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# Insecticidal Efficacy of Iranian Diatomaceous Earths on Adults of *Oryzaephilus surinamensis*

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## ABSTRACT

**Diatomaceous Earth (DE) is a dust composed of fossilized bodies of unicellular algae used as an insecticide. In this study, the insecticidal potential of four Iranian DE deposits was assessed in adults of *Oryzaephilus surinamensis* (L.). Three of DE deposits were collected from Maragheh, Mamaghan, and Khorasan Jonoobi mines and Sayan® formulation was obtained from Kimia Sabz Avar Company. The insect adults were exposed on wheat, peeled barley, and rice treated with 300, 600, 1,000, 1,500, and 2,000 ppm of the DE deposits. Experiments were conducted at 27±1°C and 55±5% RH in continuous darkness. Mortality of the exposed individuals was counted after 2, 5, 10, and 14 days of exposure on the treated substrate. Mortality increased with increasing exposure intervals and concentration level. Satisfactory level of protection was observed on wheat grain; as 100% mortality was recorded on wheat treated with 600 ppm of all tested DE deposits after a 10-day exposure. However, for rice, even after 10 days of exposure to 2000 ppm mortality didn't reach 100%. The lowest $LC_{50}$ value (39 ppm) was obtained in wheat treated with Khorasan DE sample and the highest one (908 ppm) was achieved in rice treated with Sayan®. The present study suggests that Iranian DEs may be used as potential grain protectants in stored-product pest management programs.**

**Keywords:** Barley storage, Pest management, Rice storage, Stored grain protectant, Wheat storage.

## INTRODUCTION

Diatomaceous Earth (DE) is amorphous silicon dioxide, made from the fossilized remains of diatoms (Korunic, 1998). DE has long been known as a potentially useful grain protectant because it is considered as safe and is registered as food additive in the USA and Canada. DE has low mammalian toxicity, does not affect grain end-use quality, and provides long-term protection of stored grains (Fields, 1998). Although several modes of action of DE have been proposed, it is generally accepted that DEs absorb wax layer of the insect exocuticle, resulting in death due to desiccation and, to a lesser degree, abrasion (Athanassiou *et al*., 2003; Ebeling, 1971).

The insecticidal effectiveness of different DE deposits has been studied. Vayias *et al.* (2009) evaluated the insecticidal activity of DE samples collected from different locations of south-eastern Europe against adults of *Cryptolestes ferrugineus* (Stephens), *Sitophilus oryzae* (L.), and *Rhyzopertha dominica* (F.). Golestan-Hashemi *et al.* (2011) assessed toxicity of Sayan® formulation of Iranian DE against adults of *Tribolium confusum* Jacquelin du Val. The

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efficacy of two Iranian deposits of DEs obtained from Maragheh and Mamaghan mines, at four particle size (0–149, 74–149, 0–74, 0-37 µm) was investigated on *T. confusum* adults (Ziaee and Moharramipour, 2012) and *Sitophilus granarius* (L.) (Ziaee *et al.*, 2013). The potential insecticidal effectiveness of Croatian DE at different particle sizes (0-20, 0-45, 0-150, 20-45 and 45-150 µm) was assessed on adults of *C. ferrugineus*, *S. oryzae*, *R. dominica* and *Tribolium castaneum* Herbst (Korunic *et al*., 2009).

Several factors including DEs $SiO_2$ content, particle size, tapped density, oil absorbency, pH, insect species, grain moisture content, temperature, and method of application influence the insecticidal effectiveness of DE samples (Shah and Khan, 2014). In addition, DEs efficacy varies with the grain type and grain classes (Ziaee, 2015, Ziaee *et al.*, 2007). The origin of diatoms can also affect their insecticidal potential. DEs from different geological origins have different physical characteristics ($SiO_2$ content, tapped density, oil absorbency, particle size and pH) that make a difference in their insecticidal efficacy (Shah and Khan, 2014). Iran has large deposits of diatoms, but a few of them are mining. Maragheh, Mamaghan and Khorasan Jonoobi mines are important diatomite mines of Iran with marine origin. Several techniques such as the use of ozone (Hansen *et al*., 2012), insect pathogens (Khashaveh *et al*., 2010, Rumbos and Athanassiou, 2012), and essential oils (Rajendran and Sriranjini, 2008) are used to control insect pests in stored products. But, most of them are impractical for large-scale applications. Diatomaceous earth is already a practical alternative to synthetic pesticides and has been used in large scale grain storages and silos for controlling stored grain insects (Korunic, 2013).

*Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), saw-toothed grain beetle, is found worldwide and shows preference to cereals and cereal products (Rees, 1996). The insects are also found on copra, nuts, spices, and dried fruits. They cause direct damage by eating. However, the larvae bore into damaged grains to feed selectively on the germ, and they attack the germ region of intact grains and packing materials may be damaged (Hill, 2002). Marouf *et al*. (2004) expressed that *Trogoderma granarium* Everts, followed by *T. confusum*, *O. surinamensis*, *R. dominica*, and S. *granarius* have the highest frequency in wheat and barley storages and silos of Iran. Their damage to stored wheat in storages of Tehran, Hamdan, Zanjan, Khouzestan and Sistan-Baluchestan Provinces was recorded at 6.85, 1.86, 3.20, 12.64, and 44.03%, respectively. Besides, *O. surinamensis* have also been found in nuts, and dried fruits warehouses of Iran; but their damage has not been determined (Personal communication).

The aims of the current study were to investigate the insecticidal potential of Iranian DEs against adults of *Oryzaephilus surinamensis* on wheat, peeled barley, and rice and to assess the effects of concentration level and time intervals on the efficacy of DE deposits.

## MATERIALS AND METHODS

### Insects

Adults of *O. surinamensis* were reared on rice plus 5% (by weight) brewer's yeast; at 28°C, 65±5% RH under continuous darkness in Entomology Laboratory, Shahid Chamran University. Adults used in the experiments were 7-14 days old of mixed sex (Arthur, 2000).

### Commodity

Wheat (variety Chamran), peeled barley (variety Jonoob), and rice (variety Anbar) were used in the experiments. Wheat, barley and rice kernels were obtained from Safiabad Agricultural Research Center of Dezful and maintained at -24°C for at least 2 days. Before the experiments started, the kernels were kept for a week in incubators set at 27±1°C and





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55±5% RH to raise the moisture content to near-the-environmental relative humidity. The moisture content of wheat was measured by milling, then, drying 10 g of wheat in a ventilated oven set at 110°C. The moisture content of wheat, barley, and rice was 11.5, 11.6 and 12%, respectively. For all commodities tested, 5% cracked seeds were included in the sample both to represent actual practice and to ensure food was accessible for the beetles.

### DE Deposits

Four Iranian DE deposits from Maragheh, Mamaghan, and Khorasan Jonoobi mines and Sayan® formulation were used in the experiments.

Maragheh DE was obtained from Azerbaijan Mineral Region Cooperation Company. Diatomite mine Aygoosh Maragheh is located in northwestern Iran, 20 km from Kamel Abad village (37° 22' 41.39" N 46° 19' 28.16" E). Mamaghan DE was collected from a mine in Mamaghan region, north-west of Iran five km south of Tabriz (37° 50' 18.04" N 46° 2' 25.70" E) and 1,468 m above sea level. Khorasan Jonoobi DE was obtained from Rahim Zadeh and Saboori Company. Diatomite mine is located in Khorasan Jonoobi, Birjand, Sarbishe region, Mud district, Esfezar village (32° 42' 31.92" N 59° 31' 27.68" E) and 1,839 m above sea level. Sayan® DE was obtained from Kimia Sabz Avar Co. (Tehran, Iran).

The method of preparing DE samples was the same as Ziaee and Moharramipour (2012) with some modifications. DE deposits were milled, dried in an oven set at 100°C for 24 h to achieve about 3-6% moisture content. Then, DE samples were sifted using Damavand lab 170 mesh sieve to obtain particles less than 88 µm.

