Influence of temperature and relative humidity on the efficacy of diatomaceous earth and *Metarhizium anisopliae* (Metschinkoff) Sorokin (Hyphomycetes: Deuteromycotina) against *Tyrophagus fatimii* F. (Astigmata: Acaridae)

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Abstract

The combined as well as the alone effect of diatomaceous earth (DE) and entomopathogenic fungi were evaluated against *Tyrophagus fatimii* (Astigmata: Acaridae). Two different dose rates of DE (1 g and 1.5 g/kg of wheat) and three of the fungus *Metarhizium anisopliae* (Hyphomycetes: Deuteromycotina) (3.6 x 10^7, 3.6 x 10^8 and 3.6 x 10^9 conidia/kg of wheat) were taken and studied at 20°C and 25°C with 45% and 55% r.h. under three exposure intervals. It was found that the combined effect of DE diatomaceous earth and *M. anisopliae* was maximum at 25°C and 55% r.h. which gave 75% adult mortality at their highest dose rates, however, DE alone exhibited the highest mortality (61.3%) at 25°C and 45% r.h. On the other hand, *M. anisopliae* gave maximum mortality of mites (48.7%) at 20°C and 55% r.h. at 3.6 x 10^9 conidia/kg of wheat. It was concluded that the efficacy of both DE and *M. anisopliae* increased with the increase of the exposure interval. Moreover, the increase of dose increased the mortality. In addition, temperature and r.h. are the key factors for determining the effectiveness of both DE and *M. anisopliae*.

Keywords: Diatomaceous earth, *Tyrophagus fatimii*, *Metarhizium anisopliae*, Stored wheat.

1. Introduction

Stored products protection is of utmost importance to secure a continuous and safe food supply all over the world (Ferizli et al., 2005). Many chemicals are used as selective pesticides for different pests in stored grain which include insect growth regulator's (IGR's), organophosphates, pyrethroids and plant extracts (Sanchez-Ramos and Castanera, 2003). Organophosphates have been used since 1960's as one of the main sources of stored-product pest control, as admixture with grains or applied directly to the storage structures (Cook and Armitage, 2000). Nevertheless, today the control is focused on the use of pyrethroids as an alternative to some of the traditional organophosphates due to their quick action and low toxicity to humans (Hubert et al., 2007). On the other hand, injudicious use of chemicals leads to resistance development in stored grain mites (Zdarkova, 1994) and also increases residues in the stored product commodities.

Some new control strategies are under consideration for the last few decades which includes diatomaceous earth (DE) and entomopathogenic fungi, they are ecologically sound and may be used alone or combined for the control of stored grain insect pests (Wakefield et al., 2002; Ferizli et al., 2005; Athanassiou and Steenberg, 2007; Batta, 2008). DE is a naturally occurring substance that is mined from geological deposits of fossilized diatoms and is composed of mainly by silicon dioxide (Korunic, 1998; Cook et al., 2008) with low mammalian toxicity and provide grain protection with no toxic residues (Mahdi and Khalequzzaman, 2006). Entomopathogenic fungi are also valuable tools for IPM strategy (Sivasundaram et al., 2008) as many isolates have been shown effective against several insect pests (Batta, 2004; Hong et al., 2005; Cherry et al., 2007).

The idea of synergistic interaction between DE and entomopathogenic fungi is an interesting alternative to traditional pesticides in stored grain insect control (Michalaki et al., 2007). Synergistic interaction of DEs and entomopathogenic fungi has been observed against many species (Akbar et al., 2004; Kavallieratos et al., 2006; Athanassiou et al., 2008; Batta, 2008).

There are many data available for he combined use of DEs with fungi against stored-grain insects; however, there are few reports for the efficacy of this combination against stored-product mites. The present study investigates the sole and collective effect of DE and the entomopathogenic fungi

Metarhizium anisopliae (Metschinkoff) Sorokin (Hypomycetes: Deuteromycotina) in wheat against one stored-grain mite species.

