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Research Article

EFFECT OF ABIOTIC FACTORS ON THE EFFICACY OF *Beauveria bassiana* AND DIATOMACEOUS EARTH AGAINST *Rhyzopertha dominica* (F.)

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ABSTRACT. The herein reported study was performed to assess the insecticidal efficacy of diatomaceous earth (DE) Concern® and *Beauveriabassiana* under three levels of temperature (i.e., 25, 30 and 35°C) and three levels of relative humidity (RH; 45, 60 and 75%) against *Rhyzoperthadominica* (F.). Three dose rates of DE was 200, 400 and 800ppm while for *B. bassiana*was $1x10^8$, $1.5x10^8$ and $2x10^8$ spores/kg grain that were applied on wheat grains and mortality was noticed after 7, 14 and 21 days after treatment, whereas adult progeny emergence was observed 60 days of post exposure. Results regarding DE have showed thatincrease of temperature and decrease of RH have resulted into significantly increase in adult mortality. Maximum mortality of 74.33% was assessed with a highest tested dose of 800ppm after 21 days exposure at 35°C and 45% RH, progeny development was also greatly suppressed under same conditions. For *B. bassiana,* efficacy was highly dependent on temperature and RH, as lower level of temperature (25°C) and moderate RH (60%) showed maximum mortality of 82.5% with dose rate of $2x10^8$ spores/kg grains after 21 d. Emergence of offspring in fungus treated grains was highly suppressed at 25°C with combination of both 45 and 60% RH whereas at 30 and 35°C significant reduction of progeny was noticed only with 45% RH. The present study divulge that in current scenario of climate change, that alter the patterns of environment, abiotic factors specifically temperature and humidity must be seriously considered when planning an IPM strategy based on the application of *B. bassiana* and DE Concern®.

Keywords: *Relative humidity, Temperature, DE Concern, B. bassiana, Rhyzopertha dominica*

INTRODUCTION

Post-harvest infestations by insects contribute to significant losses of cereal production [1]. Stored product insects are responsible for 9 to 20% or more post-harvest losses in different parts of world [2]. The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is a major insect pest of stored kernels which feed inside the grains and responsible for severe losses of cereals during storage [3]. It is most common and damaging in the tropic, sub-tropic [4] and moderate environments [5]. At present, insect pest management tactics in grain storage relied mainly on fumigants and synthetic insecticides that led to some serious problems such as environmental pollution [6,7], development of resistance in insect pests [8,9,10,11], toxic impacts on non-target entities [12,13] and pesticide residues in treated commodities [14,15]. Therefore, these hazardous insecticidal impacts and awareness of the public about these risks demand to progress towards more efficient, sustainable and non-chemical strategies.

Diatomaceous earth (DE) based insecticides are gaining high importance in the recent era of integrated insect pest management of stored products [16]. These have many advantages over conventional pesticides as, they are environment-friendly, non-toxic to mammals, easy to handle, provide long-lasting protection and have no effect on the end product [17,18,19]. DE is a porous sedimentary and a lightweight rock made up of remaining fossils of algae known as diatoms and the major component of all DE deposits is amorphous silicon dioxide (SiO_2) [20,21]. Tiny particles of DE stick to insect body then induce scratch on the cuticular wax layer to cause desiccation and loss of body contents which ultimately results in insect's death [22].

Management of the stored products insect pests through biological control particularly by the use entomopathogenic fungi (EPF) is another sustainable and successful replacement of synthetic chemicals [23,24]. EPF have very limited impact on environment, possess low mammalian toxicity and safer to the natural enemies of the insects [25]. The most commonly evaluated entomopathogenic fungus against insect pests of stored grains is *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) [26,27,28,29]. Mode of infection of entomopathogenic fungi includes the attachment of asexual conidia of the fungus to the insect cuticle to form appressoria, enter into the insect body, proliferate, produce toxins and eventually kill the insect [30,31]. After the death of insect, inner mycelium breeds out of host insect's cadaver, undergoes sporulation and literally raises the number of inoculums in insect-fungus system [32,33].

