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RESEARCH ARTICLE

Persistence and Efficacy of Four Iranian Diatomaceous Earths Against Three Stored Grains Beetles

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Abstract Persistence and efficacy of four Iranian diatomaceous earth formulations was assessed against *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jacquelin du Val. on barley, rice and wheat. Three diatomaceous earth samples were collected from Maragheh, Mamaghan, and Khorasan Jonoobi mines and Sayan® formulation was obtained from Kimia Sabz Avar Company. Mortality of the exposed individuals was recorded after 2, 5, 10 and 14 days of exposure. The mortality of all tested species was higher on wheat followed by barley. The insect mortality increased with increasing concentration level. In most cases, Khorasan was the most effective diatomaceous earth deposit. LC_{50} values of Khorasan deposit was 155.3, 201.5 and 293.7 ppm for *S. oryzae, T. castaneum* and *T. confusum,* respectively when exposed for 7 days on treated wheat. Adults of *S. oryzae* were the most susceptible species to diatomaceous earths. Subsequently, the persistence of diatomaceous earths was assessed after 1, 2, 3, 4, 6 and 8 months of storage. Mortality of all the tested species was high in first four months of storage but decreased rapidly overtime.

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Introduction

Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the serious primary pests of stored grains worldwide. The pest mostly attacks all cereal grains and reduces the quality of stored grains. The red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and the confused flour beetle, *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae) are major international pests of stored cereals and various foodstuffs. Both adult and larvae of the two species feed on various commodities causing considerable financial losses. They are one of the most injurious pests of flours and other milled products [1].

Diatomaceous earths (DEs) have been known as stored-grain protectants. DE consists of fossilized skeletons of diatoms which are composed almost entirely of amorphous silicon dioxide. DEs absorb wax from the insect cuticle resulting in their death through desiccation and to a lesser degree by abrasion [2]. Low mammalian toxicity and long-lasting effect of DE make it more compatible for stored product protection [3]. DE has also been recognized as safe, and is registered as a food additive in the USA and Canada [4]. DE is recognized as a persistent control agent, leaving no residue in stored products but a few pest resistant problems have been reported to it [5].

Therefore, persistence of DE is important in order to increase its effectiveness in protecting products in small-scale storages. The insecticidal efficacy and persistence of two DE formulations Dryacide® and Protect-It® were investigated against four storage pests [6]. Athanassiou et al. [7] assessed toxicity and persistence of Insecto®, SilicoSec®, and PyriSec® against *S. oryzae* on wheat and barley. In the other case Vayias, Athanassiou, Kavallieratos, Tsesmeli and Th Buchelos [8] evaluated persistence of Insecto®, PyriSec® and SilicoSec® DE formulations against *T. confusum* on wheat and maize. However, in all cases, persistence of DEs has been reported, but their duration will depend on the formulation, storage conditions (temperature, humidity, etc.), pest species and treated commidity [5].

DEs are produced in more than 30 countries and most of their products are related to USA, France and Republic of Korea. These countries accounted for 61 % of the world production [9]. Iran has natural mines of DE, although a few of them are quarried and processed for water purification, commercial fluids filtration, clarification, and other industrial uses [10]. In the present study, the insecticidal potential of Iranian DEs has been evaluated in order to intoduce the most efficacoues one as a natural native insecticide for controlling insect pests of stored products. Therefore, the aim of the current study was to evaluate the insecticidal activity of four different Iranian DEs against adults of *S. oryzae*, *T. castaneum* and *T. confusum*; and to investigate the persistence of DEs in protection of wheat, barley and rice grains over an 8-month period.

Material and Methods

Used DE Deposits

Four Iranian DE samples were tested in the experiments. Three of DE deposits were collected from Maragheh, Mamaghan, and Khorasan Jonoobi mines.

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 5 km south of Tabriz (37°50′18.04″N 46°2′25.70″E). 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). Sayan® DE was obtained from Kimia Sabz Avar Company, Tehran-Iran. DEs tested in the experiments are from marine origins. DE samples were dried in an oven set at 100 °C for 24 h to obtain about 6 % moisture content and sieved using Damavand lab 170 mesh sieve to obtain particles less than 88 μm [11].

Insect Species

Sitophilus oryzae was reared on whole wheat, Chamran variety, while *T. castaneum* and *T. confusum* were reared on wheat flour plus 5 % brewer yeast (by weight). All the species were cultured at 27 ± 1 °C and 65 ± 5 % RH and held in continuous darkness in Entomology Laboratory, Shahid Chamran University, Ahvaz-Iran. The insects used in the experiments were 7–14 days old and of mixed sexes.