2. Materials and methods

2.1. DE and fungal formulation

The DE used was Protect-It (Hedley Technologies, Mississauga, Ontario, Canada), which contains 83.7% SiO_2 with 10% silica aerogel. The *M. anisopliae* strain (WG01) was firstly isolated from *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) which was collected from a local rice storage structures in the periphery of District Faisalabad, Punjab, Pakistan and it was sub-cultured on synthetic media.

2.2. Commodity

Fresh, clean and infestation-free wheat was taken from the grain market of Faisalabad, Pakistan. The moisture content of the grain, as measured by a Dickey-John moisture meter, was 12.1%.

2.3. Mites

Infested samples of wheat grains were collected from different storage structures and flour mills of Faisalabad district and the stored grain mites were separated by using Berlese funnel. They were reared on the diet containing yeast and organic whole meal wheat flour at the ratio of 3:1. The mites were taken into a conical flask with 2-3 spatulas of diet. The top of the flask was closed by using cotton plug in such a way that the mites could not escape from the conical flask. When the culture was prepared, it was placed into the incubator at 20°C and 80% r.h. The culture was inspected after one week and shifting was carried out after 15 d in a new flask.

2.4. Bioassay

Three dose rates of *M. anisopliae* used in the experiment were 3.6×10^7, 3.6×10^8 and 3.6×10^9 conidia/kg of grain, while the DE doses were 1 and 1.5 g/kg. The wheat was treated with each dose rate of both DE and entomopathogenic fungi alone and also in their possible combinations. Samples of 60g of treated wheat for each dose rate were taken into three separate plastic jars (replicates) also three jars with untreated wheat were prepared which served as a control. Then, 30 adult mites of same age were added in each jar and the top of the jars were closed with muslin cloth to maintain the aeration. The treatments were kept into incubators and experiments were conducted at different sets of temperature (20 and 25°C) and r.h. (45 and 55%). The r.h. levels of 45 and 55% were kept constant in the incubator at 20°C by using the salts of K_2HPO_4 and $NiCl_2.6H_2O$ respectively; similarly, the conditions were maintained at 25°C by using the salt of Potassium Carbonate for 45% r.h. and Magnesium Nitrate for 55% r.h. (Winston and Bates, 1960; Greenspan, 1977). Mite mortality was assessed after 5, 10 and 15 d of exposure. The data for mortality was recorded by counting all the live and dead mites in each replication.

2.5. Statistical analysis

The mortality of the mites was tested statistically by using the GLM procedure through MINITAB software after correcting the mean mortalities by using Abbott's (1925) formula. Every exposure interval was subjected to one-way analysis of variance separately; the mortality for the control was very low so not included in the analysis. Means were compared by using Tukey-Kramer test at $P \leq 0.05$ (Sokal and Rohlf, 1995).

3. Results

3.1. Mortality (%) of T. fatimii after 5 days

Main effects and interactions were significant at $P<0.05$ (treatments df=10,131; F=69.4; temperature df=1,131; F=56.7; r.h. df=1,131; F=24.2; treatment x temperature df=10,131; F=5.7; treatment x r.h. df=10,131; F= 3.2) with the exception of temperature x r.h. (df=1,131; F=0.1; P=0.7) and treatment x temperature x r.h. (df=10,131; F=0.1; P=1.0). The highest mortality (66.7%) was obtained at 25°C and 55% r.h. with the higher dose rates of DE and *M. anisopliae*. However, in the case of DE alone the highest mortality obtained was 41.1% at 25°C and 45% r.h. combination, while in the case of *M. anisopliae*, the highest mortality was 38.9% at 20°C and 55% r.h. (Table 1).