### Experiments

Each DE deposit was applied at five rates 300, 600, 1,000, 1,500 and 2,000 ppm and each rate was replicated six times. Twenty grams of each commodity (whole+5% cracked) were taken and placed in a small glass vial (2 cm diameter and 4 cm height). The samples were treated individually with the respective quantities of DEs. Subsequently, the vials were shaken for 5 min to obtain an even distribution of the DE on the seed samples. Twenty adults of *O. surinamensis* were introduced into each glass vial which was covered with muslin cloth to provide sufficient ventilation. The untreated commodities served as the control treatment with six replicates for each commodity. The vials were then placed in incubator set at 27±1°C and 55±5% RH. Mortality was measured after 2, 5, 10 and 14 days of exposure. When no leg or antennal movements were observed, insects were considered dead.

In order to measure the lethal concentration of DE samples that causes 50% mortality on *O. surinamensis* adults, another experiment was conducted. Condition of this experiment was the same as the previous one, except that different concentrations of the DEs were applied. Concentration setting experiment was carried out to determine a range of concentrations that cause between 20–80% mortality (Robertson *et al.*, 1984). The mortality was counted after 7 days of exposure.

### Data Analysis

No mortality was observed in the control group, so there was no need for data correction. Data of the first experiment was transformed to square root of arcsine (Sokal and Rohlf, 1995) to standardize means and normalize variances. But, non-transformed data are presented in the figures. A one-way Analysis of Variance was performed to determine the effects of DE deposits at different exposure intervals at each concentration level on adult’s mortality (SPSS, 2007). Means were separated using Tukey Multiple Range Test at $P< 0.05$. Data obtained from concentration-response bioassay were subjected to Probit analysis (Finney, 1971) to estimate lethal concentration ($LC_{50}$) and 95% confidence limits using SPSS software version 16.0 at *P*< 0.05 (SPSS 1999).

## RESULTS

Adult's mortality was increased as time of exposure to the DE samples increased. The mortality was recorded at 27.5, 54, 68, and 34% at the lowest concentration of 300 ppm of Maragheh, Mamaghan, Khorasan and Sayan®, respectively; which increased to100% after 14 days exposure to all tested DE samples on wheat. Regardless of high exposure time, the mortality of adults increased with increasing concentration level. Almost, complete mortality was observed on wheat treated with 2,000 ppm of each DE samples (except for Khorasan with 99% mortality) within 2 days of exposure (Figure 1).

Similar trends were observed in barley of which the mortality increased for all DE samples with increasing time of exposure to each concentration. Adults mortality on barley treated with 300 ppm of Maragheh, Mamaghan, Khorasan and Sayan® DE samples at the 2-day exposure interval was recorded at 9, 6, 10, and 2%, respectively. However, the mortality reached 55, 66, 86, and 71% after 14 days of exposure. At the highest concentration (2000 ppm) on barley, mortality was significantly higher even after a 2-day exposure (98, 100, 75, and 93%, respectively) and reached 100% after 14 days of exposure (Figure 2).

Generally, the mortality was too low when adults were exposed to rice treated with 300 ppm of DE after 2 days from commencement of exposure, and there were no significant differences among the four DEs tested. However, the mortality increased with increasing time interval and reached 75, 87, 72, and 39% after 14 days exposure to Maragheh, Mamaghan, Khorasan, and Sayan® DEs, respectively. Mortality of adults after a 2-day exposure to rice treated with 2,000 ppm of Maragheh, Mamaghan, Khorasan, and Sayan® DEs was 51, 67, 59, and 45%, respectively. It must be noted that 100% mortality was recorded on the adults after 14 days of exposure (Figure 3).

Based on $LC_{50}$ values and their confidence limits, *O. surinamensis* adults were more susceptible in wheat kernel. Sayan® followed by Khorasan were the least effective DE samples in rice and 908 ppm of Sayan® was needed to achieve 50% mortality after 7 days of exposure (Table 1).