Commodity

The grains tested in the bioassays were wheat (Chamran variety), peeled barley (Jonoob variety) and rice (Anbar variety). The grains were obtained from Safiabad Agricultural Research Center of Dezful and maintained at −24 °C for at least 2 days. The grains were kept in an incubator set at 27 ± 1 °C and 55 ± 5 % RH for a week to achieve the moisture content related to environmental relative humidity. The moisture content of the grains was measured by milling then drying 10 g in a ventilated oven set at 110 °C. It was 11.5 % for wheat, 11.6 % for barley and 12 % in the case of rice. For *T. castaneum* and *T. confusum* species, 5 % cracked plus 95 % whole kernels were used in the test samples, both to represent actual practice and to ensure food accessible for the beetles. However, whole kernels were used for *S. oryzae* bioassays.

Bioassays

Insecticidal Efficacy Bioassay

Five concentrations of the DE deposits: 300, 600, 1000, 1500 and 2000 mg/kg of wheat, barley and rice (twenty DE deposits and concentration combinations for each type of commodity) were applied in the experiments. Each of DE deposits at five concentrations was separately mixed with the grains held in jars containing 300 g of the commodity. The jars were tightly sealed with lids and thoroughly shaken for 5 min to obtain an even distribution of the DE on the grain samples. Subsequently, the grains in the jars were divided in six glass vials with 50 g treated grains. Twenty adults of each species were introduced into each glass vial, which was covered with muslin cloth to provide sufficient ventilation. The untreated commodities served as the control treatment. The vials were then placed in incubator set at 27 ± 1 °C and 55 ± 5 % RH in continuous darkness. Mortality was recorded after 2, 5, 10 and 14 days of exposure. When no leg or antennal movements were observed, insects were considered dead.

Concentration–Response Bioassay

Another experiment was carried out to assess lethal concentrations which caused 50 % (LC_{50}) and 90 % mortality (LC_{90}). Preliminary test was conducted for each DE samples in each commodity to evaluate the concentrations (five concentrations) that caused 20–80 % mortality [12]. Therefore, different concentrations were applied in the bioassays. The conditions of the experiment were as above. The mortality was counted after 7 days of exposure.

Persistence Bioassay

The persistence of the tested DEs was evaluated for a period of 8 months. For this purpose, 1200 g of each grain was poured in a jar treated with DE deposits corresponding to LC_{90} values obtained from pervious experiment. The jars were shaken for 5 min and then sealed and were kept in the incubator set at 27 ± 1 °C and 55 ± 5 % RH until testing. The first persistence bioassay was carried out one month after treatment and continued after 2, 3, 4, 6 and 8 months of first treatment. Therefore, six bioassays were performed. In each bioassay, four samples of 50 g each were taken from each jar and poured to the vials (four replications). Untreated grains were applied as control. Then, 25 adults of each species were introduced to each vial separately and the vials were placed in the incubator with above conditions. The mortality was counted 7 days after exposure.

Data Analysis

There was no mortality in control groups, so, there was no need to correct the mortality data. Mortality percentages were analyzed by using one-way analysis of variance to determine significant differences. Means were separated by Tukey test at $P = 0.05$.

Data obtained from concentration–response bioassay were subjected to Probit analysis [13], to estimate lethal concentration (LC_{50} and LC_{90}) using SPSS software version 16.0 at $P = 0.05$ [14].

Results and Discussion

For *S. oryzae*, all main effects (type of commodity: $F_{11,\ 1428} = 157.2$; concentration: $F_{19,\ 1420} = 155.0$; DE deposit: $F_{15,\ 1424} = 62.9$) were significant at the $P < 0.0001$ level (Fig. 1). The mortality of *S. oryzae* was higher in wheat grains rather than barley and rice. The mortality was highly influenced by concentration level of DE and time of its exposure. The lowest rate of mortality was observed when insects were exposed to 300 mg/kg of