In the grain storage ecosystem, abiotic factors such as temperature, gas composition, and RH play a vital role for the development of insects [34,35,36]. The association of these environmental variables with insecticides is an important concern for effective pest management program [37,38]. For most of the stored grain insect species, various examinations have shown that DEs are more viable at increased temperatures and reduced level of RH, as it overcomes the proportion of water loss by insect body [39,40,41,42]. Likewise for entomopathogenic fungi environmental conditions specifically humidity and temperature are also considerably important factors due to their effects on successful fungal treatments [43]. As far as concern of temperature, it is mostly acknowledged that the conidial viability and germination has negative effect of high temperature [44]. The accessible literature so far has given contradictory outcomes for the impact of relative humidity on pathogenicity of EPF [45] Such as, Searle and Doberski [46] stated that *B. bassiana* caused infection to *Oryzaephilus surinamensis* (L.) at 100% RH but not at 90% RH, while for the same fungus Akbar *et al.*[45] found no statistically significant difference in the larval mortality of *Tribolium castaneum* at 75 or 54% RH similarly, Lord [47] that pathogenicity of *B. bassiana* to *R. dominica* was not significantly different between RH of 43, 56, 75 and 85%.

In the current scenario of climate change, as adverse environmental events occur that are responsible for fluctuation of temperature and RH, therefore present research was designed to check the impact of diversified RH and temperature on the efficacy of DE and *B. bassiana* against *R. dominica*.

MATERIALS AND METHODS

Rearing of Insects

Adults of *R. dominica* were collected from grains market and cereal storage structures of Punjab Food Department located at district Faisalabad, Pakistan. The collected insects were cultured in laboratory at optimum conditions of 30 ± 2°C and 65 ± 5% RH inside the incubator (Model MIR-254, SANYO). To attain uniform population, 60 adults of *R. dominica* were put in the plastic jars, having 300g of sterilized wheat with moisture content up to 12% measured by Dickey-John moisture meter (Multigrain CACII; Dickey–John Co., Auburn, IL, USA). These jars were concealed with muslin fabric and fastened with rubber bands to prevent insect escape and were kept in an incubator at 30 ± 2°C and 65% RH. Beetles were permitted to breed and infestation for 3 days, after that beetles were removed from the jars by sieving out and jars containing infested grains were again placed in incubator at aforementioned growth conditions to get homogenous population. Then 3 days old adults of test insect were used for further experimentation.

Formulations

The commercial formulation of fresh water diatomaceous earth i.e. DE Concern® (85% SiO_2, 10% other elements oxides and 5% moisture) and *B. bassiana*i.e. Racer BB® (1×10^8 conidia/gram) were used for the experimentation. The DE Concern® was procured from Necessary Organics, USA while the formulation of *B. bassiana* (Racer BB®) was imported from Agri Life, Medak District. Hyderabad, India.

Bioassay

Three concentrations ($1x10^8$, $1.5x10^8$ and $2x10^8$ spores/kg grain)of *B. bassiana* and three dose rates of diatomaceous earth (200, 400 and 800 ppm) were applied on lots of 1kg of wheat grains. As both, DE and *B. bassiana* formulations were in powder form therefore these were admixed with grains by shaking manually for 5-8 minutes. The treated grains of 100g were put into experimental jars and in each jar 40 adults of test insect were released. These jars were placed in incubator at three levels of temperature (25, 30 and 35°C) and RH (45, 60 and 75%). The desired RH level was sustained by using saturated salt solutions of sodium chloride (NaCl) and sodium bromide (NaBr), as described by Greenspan [48]. The control treatment was also tested on each combination of temperature and relative humidity. Data regarding mortality was collected after exposure intervals of 7, 14 and 21 days. After 21days all alive and dead adults were discarded and jars were maintained at the similar abiotic conditions for 60 days to assess the F_1 adult emergence [49].

Statistical Analysis

Completely Randomized Design with three replications of each treatment was used for experiment and data was collected for mortality and adult progeny emergence. Observed mortality was corrected by using Abbott's formula [50]. Separately for each biopesticide, four way-ANOVA was used for data regarding adult mortality with Temperature, RH, Dose and Exposure as main factors while for progeny production, data was submitted to three way-ANOVA with Temperature, RH and Dose as main effects. All analysis were statistically examined with R-Software (version 3.5.1) [51] and Tukey-Kramar HSD test ($P \leq 0.05$) was used for multiple comparisons of treatments means [52].