## DISCUSSION

Findings of the current study indicate that Iranian DE samples have potent toxicity against adults of *O. surinamensis.* The insecticidal activity of DEs varied with commodity, concentration level, and extension of exposure times. Our results are in accord with reports by Golestan-Hashemi *et al.* (2011) that long exposed time is essential to achieve desirable results by Iranian DE deposits. They stated that 14 days of exposure to 315 ppm of Irainan DE, Sayan®, caused complete mortality on *T. confusum* adults on wheat. Results of this study showed that 300 ppm of all tested DE samples caused more than 95% mortality on *O. surinamensis* on wheat after 10 days, which increased to 100% after 14 days. Therefore, mortality increased as the exposure interval increased.

According to the literature review, the efficacies of DE formulations differ on different commodities. Among cereals, DE formulations are more effective on wheat and less effective on paddy rice and barley, followed by maize (Athanassiou *et al.*, 2003, Athanassiou *et al.*, 2008, Kavallieratos *et al.*, 2005). Lower effectiveness of DEs in rice and barley may be explained partly by smoother external surface and lower degree of DE particles adhesion to the kernels (Athanassiou *et al.*, 2003). Thus, long-term protection of these kernels is essential to obtain a satisfactory level of protection.





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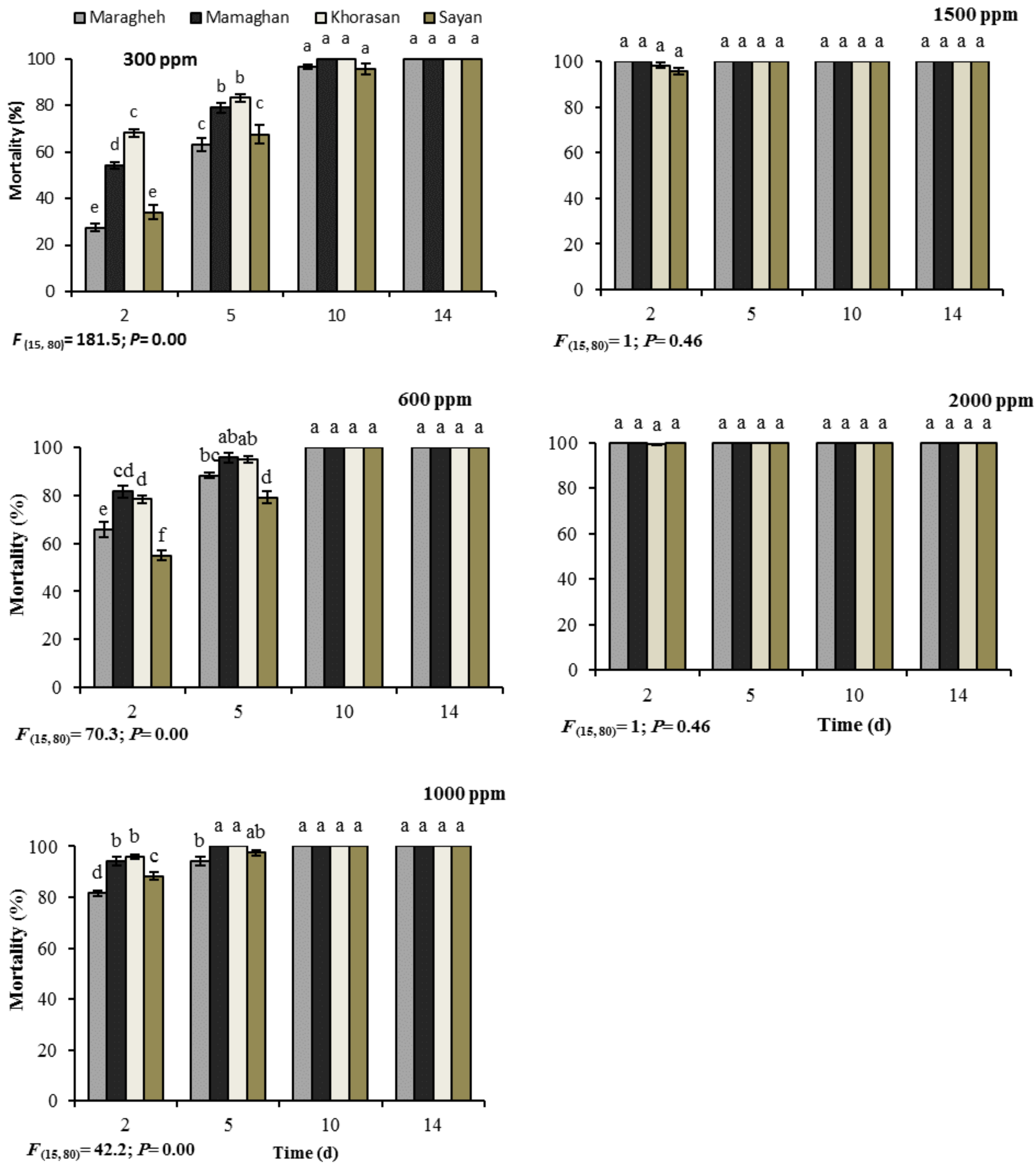