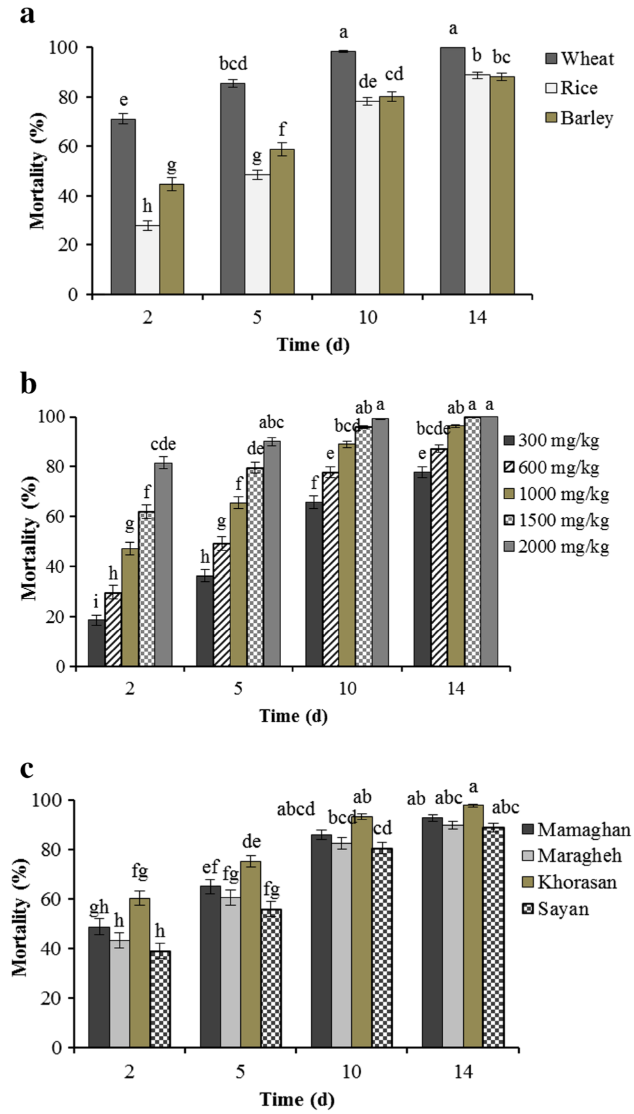

Fig. 1 Mean mortality (%) ± SE of *Sitophilus oryzae* adults exposed to wheat, rice, and barley (mean of all concentrations of all the DEs) (**a**) treated with DE at five concentrations (mean of all the DEs on all the crops) (**b**) and four DE deposits (mean of all concentrations on all the crops) (**c**) after 2, 5, 10 and 14 days of exposure. Means followed by the same letter are not significantly different using Turkey's Test at $P < 0.05$

DE samples. However, the efficacy of 1500 and 2000 mg/kg was more or less the same. At 2 and 5 days exposure time, the insecticidal efficacy of Khorasan DE sample was more than the rest. However, after 14 days of exposure, no significant differences were noted in mortality levels among the tested DE deposits (Fig. 1).

The mortality percent of *T. castaneum* is presented in Fig. 2. All main effects (type of commodity: $F_{11,\ 1428} = 388.4$; concentration: $F_{19,\ 1420} = 204.0$; DE deposit: $F_{15,\ 1424} = 224.5$) were significant at the $P < 0.0001$ level. After 14 days of exposure, wheat was the most protected commodity from *T. castaneum*

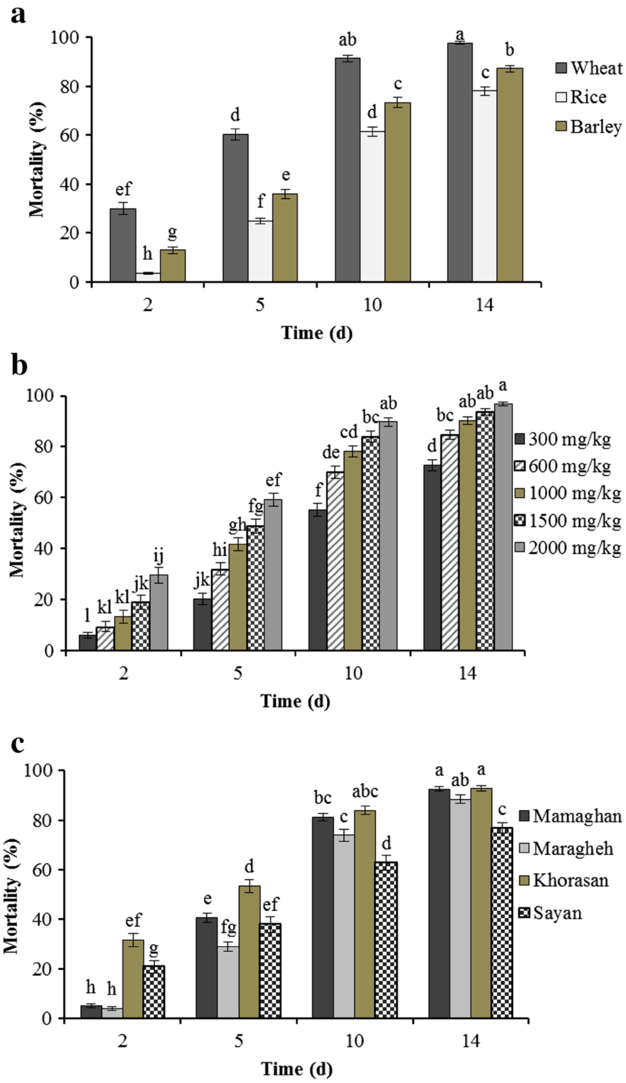

Fig. 2 Mean mortality (%) ± SE of *Tribolium castaneum* adults exposed to wheat, rice, and barley (mean of all concentrations of all the DEs) (**a**) treated with DE at five concentrations (mean of all the DEs on all the crops) (**b**) and four DE deposits (mean of all concentrations on all the crops) (**c**) after 2, 5, 10 and 14 days of exposure. Means followed by the same letter are not significantly different using Turkey's Test at $P < 0.05$