Table 1 Mean mortality of *Tyrophagus fatimii* (% ± SE) after 5 days of exposure on wheat treated with *Metarhizium anisopliae* and DE at different temperatures (20ºC, 25ºC) and r.h. (45%, 55%) at different dose rates; DE_1=1g/kg DE_2=1.5g/kg Ma_1=3.6x10^7 Ma_2=3.6x10^8 Ma_3=3.6x10^9 (within each column, means followed by the same letters are not significantly different; Tukey and Kramer HSD test; df=10,32)

	Mortality (%) ± SE			
	45% r.h.		55% r.h.	
Treatments	**20º C**	**25º C**	**20º C**	**25º C**
DE_1	27.7 ± 4.01cd	38.8 ± 2.94cde	23.3 ± 1.92f	30.0 ± 1.92f
DE_2	30.0 ± 1.92cd	41.1 ± 2.94cd	26.6 ± 1.92ef	36.6 ± 1.92def
Ma_1	21.1 ± 2.94d	17.7 ± 2.94g	28.8 ± 2.94def	25.5 ± 2.94f
Ma_2	26.6 ± 1.92cd	22.2 ± 2.94fg	34.4 ± 2.94cdef	30.0 ± 1.92f
Ma_3	30.0 ± 1.92cd	26.6 ± 1.92efg	38.8 ± 2.94bcde	34.4 ± 4.01ef
DE_1 + Ma_1	24.4 ± 2.94d	33.3 ± 1.92def	27.7 ± 2.94def	38.8 ± 2.94def
DE_1 + Ma_2	28.8 ± 2.94cd	40.0 ± 1.92cde	34.4 ± 2.94cdef	44.4 ± 2.94cde
DE_1 + Ma_3	34.4 ± 4.01bcd	45.5 ± 2.94bcd	40.0 ± 1.92bcd	50.0 ± 1.92bcd
DE_2 + Ma_1	40.0 ± 1.92abc	48.8 ± 2.94abc	45.5 ± 2.94abc	54.4 ± 2.94abc
DE_2 + Ma_2	46.6 ± 1.92ab	55.5 ± 2.94ab	51.1 ± 2.94ab	61.1 ± 2.94ab
DE_2 + Ma_3	51.1 ± 2.94a	61.1 ± 2.94a	56.6 ± 1.92a	66.6 ± 1.92a
ANOVA	F = 11.4	F = 25.0	F = 16.5	F = 26.1
	P < 0.01	P < 0.01	P < 0.01	P < 0.01

3.2. Mortality (%) of T. fatimii after 10 days

Main effects and interactions were significant at P <0.05 (treatments df=10,131; F=30.9; temperature df=1,131; F=26.4; r.h. df=1,131; F=11.3; treatment x temperature df=10,131; F=2.8; treatment x r.h. df=10,131; F=1.8) with the exception of temperature x r.h. (df=1,131; F=0.1; P=0.7) and treatment x temperature x r.h. (df=10,131; F=0.1; P=1.0). The highest mortality (70.8%) was obtained at 25ºC and 55% r.h. with the collective highest dose rates of DE and *M. anisopliae*. In the case of DE alone, the highest mortality recorded was 49.5% at 25ºC and 45% r.h., while mortality for *M. anisopliae* alone was 43.9%, at 20ºC and 55% r.h., at 3.6 x 10^9 conidia/kg of wheat (Table 2).

Table 2 Mean mortality of *Tyrophagus fatimii* (% ± SE) after 10 days of exposure on wheat treated with *Metarhizium anisopliae* and DE at different temperatures (20°C, 25°C) and r.h. (45%, 55%) at different dose rates; DE_1=1g/kg DE_2=1.5g/kg Ma_1=3.6x10^7 Ma_2=3.6x10^8 Ma_3=3.6x10^9 (within each column, means followed by the same letters are not significantly different; Tukey and Kramer HSD test; df=10,32)

