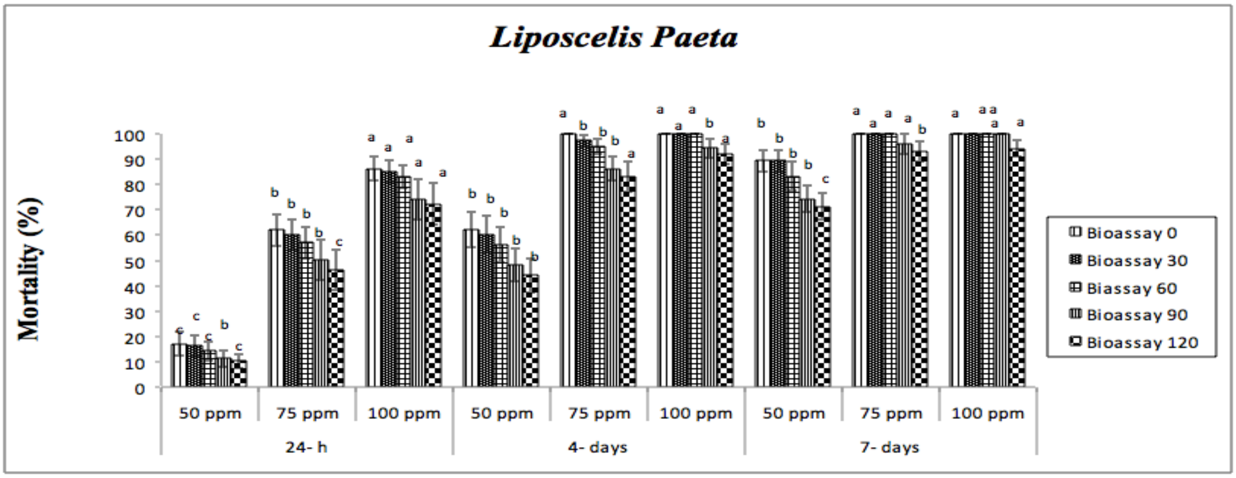

Figure 2. Mean mortality (% ± SE) of *Liposcelis paeta*, exposed for 24 h in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

Rhyzopertha dominica

Inert-PMS gave 12.3% mortality at 0 d storage, after 24 h, followed by 39.1 and 72.2% after 4 d and 7 d, respectively (Figure 3), at the same dose rate and storage. However, progeny production for *R. dominica* was adversely affected by the Inert-PMS dose rate. Newly emerged young progeny had mostly died in treated grains; progeny number was 0.41 at 100 mg Inert-PMS/kg (Figure 5) in the initial bioassay.

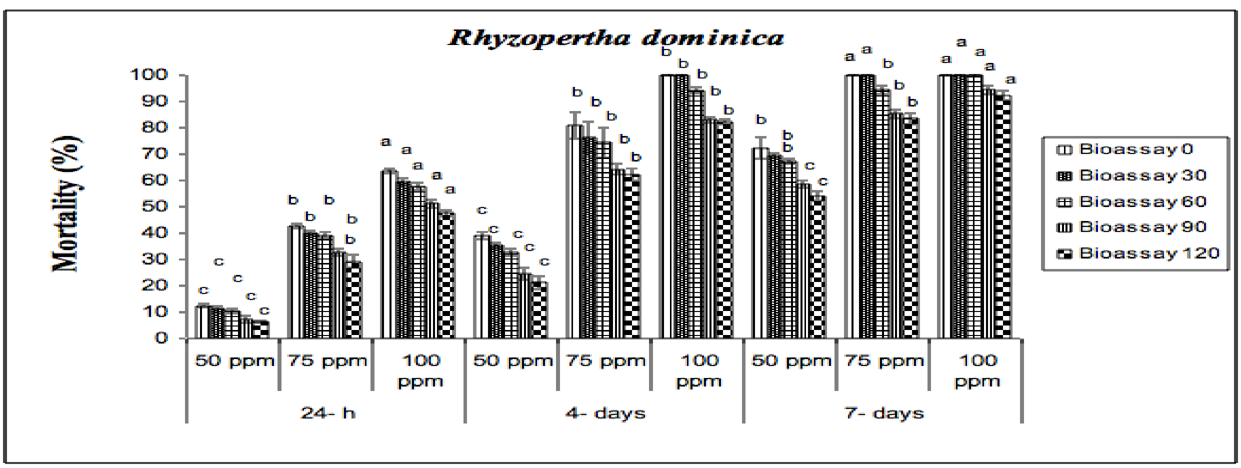

Figure 3. Mean mortality (% ± SE) of *Rhyzopertha dominica* exposed for 4 d in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by the same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

Tribolium castaneum

Mortality of *T. castaneum* increased with the exposure intervals and dose rate. Minimum mortality (4.90%) occurred at 50 mg/kg after 24 h following 120 d of storage. However, increasing the exposure interval, increased the adult mortality to 38.1 and 46.2% after 4 d and 7 d, respectively (Figure 4), at 50 mg/kg. A similar level of the mortality occurred for 60 d storage, but mortality levels decreased after 60 da. Progeny emergence (Figure 5) after exposure of this beetle was generally low (0.83 mean numbers of emerged adults) at 50 mg/kg and decreased with increase of dose (0.58 mean numbers of emerged adults) at 100 mg/kg in the initial bioassay.