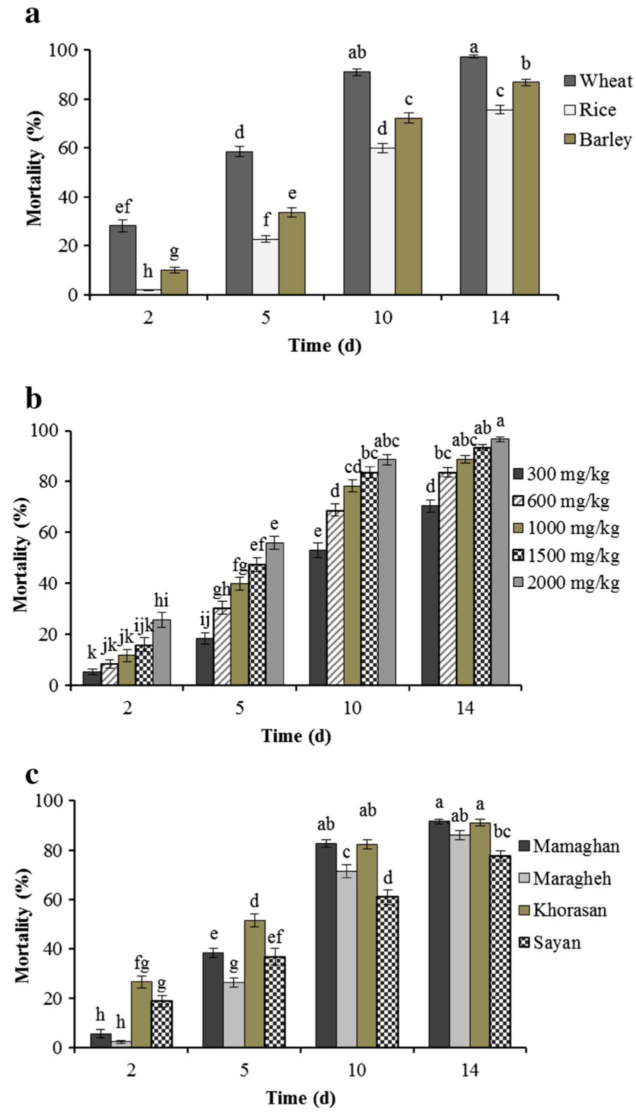

Fig. 3 Mean mortality (%) ± SE of *Tribolium confusum* adults exposed to wheat, rice, and barley (mean of all concentrations of all the DEs) (**a**) treated with DE at five concentrations (mean of all the DEs on all the crops) (**b**) and four DE deposits (mean of all concentrations on all the crops) (**c**) after 2, 5, 10 and 14 days of exposure. Means followed by the same letter are not significantly different using Turkey's Test at $P < 0.05$

infestations. The mortality of *T. castaneum* adults increased with the increase of the DE concentration and time exposed to each concentration. After 2 and 5 days of exposure, Khorasan was significantly the most effective DE sample. After 14 days of exposure, there were no significant differences among Mamaghan, Maragheh and Khorasan DEs. The insecticidal efficacy of Sayan® was always the lowest (Fig. 2).

For *T. confusum*, all main effects (type of commodity: $F_{11,\ 1428} = 386.0$; concentration: $F_{19,\ 1420} = 198.0$; DE deposit: $F_{15,\ 1424} = 213.8$) were significant at the $P < 0.0001$ level. Just like *S. oryzae* and *T. castaneum*, for *T. confusum* also DE samples were more effective on wheat followed by barley. In early time intervals after treatment (2 and 5 days), the efficacy of Khorasan DE deposit was more than that of others. But after 10 days, there was no significant difference between Khorasan and Mamaghan. However, after 14 days, all DE samples showed the same levels of mortality against *T. confusum* adults (Fig. 3).

Lethal concentrations of different Iranian DE deposits which caused 50 and 90 % mortality on *S. oryzae* adults are presented in Table 1. Susceptibility of *S. oryzae* was more to Khorasan ($LC_{50} = 351.5$ ppm for barley, 492.7 ppm for

Table 1 Lethal concentration of different Iranian DE deposits on *Sitophilus oryzae* ($df = 3$)