RESULTS AND DISCUSSION

Adult Mortality

DE Concern®

Results revealed that adult mortality of *R. dominica* by application of DE Concern® was significantly impacted by all main effects i.e. Temperature, RH, Dose and Exposure (Table 1). After exposure period of 7 days, mortality of *R. dominica* on wheat grains treated with DE Concern® was usually low at low temperature regime (25°C) with all tested levels of RH for all doses and hardly reached up to (12.5%) at 25°C with combination of 45% RH at higher dosage of 800ppm. It was observed that the mortality rate increased, with the rise of temperature and decrease in RH levels and maximum mortality (30%) was noted at 35°C and 45% RH followed by 29.16% at 35°C with the RH level of 60% at highest tested dose rate of 800ppm. While the minimum mortality rate of 5.83% was registered at 25°C and 60% RH with 200ppm. After fortnight application of DE Concern®at 25°C, the percentage of dead adults ranged from (18.16%) to (22.5%) at 45% RH level and from (10.33%) to (20%) at 60% RH level and from (13.33%) to (22%) at 75% RH level with significant differences among concentrations. For the same exposure period, the higher level of mortality (54.66%) was noticed at 35°C and 45% RH followed by (53.33%) at 35°C and 60% RH with the highest concentration of DE Concern®. Generally, low mortality percentage was observed at 75% RH with all temperature regimes and doses. The highest levels of mortality were noticed after an exposure interval of 21 days.Highest mortality (74.33%) of exposed adults was observed at 35°C and 45% RH followed by (69.16%) at 35°C and 60% RH level with the highest tested concentration of 800ppm. However, the increase in temperature and decrease in RH levels positively correlated with the highest mortality at all concentrations (Table 2).

Table 1. *ANOVA parameters for adult mortality of R. dominica by the application of DE Concern® and B. bassiana*

		DE Concern®		***Beauveria bassiana***	
Source	**Df**	***F***	***P***	***F***	***P***
Temperature	2	312.3	<0.01	218.4	<0.01
RH	2	235.2	<0.01	208.4	<0.01
Exposure	2	470.7	<0.01	647.0	<0.01
Dose	2	390.0	<0.01	464.2	<0.01
Temperature × RH	4	230.4	<0.01	114.88	<0.01
Temperature × Exposure	4	179.4	<0.01	104.5	<0.01
Temperature × Dose	4	62.4	<0.01	23.42	<0.01
RH × Exposure	4	144.4	<0.01	90.3	<0.01
RH × Dose	4	32.5	<0.01	86.7	<0.01
Exposure × Dose	4	72.2	<0.01	205.6	<0.01
Temperature × RH × Exposure	8	28.3	<0.01	45.7	<0.01
Temperature× RH × Dose	8	15.5	<0.01	31.4	<0.01
Temperature × Exposure × Dose	8	3.9	<0.01	0.63	0.7
RH × Exposure × Dose	8	2.3	0.02	4.02	<0.01
Temperature × RH × Exposure × Dose	16	0.9	0.4	1.64	0.06

Table 2. *Mortalityof R. dominica on wheat treated with various dose rates of DE Concern® at different levels of temperature (T) and relative humidity (RH)*

			Mortality (%) ± SE		
Exposure (Days)	**T(˚C)**	**RH (%)**	**200 ppm**	**400 ppm**	**800 ppm**
7	25	45	9.16± 0.13bc A	10± 0.12c A	12.50± 0.13b A
		60	5.83± 0.10c A	7.50± 0.08c A	10.83± 0.14b A
		75	7.50± 0.13bc A	8.33± 0.11c A	12.50± 0.13b A
	30	45	16.83± 0.14a B	21.66± 0.15a AB	27.50± 0.18a A
		60	14.16± 0.13ab B	19.16± 0.15ab AB	23.33± 0.16a A
		75	6.66± 0.10c A	10.83± 0.10bc A	11.66± 0.15b A
	35	45	18.50± 0.17a B	23.50± 0.18a AB	30± 0.17a A
		60	18.33± 0.15a B	23.33± 0.16a AB	29.16± 0.16a A
		75	9.16± 0.07bc A	11.66± 0.13bc A	14.16± 0.13b A
14	25	45	18.66± 0.17cd A	18.83± 0.13cd A	22.50± 0.19b A
		60	10.83± 0.15d B	13.33± 0.14d AB	20± 0.18b A
		75	13.33± 0.15d B	15± 0.19cd B	22± 0.14b A
	30	45	30.83± 0.20ab B	39.16± 0.2ab B	50± 0.16a A
		60	25.83± 0.13bc B	34.33± 0.12b AB	42.50± 0.16a A
		75	12.50± 0.10d B	20± 0.10cd AB	21.66± 0.18b A
	35	45	35± 0.19a B	43.50± 0.19a B	54.16± 0.16a A
		60	33.33± 0.13ab C	43.33± 0.20a B	53.33± 0.16a A
		75	16± 0.16d B	21.66± 0.13c AB	25.83± 0.13b A
21	25	45	22.50± 0.14c B	27.16± 0.19c AB	32.33± 0.16c A
		60	16.66± 0.16c B	19.16± 0.13c AB	27.50± 0.14c A
		75	18.33± 0.11c B	21.66± 0.17c AB	29.16± 0.21c A
	30	45	41.66± 0.12ab B	54.16± 016ab AB	68.33± 0.21a A
		60	35± 0.20b B	48.16± 0.21b B	57.50± 0.11b A
		75	16.66± 0.16c B	27.50± 0.20c A	28.33± 0.16c A
	35	45	48.33± 0.16a C	60.00± 0.16a B	74.33± 0.23a A
		60	45.83± 0.19ab C	57.66± 0.13ab B	69.16± 0.22a A
		75	22.50± 0.19c B	29.16± 0.17c AB	35.83± 0.21c A