	Mortality (%) ± SE			
	45% r.h.		55% r.h.	
Treatments	**20°C**	**25º C**	**20° C**	**25° C**
DE_1	33.6 ± 2.17bcd	46.0 ± 6.93abcd	30.5 ± 2.90d	35.0 ± 2.51cd
DE_2	39.8 ± 2.64abcd	49.5 ± 6.35abcd	34.9 ± 2.32cd	43.7 ± 3.81bcd
Ma_1	25.2 ± 1.53d	21.7 ± 2.17e	34.3 ± 0.52cd	29.8 ± 0.99d
Ma_2	30.4 ± 2.21cd	27.0 ± 2.27de	38.9 ± 0.55bcd	34.9 ± 1.81cd
Ma_3	34.9 ± 0.88bcd	30.2 ± 2.32cde	43.9 ± 4.51bcd	39.2 ± 2.62bcd
DE_1 + Ma_1	29.7 ± 4.20cd	38.3 ± 0.92bcde	33.9 ± 1.79cd	43.5 ± 1.19bcd
DE_1 + Ma_2	34.3 ±0.52bcd	44.3 ± 1.79abcde	39.2 ± 3.23bcd	49.8 ± 1.81abcd
DE_1 + Ma_3	39.2 ± 2.62abcd	50.7 ± 2.59abc	44.4 ± 2.78bcd	55.8 ± 4.32abc
DE_2 + Ma_1	44.6 ± 4.25abc	54.6 ± 5.16ab	49.2 ± 2.59abc	58.4 ± 0.83ab
DE_2 + Ma_2	50.0 ± 3.13ab	60.8 ± 7.13ab	55.4 ± 7.43ab	67.3 ± 8.67a
DE2 + Ma3	55.3 ± 7.03a	66.2 ± 6.38a	61.3 ± 1.72a	70.8 ± 9.08a
ANOVA	F = 7.4	F = 9.3	F = 8.5	F = 9.5
	P < 0.01	P < 0.01	P < 0.01	P < 0.01

3.3. Mortality (%) of T. fatimii after 15 days

Main effects and interactions were significant at P<0.05 (treatments df=10,131; F=12.4; temperature df=1,131; F=13.3; r.h. df=1,131; F=4.4) with the exception of treatment x temperature (df=10,131; F=1.5; P=0.1); treatment x r.h. (df=10,131; F=0.7; P=0.7); temperature x r.h. (df=1,131; F=0.2; P=0.6); treatment x temperature x r.h. (df=10,131; F=0.0; P=1.0). The highest mortality due to the application of DE alone was 61.3% at 25°C and 45% r.h., while in the case of *M. anisopliae* alone the maximum mortality was 48.7% at 20°C and 55% r.h. at their highest dose rates. The results for the highest dose rate of DE and *M. anisopliae* exhibited the highest mean mortality (75%) at 25°C and 55% r.h. (Table 3).

Table 3 Mean mortality of *Tyrophagus fatimii* (% ± SE) after 15 days of exposure on wheat treated with *Metarhizium anisopliae* and DE at different temperatures (20°, 25°C) and r.h. (45%, 55%) at different dose rates; DE_1=1g/kg DE_2=1.5g/kg Ma_1=3.6x10^7 $_{Ma2}$=3.6x10^8 Ma_3=3.6x10^9 (within each column, means followed by the same letters are not significantly different; Tukey and Kramer HSD test; df=10,32)

Treatments	Mortality (%) ± SE			
	45% r.h.		55% r.h.	
	20°C	25°C	20°C	25°C
DE_1	39.6±5.20abc	57.0±1.78ab	35.1±1.85c	48.4±4.56abc
DE_2	45.2±4.80abc	61.3±6.21ab	39.6±1.65bc	53.3±8.82abc
Ma_1	30.1±1.65c	25.7±2.11b	39.9±6.85bc	34.2±3.09c
Ma_2	35.0±3.07bc	31.1±4.10ab	44.8±4.91abc	38.9±4.47bc
Ma_3	39.0±2.08abc	36.1±5.42ab	48.7±3.77abc	43.0±8.52abc
DE_1 + Ma_1	33.1 ± 1.31bc	43.3 ± 3.44ab	39.1 ± 4.19bc	48.1 ± 4.30abc
DE_1 + Ma_2	38.2 ± 2.75bc	50.0 ± 5.77ab	44.8 ± 2.60abc	56.0 ± 3.62abc
DE_1 + Ma_3	44.8 ± 2.60abc	54.1 ± 8.33ab	49.6 ± 2.92abc	59.8 ± 1.55abc
DE_2 + Ma_1	50.0 ± 2.62abc	61.6 ± 7.26ab	56.5 ± 3.62abc	65.5 ± 8.68abc
DE_2 + Ma_2	54.8 ± 9.80ab	66.6 ± 8.33ab	61.5 ± 9.63ab	71.1 ± 4.44ab
DE_2 + Ma_3	62.5 ± 7.22a	72.2 ± 20.03a	66.6 ± 6.67a	75.0 ± 14.43a
ANOVA	F = 4.4	F = 3.4	F = 4.0	F = 3.5
	P < 0.01	P = 0.007	P = 0.0031	P = 0.0069