Iranian DEs	Commodity	LC_{50} (ppm)	Cl (ppm)		LC_{90} (ppm)	Slope	κ^2	*P* value
			Lower	Upper				
Maragheh	Barley	634.2	570.7	696.7	1863.3	2.73	0.66	0.88
	Rice	688.3	590.3	788.8	3249.7	1.90	2.24	0.52
	Wheat	221.8	202.3	241.3	577.4	3.08	3.70	0.29
Mamaghan	Barley	543.3	487.5	596.2	1445.2	3.01	0.17	0.98
	Rice	602.1	509.6	695.3	2952.5	1.85	2.21	0.53
	Wheat	191.01	172.8	208.6	488.6	3.14	0.18	0.98
Khorasan	Barley	351.5	303.6	402.5	1775.8	1.82	4.03	0.25
	Rice	492.7	416.4	577.6	3301.1	1.55	3.36	0.33
	Wheat	155.3	141.3	168.2	396.5	3.14	0.52	0.91
Sayan	Barley	663.1	603.7	722.1	1750.2	3.04	0.58	0.90
	Rice	726.6	620.4	836.7	3748.6	1.79	0.16	0.98
	Wheat	212.1	192.1	231.7	575.8	2.95	0.76	0.85

Cl confidence limit (95 %)

Table 2 Lethal concentration of different Iranian DE deposits on *Tribolium castaneum* ($df = 3$)

Iranian DEs	Commodity	LC_{50} (ppm)	Cl (ppm)		LC_{90} (ppm)	Slope	κ^2	*P* value
			Lower	Upper				
Maragheh	Barley	756.6	657.4	854.7	2964.1	2.16	0.31	0.95
	Rice	920.4	804.7	1033.6	3302.6	2.31	4.29	0.23
	Wheat	580.5	517.1	645.7	1999.2	2.38	0.18	0.97
Mamaghan	Barley	435.6	347.4	528.1	3665.4	1.38	0.93	0.81
	Rice	764.9	615.8	920.0	6479.2	1.38	0.17	0.98
	Wheat	356.8	298.3	416.7	2060.2	1.68	0.61	0.89
Khorasan	Barley	311.1	263.3	360.4	1784.6	1.68	0.46	0.92
	Rice	744.2	601.2	892.5	5979.6	1.41	6.13	0.10
	Wheat	201.5	176.3	225.8	763.8	2.21	0.48	0.99
Sayan	Barley	866.1	330.2	1696.4	16279.0	1.00	8.12	0.04
	Rice	1078.0	932.3	1229.6	4941.0	1.93	0.43	0.93
	Wheat	476.2	424.5	529.9	1730.0	2.28	6.43	0.092

Cl confidence limit (95 %)

rice, and 155.3 ppm for wheat) followed by Mamaghan DE deposit ($LC_{50} = 543.3$ ppm for barley, 602.1 ppm for rice, and 191.01 ppm for wheat). However, in some cases the confidence limits (95 %) of LC_{50} values were overlapped. All tested DE samples were more effective on wheat followed by barley and rice (Table 1).

For *T. castaneum*, Khorasan DE sample ($LC_{50} = 201.5$ ppm) was most effective in protecting wheat followed by Mamaghan ($LC_{50} = 356.8$ ppm) deposit. However, Sayan® ($LC_{50} = 476.2$ ppm) and Maragheh ($LC_{50} = 580.5$) were the least effective DE deposits against *T. castaneum* adults. The same trend was almost true in case of rice and barley. Among three tested commodities, DE samples were more effective on wheat followed by barley (Table 2).

Based on LC_{50} values, *T. confusum* adults were significantly more susceptible to Khorasan and Mamaghan DEs on different commodities. LC_{50} values were calculated 293.7, 433.5 and 795.7 ppm for Khorasan on wheat, barley and rice, respectively; whereas 386.3, 482.7, and 805.5 ppm were calculated in the case of Mamaghan DE deposit, respectively (Table 3).

Results of this study showed that Iranian DEs could be successfully applied to protect wheat, barley and rice against *S. oryzae*, *T. castaneum* and *T. confusum*. The effectiveness of DEs is influenced by type, quality and properties of commodity [2, 7, 15, 16]. In the present study, all tested DE deposits have more insecticidal toxicity on wheat followed by barley. However, in most cases the protection efficacy of DEs on wheat was reduced during

Table 3 Lethal concentration of different Iranian DE deposits on *Tribolium confusum* ($df = 3$)

Iranian DEs	Commodity	LC_{50} (ppm)	Cl (ppm)		LC_{90} (ppm)	Slope	κ^2	*P* value
			Lower	Upper				
Maragheh	Barley	843.1	753.2	931.7	2629.2	2.59	0.77	0.85
	Rice	973.3	843.8	1106.9	4369.5	1.96	0.41	0.93
	Wheat	658.6	591.5	725.3	2034.5	2.61	0.13	0.98
Mamaghan	Barley	482.7	385.7	585.5	4354.3	1.34	0.33	0.95
	Rice	805.5	679.8	930.5	4123.5	1.80	1.42	0.70
	Wheat	386.3	317.8	456.8	2401.1	1.61	0.79	0.85
Khorasan	Barley	433.5	376.4	488.8	1702.5	2.15	2.23	0.52
	Rice	795.7	647.2	951.3	6362.4	1.41	3.73	0.29
	Wheat	293.7	258.4	330.6	1203.5	2.09	5.61	0.13
Sayan	Barley	918.7	819.6	1016.4	2866.0	2.59	0.94	0.81
	Rice	1257.5	1107.9	1596.7	4629.3	2.26	1.16	0.76
	Wheat	511.5	454.3	568.8	1689.0	2.47	5.03	0.16