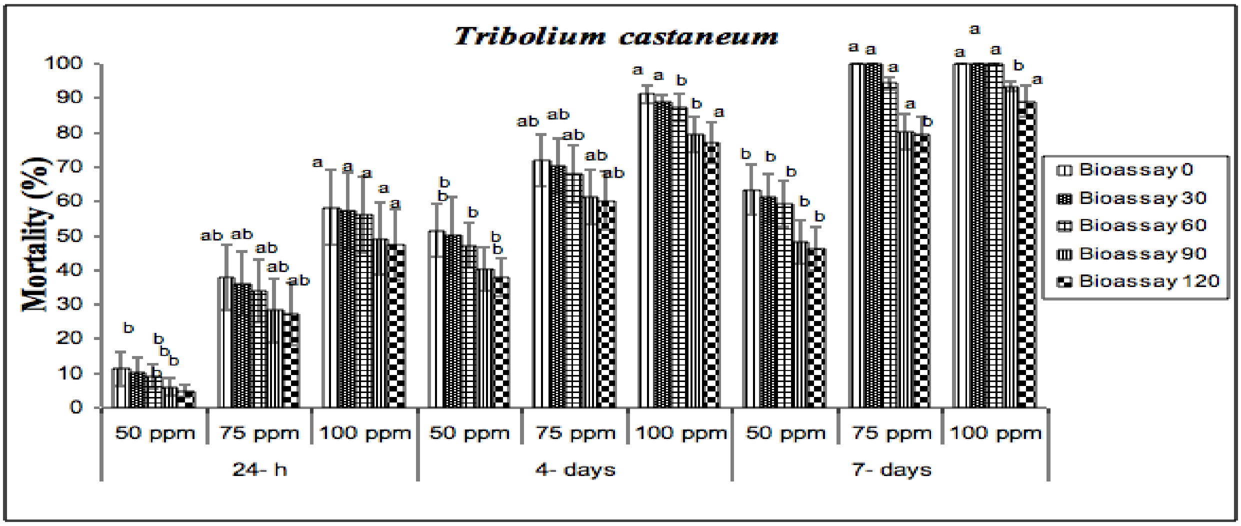

Figure 4. Mean mortality (% ± SE) of *Tribolium castaneum* exposed for 7 d in wheat grain treated with a diatomaceous earth product, Inert-PMS, at three doses in five bioassays conducted from 0 to 120 d after application. Means followed by the same letter are not significantly different from each other as indicated by the HSD test at 0.05%.