4. Discussion

There are numerous studies available on the efficacy of DE and entomopathogenic fungi for the control of stored grain insect pests but there are few studies available for stored-grain mites (Cook and Armitage, 1999, 2000; Wakefield et al., 2002; Palyvos et al., 2006; Athanassiou and Palyvos, 2006; Cook et al., 2008). In the present experiment, the effectiveness of both DE and entomopathogenic fungi was evaluated against *T. fatimii* alone or in combination in various doses.

Mortality of *T. fatimii* was increased with the increase in the dose rate of *M. anisopliae.* However, the efficacy was increased by the addition of the lowest dose of DE at 25°C and 55% r.h. This was also confirmed by Michalaki et al. (2006) who suggested that the DE promoted the action of fungi when the conidial concentration was at a certain threshold level. In fact, the presence of the DE particles may damages the conidia due to their abrasive action, but by increasing the dose rate of fungi, the number of undamaged conidia can increased the insecticidal efficacy.

The temperature plays a key role in determining the effectiveness of DE and entomopathogenic fungi (Arthur, 2000; Michalaki et al., 2006). At high temperature water loss from the insect's body occurs quickly; also, the insects move faster, and more DE particles are attached with the cuticle (Fields and Korunic, 2000). Our results are contradictory to Michalaki et al. (2006) who studied the effectiveness of *M. anisopliae* against *T. confusum* and found that the increase of temperature increases the efficacy of *M. anisopliae*. Our results are in accordance with the results reported by Moore et al. (1996), where the increase of temperature negatively affected the germination of conidia. Moreover, Hedgecock et al. (1995) and Moore and Higgins (1997) reported the viability of the conidia was seriously reduced at temperature 30°C as compared to -10°C during storage. High temperature (35°C) reduces the fungal germination (Sun et al., 2003), while Ouedraogo et al. (1997) reported 25°C as optimum temperature for

M. anisopliae. In our case, 25°C was the best for the germination of conidia of *M. anisopliae*. The influence of temperature on the effectiveness has yet not been explored and needs additional attention.

Another abiotic factor, the relative humidity is also very important as our results indicated that the mortality of *T. fatimii* was maximum at 55% r.h. when the DE and *M. anisopliae* was used collectively. These results are in agreement with Michalaki et al. (2006) who proved that *M. anisopliae* was more effective at 55% r.h. against *T. castaneum*. The efficacy of DEs is also affected by the change of r.h. in the commodity as DE particles absorb moisture from the air and their ability to attach with the insect cuticle decreases (Stathers et al., 2004). Shi et al. (2008) reported that conidial germination depends on the r.h. and temperature as they proved that the fungal action was significant at 20-25°C but not at 30°C, also, the effect of temperature was pronounced at 51-74% r.h.

There are not many data available for the efficacy of DE and entomopathogenic fungi alone and in combination under different temperature and r.h. regimes against stored-grain mites. It was concluded that combined dose rates of both DE and *M. anisopliae* gave highest mortality at 25°C and 55% r.h. after 15 d of exposure. It was also observed that mite mortality increased with the increase of temperature and r.h. The results may provide the basic information for a reduced-risk control of stored-grain mites but more focused research is needed to exploit the best alternatives for a successful IPM program.

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