Cl confidence limit (95 %)

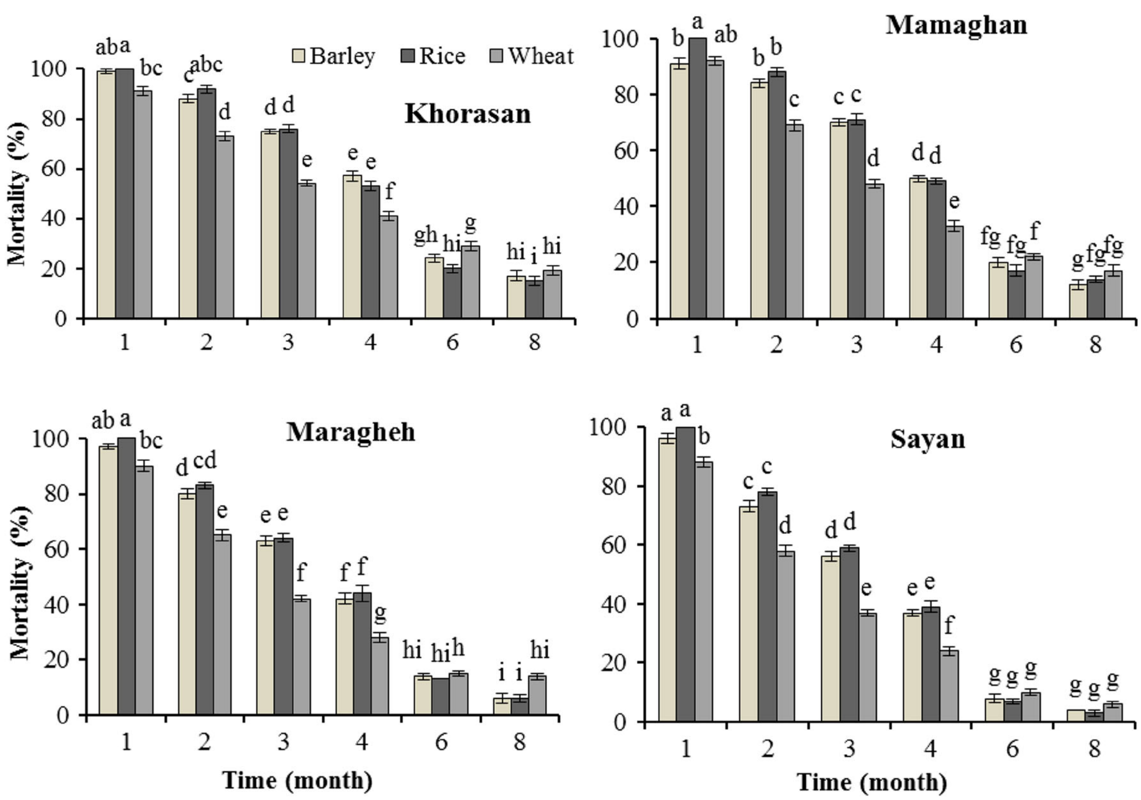

Fig. 4 Persistence of Iranian DE samples in barley, rice and wheat against *Sitophilus oryzae*. Means followed by the same letter are not significantly different using Turkey's Test at $P < 0.05$

the storage period. This trend was also observed by Athanassiou et al. [7] who reported that in the first 45 days of treatment with Insecto®, SilicoSec®, and PyriSec® DE formulations, *S. oryzae* mortality was significantly more on wheat than barley. However, similar mortality was observed in both commodities with increasing time of storage. The possible reasons for the reduced *S. oryzae* mortality in wheat than barley over time may be attributed