**Figure 1.** Mean mortality (%)±SE of *Oryzaephilus surinamensis* adults exposed to different DE deposits on wheat. Means followed by the same letter are not significantly different using Turkey's test at $P$< 0.05.



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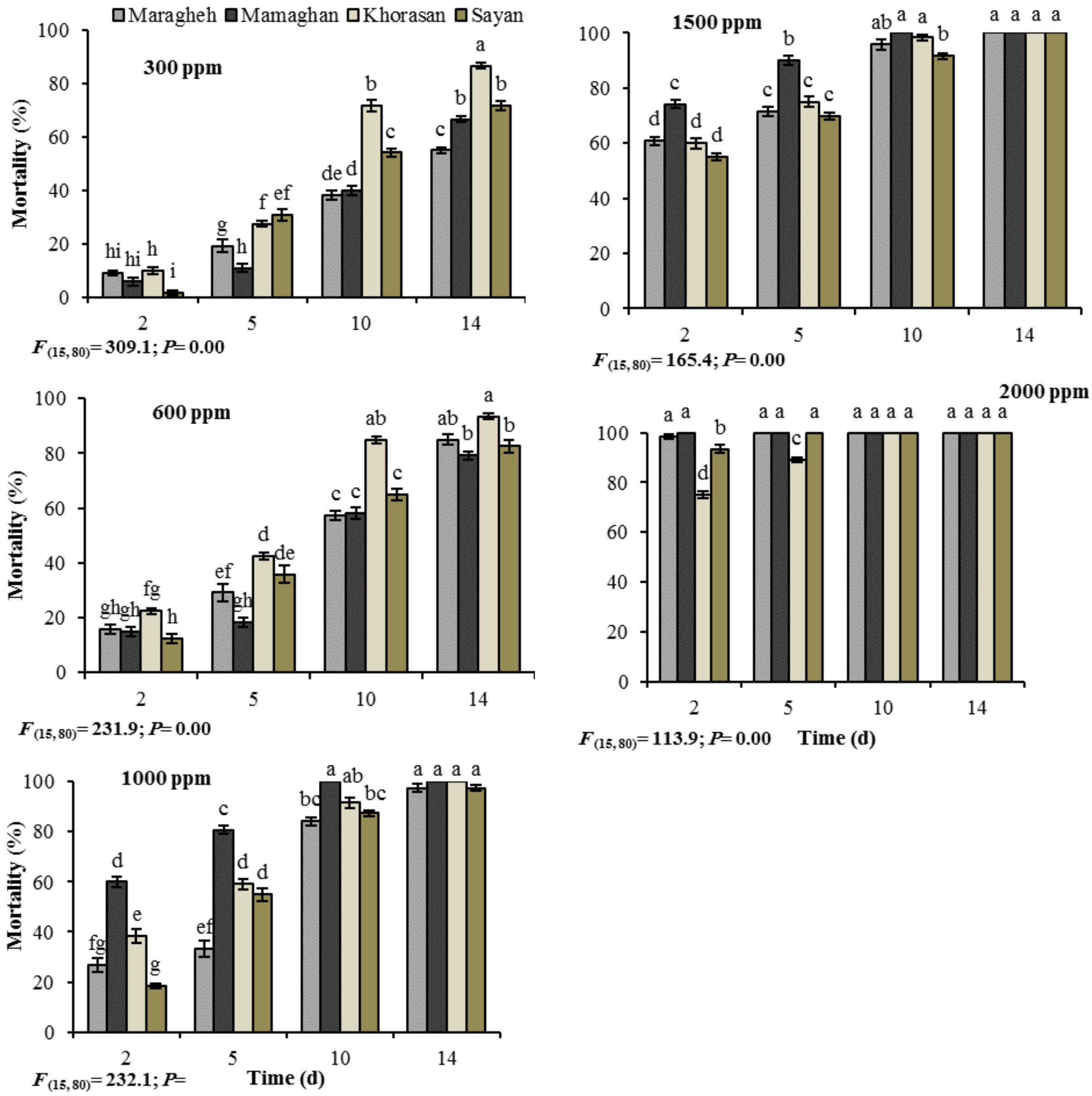