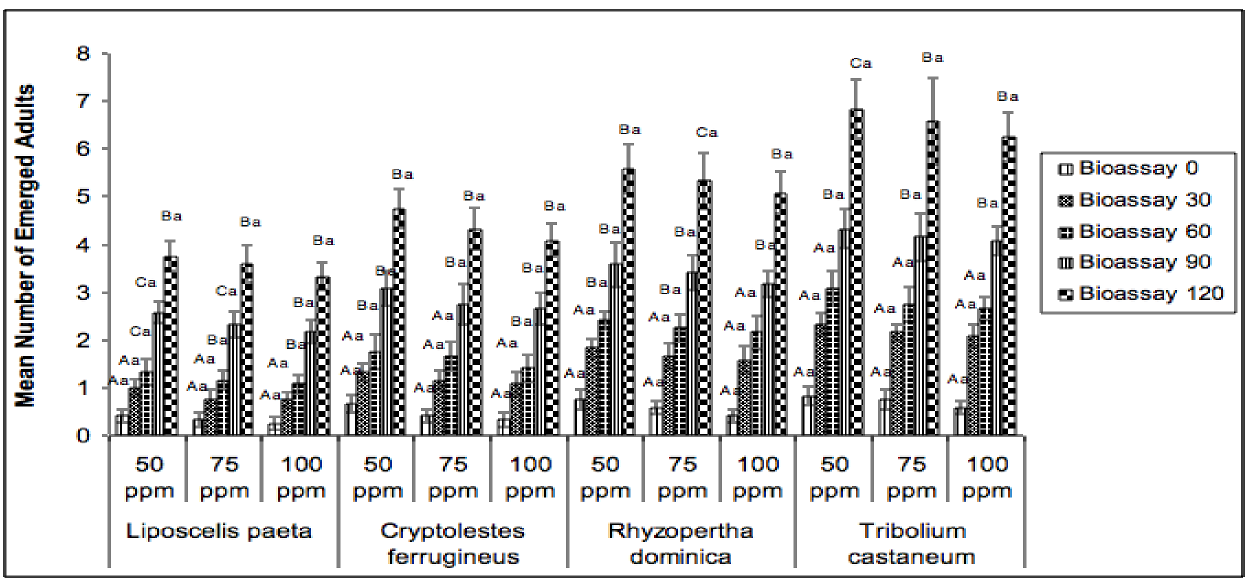

Figure 5. Mean number of emerged adults (±SE) of beetles, *Liposcelis paeta*, *Cryptolestes ferrugineus and Rhyzopertha dominica,* 63 d after the removal of adult insects from grain treated with Inert-PMS at three doses, and a psocid, *Tribolium castaneum,* after 30 d after the removal of adult insects. Means followed by the same lowercase letters (within each dose) and uppercase letters (within each bioassay) are not significantly different from each other as indicated by the HSD test at 0.05%.

Discussion

Earlier studies have shown that stored grain insect pests can be controlled by commercially available DE formulations. For example, Kavallieratos et al. (2005) used DEs Insecto and SilicoSec on eight different grain commodities, at 750 mg/kg and recorded mortality after 14 d of exposure ranging from 63 to 97%. Similarly, Vardeman et al. (2006) reported that Protect-It at the same exposure found that 400 mg/kg gave 85% adult mortality for *R. dominica*. However, our findings are consistent with the results reported in other studies (Fields & Korunic, 2000; Athanassiou & Kavallieratos, 2005). In addition to this, the dose rates used in the above studies were considerably higher. Korunic et al. (1998) noted that 500 mg DE/kg caused an 8% reduction in the bulk density of grain, when it was mixed with the whole grain mass. DE may be useful as a surface treatment to reduce the contact upon grain bulk density (Subramanyam et al., 1994). Our results clearly revealed that Inert-PMS can be used at doses that are significantly lower that older DEs, which in some cases, would be a reduction to 50 mg/kg. The increased efficacy of Inert-PMS can be attributed to the presence of the chemicals, spinosad and pirimiphos-methyl, included in its formulation. Our results indicated that Inert-PMS applied to stored grain is most effective against *L. paeta,* followed by *C. ferrugineus*, *R. dominica* and *T. castaneum*.

Athanassiou et al. (2006) reported 100% mortality of the exposed *R. dominica* adults after 14 d in wheat with 75 mg DEBBM (a diatomaceous earth formulation enhanced with bitterbarkomycin)/kg used as powder. However, our study indicated that Inert-PMS gave 100% mortality at 75 mg/kg after 4 d against all insects tested.

From our findings, Inert-PMS can be used successfully in stored wheat against stored grain insect pests, but its effectiveness could be influenced by several factors, such as the type of commodity, the application rate and the exposure interval. Treatment or wheat grain gave promising results, with the DE tested persisted for 60 d. It appears that the decline in persistence occurs gradually. This decline in persistence might be due to environmental conditions during the experiment as well as to kernel oil absorption by DE particles. According to Subramanyam & Roesli (2000), DEs provide safeguard to the grain as long as they remain dry and DEs are generally less effective under humid conditions (Arthur, 2000). For instance, Vayias & Athanassiou (2004) reported that SilicoSec was more effective against *Tribolium confusum* Jacquelin du Val adults and larvae at 55% than at 65% RH. Insects can moderate water loss under humid conditions, and this indirectly reduces DE efficacy (Subramanyam & Roesli, 2000; Mewis & Ulrichs, 2001).

In our study, Inert-PMS was effective in wheat for all insect species tested. Mortality was low at short exposure intervals (24 h) and increased with longer exposure. This shows that the chance of insects coming into contact with DE particles is improved with increasing exposure time leading to increased efficacy (Athanassiou et al., 2003, 2005). Our study shows that Inert-PMS is effective and could offer long-term protection of stored grain against the pest species tested.

DEs are generally slower acting than other grain protectants (Golob, 1997; Korunic, 1998), which may permit adults of insects to oviposit before dying and persist to cause grain damage (Subramanyam & Roesli, 2000). Hence, even if 100% parental mortality occurs, progeny emergence needs to be prevented due to have a zero threshold level for this pest (Athanassiou et al., 2003; Vardeman et al., 2006). With Inert-PMS parental mortality was high even after 4 d of exposure and this resulted in reduced progeny production. Furthermore, we observed that most of the individuals exposed to Inert-PMS during the first exposure interval had little or no activity.

In summary, Inert-PMS is effective even at low dose rates and potentially could become a vital element in an IPM-compatible strategy. However, there are numerous DEs that contain low to high concentrations of insecticides that cause both progeny suppression and adult mortality (Athanassiou et al*.,* 2004, 2006). Additional experimental work is needed to determine if Inert-PMS and other DE formulations are useful for on-farm insect management.

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