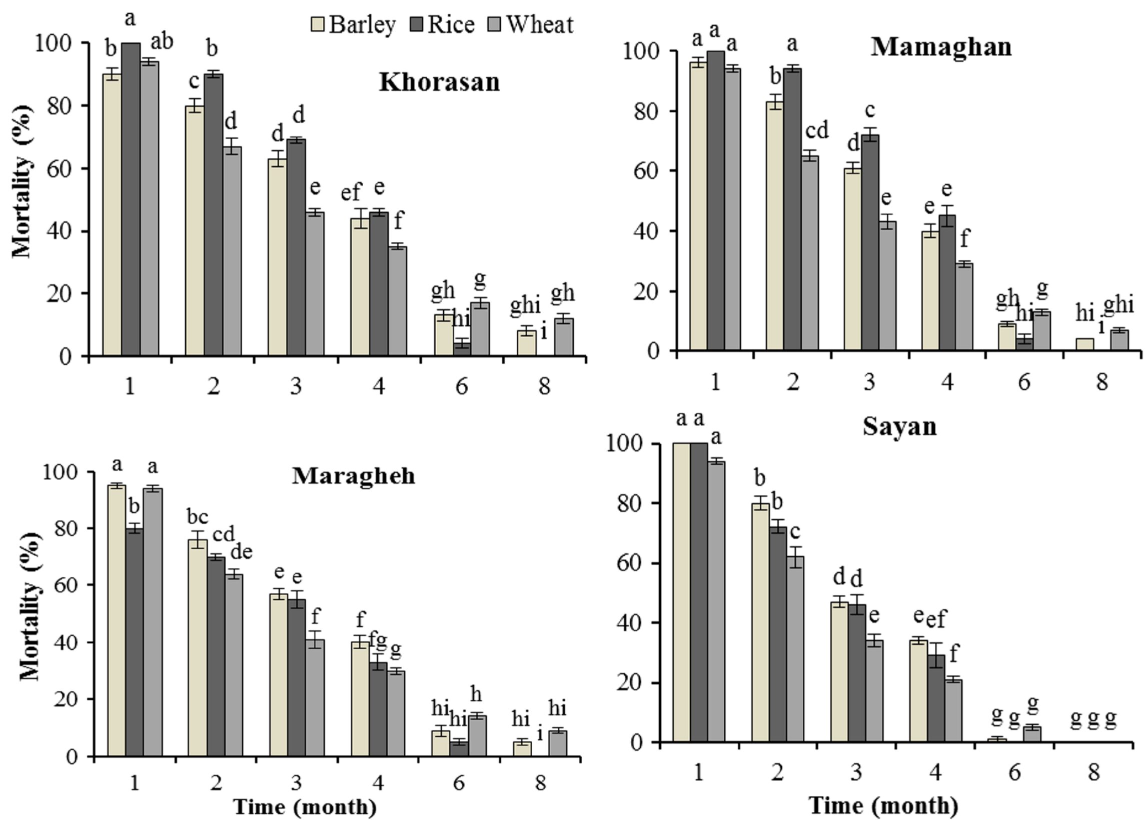

Fig. 5 Persistence of Iranian DE samples in barley, rice and wheat against *Tribolium castaneum.* Means followed by the same letter are not significantly different using Turkey's Test at $P < 0.05$

to the faster oil absorption on wheat than on barley, or the high oil composition of wheat kernels. According to the present findings, regardless of the commodity type, higher concentration level and longer exposure time is required to obtain desirable control of the pests. The insecticidal effectiveness of DE formulations increased as concentration level and time exposed to each concentration increased. These findings are in accordance with previous studies with different DE formulations [6, 7, 10, 17, 18] (Supplementary Table).

The susceptibility of stored-product insects is different to DEs [19]. In the current study, adults of *S. oryzae* seem to be more susceptible than two tested species. Therefore, low concentration level may provide a satisfactory level of protection. However, in more cases the susceptibility of *T. castaneum* and *T. confusum* adults to different DEs was similar. *Tribolium* sp. was reported the least susceptible stored-product insects to DEs [19]. Between these two species, Arthur [20] stated that *T. confusum* was significantly less susceptible to Protect-It DE formulation than *T. castaneum.* It is also evident from the present results that in some cases, LC_{50} values of different DE deposits were more for controlling 50 % of *T. confusum* adults than *T. castaneum* indicating less susceptibility of this species.

Previous studies have shown that the performance of various DEs is notably different on the same commodity and the same DE has different toxicity on the same commodity [7, 17, 18]. According to the present findings, Khorasan was the most effective followed by Mamaghan and Sayan® was the least effective DE deposit in most cases. This can be attributed to DEs origin, physical and chemical properties that could affect their insecticidal potential [10]. All DEs tested in the present study were of marine origin collected from different locations. Snetsinger [21] represented that the source of DEs provides strong impact on the ability of its insecticidal activity. DEs from salt water are weak and cheaper than DEs of fresh water origin. However, other studies showed that the insecticidal efficacy of DEs depends on their physical properties rather than on their origin [19, 22].