**Figure 2.** Mean mortality (%)±SE of *Oryzaephilus surinamensis* adults exposed to different DE deposits on barley. Means followed by the same letter are not significantly different using Turkey's test at *P*< 0.05.

Our findings indicated that DEs effectiveness decreased in rice and barley, in accordance with Kavallieratos *et al.* (2005). They reported that the efficacy of Insecto® and SilicoSec® DE formulations against adults of *R. dominica* on peeled barley was less than whole barley. They stated that reduced efficacy of the DEs may be due to the reduction in DE adhesion to the grains because of the removal of the seed coat. Aldryhim (1993) stated that the efficacy of DEs on different commodities was related to: (1) Degree of DE particles adhesion to kernels, and (2) Rate of DE particles picked up by insects cuticle. In addition, high oil content of some kernels such as maize





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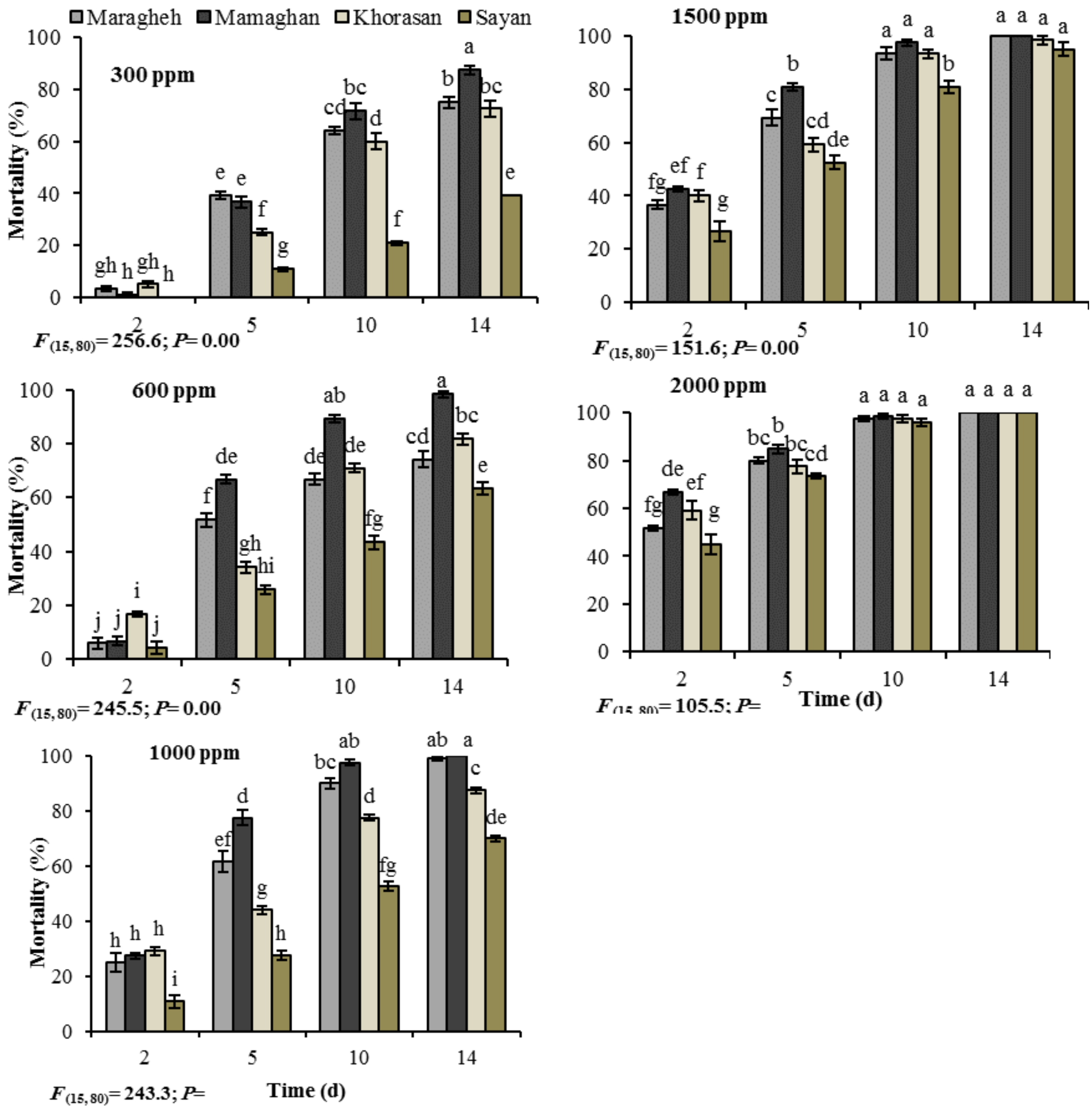


**Figure 3.** Mean mortality (%)±SE of *Oryzaephilus surinamensis* adults exposed to different DE deposits on rice. Means followed by the same letter are not significantly different using Turkey's test at $P< 0.05$.



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(Athanassiou *et al.*, 2008, Vayias *et al.*, 2006) and oilseeds (Ziaee *et al.*, 2007) may be a factor contributing to the toxicity of DE samples. High oil content may reduce the insecticidal activity of DE formulations due to adsorption of lipids from kernels surfaces. Apart from oil content of the kernel, other physical, chemical, structural, and compositional properties of the grain influence DE particles retention and their efficacy on different kernels (Athanassiou *et al.*, 2003, Kavallieratos *et al.*, 2005). Ziaee *et al.* (2007) speculated that absorption of lipid content of kernels would detract its ability to absorb insect wax and DEs become inactivated.

The type, origin, and $SiO_2$ content of DEs also affect their insecticidal potential (Shah and Khan, 2014). Rojht *et al.* (2010) stated that although constituents of DE maybe considered as a valuable tool for predicting its insecticidal efficiency, SiO2 or opal-content

**Table 1.** Lethal concentration of Iranian DE deposits on *Oryzaephilus surinamensis* (df= 3).

| Iranian DEs | Commodity | $LC_{50}$ (ppm) | Cl (ppm) [a] Lower | Upper | $LC_{90}$ (ppm) | Slope | $K^2$ | *P* value |
|---|---|---|---|---|---|---|---|---|
| Maragheh | Wheat | 101.9 | 92.5 | 111.1 | 264.1 | 3.10 | 0.37 | 0.94 |
| | Barley | 401.4 | 362.7 | 439.0 | 1079.2 | 2.98 | 0.33 | 0.95 |
| | Rice | 416.9 | 352.1 | 485.0 | 2442.9 | 1.66 | 0.39 | 0.94 |
| Mamaghan | Wheat | 51.7 | 25.0 | 83.2 | 291.4 | 1.70 | 10.91 | 0.01 |
| | Barley | 328.2 | 307.7 | 348.0 | 642.2 | 4.3 | 2.31 | 0.51 |
| | Rice | 343.3 | 291.3 | 396.0 | 1620.0 | 1.90 | 0.59 | 0.89 |
| Khorasan | Wheat | 39.0 | 30.1 | 48.9 | 496.7 | 1.16 | 2.49 | 0.47 |
| | Barley | 310.9 | 267.2 | 355.6 | 1494.7 | 1.87 | 0.81 | 0.84 |
| | Rice | 430.0 | 369.6 | 493.0 | 2062.8 | 1.88 | 0.48 | 0.92 |
| Sayan | Wheat | 144.1 | 126.4 | 161.8 | 514.3 | 2.32 | 2.50 | 0.47 |
| | Barley | 468.4 | 411.0 | 525.4 | 1788.2 | 2.20 | 0.02 | 1.0 |
| | Rice | 908.5 | 802.6 | 1017.8 | 3412.2 | 2.23 | 2.48 | 0.47 |