LC_{90} values obtained from first bioassay were applied for assessing persistence of DE deposits. Generally, adult mortality was high at initial period of exposure to DE deposits. However, after 4 months, the insecticidal toxicity of all tested DEs showed a decreasing trend (Figs. 4, 5, 6). Mortality of *T. castaneum* adults was more or less the same in the first month in all commodities treated with different

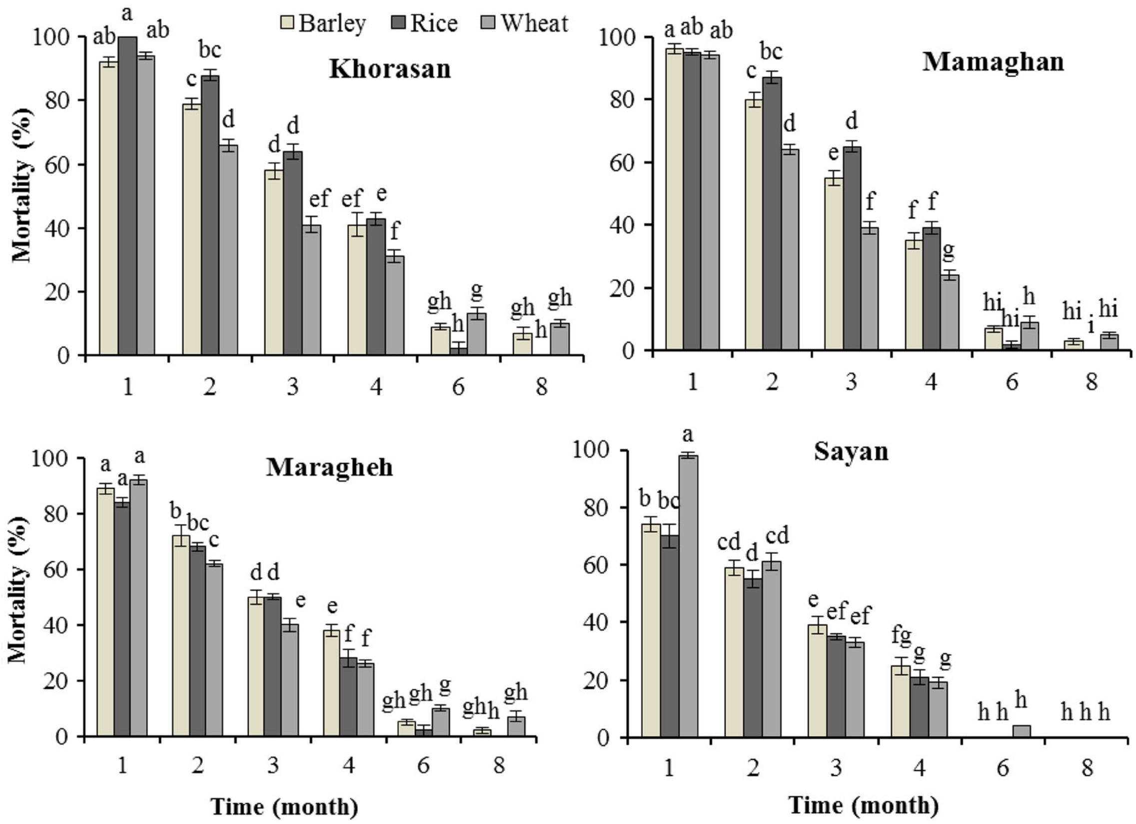

Fig. 6 Persistence of Iranian DE samples in barley, rice and wheat against *Tribolium confusum.* Means followed by the same letter are not significantly different using Turkey's Test at $P < 0.05$

DE deposits. However, mortality reduced more rapidly overtime on wheat than on barley and rice (Fig. 4).

Persistence of Iranian DE deposits against *T. confusum* is presented in Fig. 5. The rapid reduction in effectiveness of DEs was evident with increasing duration of storage in all tested commodities (Fig. 5.

Different *S. oryzae* mortality levels were recorded among different commodities exposed to each DE deposit. The mortality was 88–100 % in the first month of storage but decreased as time of storage increased (Fig. 6).

One of the main advantages of DEs is their persistence [2, 4]. Athanassiou et al. [7] found a satisfactory level of protection of wheat and barley against *S. oryzae* for the first 270 days of storage. However, after this interval they reported a gradual decrease in the mortality. In the current study, DEs were well able to maintain their stability in the first few months. However, according to the present results the protective effect of DEs declined overtime. This is in agreement with Stathers, Denniff and Golob [6] that the DEs became less effective and the commodity became less suitable for insect development over the duration of the experiments. They declared that DEs ability in absorbing moisture from air and the surrounding environment over time may cause their less effectiveness. In addition to moisture, oil absorption from commodities during the storage period can also reduce DEs insecticidal efficacy. Ziaee et al. [18] stated that adsorption of lipids from commodity surfaces reduced DEs insecticidal toxicity. The results of present experiments demonstrate that the effectiveness of DEs decreased overtime specially for Sayan® formulation.

Conclusion

In conclusion, the present results indicated that Khorasan and Mamaghan DE deposits were found more effective than Maragheh and Sayan. DEs were less effective on rice followed by barley. Adults of *S. oryzae* were more susceptible than *T. castaneum* and *T. confusum* to the tested DEs. According to this study, Iran has the potential sources of DEs that should be considered, given the importance of using locally sustainable and natural DEs. However, more studies are required to process DE deposits and make them commercially exploitable.

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Compliance with Ethical Standards

Conflict of interest None.

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