[a] Confidence limit (95%).

are better able to express DE insecticidal effectiveness rather than the remaining minerals. Our previous study indicated that Mamaghan DE was more effective than Maragheh samples against adults of *T. confusum*. $SiO_2$ content of Mamaghan and Maragheh DEs were reported as 89.87 and 75.35%, respectively. High toxicity of Mamaghan DE deposits may attribute to high silicon dioxide content of the sample. All Iranian DEs applied in these experiments had marine origin (Ziaee and Moharramipour, 2012). McLaughlin (1994) and Snetsinger (1988) declare that DEs from marine origin are weak and cheaper than DEs with freshwater origin. However, Saez and Fuentes Mora (2007) noted that the efficacy of DEs is not influenced by their origin. The results of this study demonstrate that Khorasan Jonoobi and Mamaghan samples were the most, and Sayan® the least, effective DE samples.

It is evident from the results that the level of grains protection is influenced by the type of DE sample, type of grain, application rate, and time of exposure.

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# توانایی حشره کشی خاک‌های دیاتومه ایرانی روی حشرات کامل *Oryzaephilus Surinamensis*

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## چکیده

خاک دیاتومه، گردی است که از بقایای فسیلی جلبک تک سلولی به دست آمده و به عنوان حشره کش مورد استفاده قرار می‌گیرد. در این مطالعه، قدرت حشره کشی چهار نهشته ایرانی خاک دیاتومه روی حشرات کامل *Oryzaephilus surinamensis* (L.) مورد بررسی قرار گرفت. سه نهشته خاک دیاتومه از معدن‌های مراغه، ممقان، خراسان جنوبی جمع آوری شدند و فرمولاسیون Sayan® نیز از شرکت کیمیا سبز آور تهیه شد. حشرات کامل در گندم، جو پوست کنده و برنج تیمار شده با غلظت‌های ۳۰۰، ۶۰۰، ۱۰۰۰، ۱۵۰۰ و ۲۰۰۰ پی پی ام از هر نهشته دیاتومه قرار گرفتند. آزمایش‌ها در دمای ۱ ± ۲۷ درجه سلسیوس، رطوبت نسبی ۵ ± ۵۵ درصد و تاریکی انجام شد. مرگ و میر حشرات مورد آزمایش ۲، ۵، ۱۰ و ۱۴ روز پس از تیمار شمارش شد. مرگ و میر با افزایش مدت زمان و غلظت افزایش یافت. حفاظت رضایت بخشی در گندم مشاهده شد؛ به طوری که ۱۰۰ درصد مرگ و میر در گندم تیمار شده با غلظت ۶۰۰ پی پی ام از همه نهشته‌های ایرانی، ۱۰ روز بعد از تیمار گزارش شد. اگرچه، در مورد برنج حتی پس از گذشت ۱۰ روز از تیمار با غلظت ۲۰۰۰ پی پی ام، درصد مرگ و میر به ۱۰۰ درصد نرسید. کمترین مقدار $LC_{50}$ (۳۹ پی پی ام) مربوط به گندم تیمار شده با خاک دیاتومه خراسان و بیشترین مقدار آن (۹۰۸ پی پی ام) برای برنج تیمار شده با Sayan® بود. این مطالعه پیشنهاد می‌کند که خاک‌های دیاتومه ایرانی ممکن است به عنوان حفاظت کننده غلات در برنامه‌های مدیریت تلفیقی آفات محصولات انباری به کار گرفته شوند.




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