For each exposure means with similar upper case letters within each row and with similar lower case letters within each column are not significantly different (Tukey- Kramer HSD test at $P \leq 0.05$)

B. bassiana

Results regarding the adult mortality of *R. dominica* showed that all main factors temperature, RH, Dose and Exposure were significant (Table 1). It was noted that after 7 days of exposure period the toxicity of *B. bassiana* remained less at high temperature level (35°C) and low RH (45%) with all tested dose rates. The maximum adult mortality of 37.50% was observed with higher tested dose rate (2×10^8spoers/kg grains) at a low range of temperature 25°C and moderate RH of 60% followed by 31.66% mean mortality with the same RH level at 30°C on same dose rate. The rise of temperature level up to 30° and 35°C in most instances proceeded in noticeably decreased mortality of *R. dominica* adults for all RH levels. Lowest adult mortality (7.5%) and (8.33%) were recorded at high tested regimes of temperature 35°C with 60% RH and at 30°C with 45% RH respectively. After post application period of 14 days of *B. bassiana* the adult mortality was highest at 25°C as compared to 30° and 35°C. At 25°C mortality ranged between (21.66%) to (35%), (40.83%) to (68.33%) and (27%) to (56%) for 45, 60 and 75% RH levels respectively, with significant difference among tested concentrations. Among tested RH levels maximum mortality ranges were observed at 60% on temperature regimes of 25 and 30°C but the same trend was not noticed in case of 35°C where mortality was minimum (13.33%) at 60% RH as compared to (18.50%) at 75% with lowest tested concentration (1×10^8Spores/kg grains). The results of the experiments revealed that the maximum mortality rates were obtained after 21 days of post treatment application period. Overall mortality of tested insect decreased with the increase in temperature levels, maximum mortality was achieved (82.50%) at 25°C and 60% RH followed by (69.16%) at 30°C with same RH level at highest concentration of 2×10^8spoers/kg grains. While, the lowest mortality after 21 days exposure was (16.33%) recorded at 35°C and 60% RH with lowest dose. Response of RH was same at 25 and 30°C, as on both levels maximum mortality was achieved with 60% RH but in case of 35°C the trend was changed and higher mortality was attained with 45% RH. It was obvious from the result that efficacy of *B. bassiana* highly depends on temperature and RH levels (Table 3).

Table 3. *Mortality of R. dominica on wheat treated with various dose rates of B. bassiana (conidia) at different levels of temperature (T) and relative humidity (RH)*

Exposure (Days)	T (˚C)	RH (%)	Mortality % ± SE		
			1×10^8	1.5×10^8	2×10^8
7	25	45	11± 0.10bc B	14.16± 0.10cd AB	19.16± 0.15de A
		60	22.16± 0.13a B	27.50± 0.13a B	37.50± 0.14a A
		75	15± 0.10abc B	21.83± 0.10abc AB	30.83± 0.13abc A
	30	45	8.33± 0.06c A	13.33± 0.12cd A	15.16± 0.13e A
		60	19.16± 0.11ab B	24.83± 0.13ab AB	31.66± 0.10ab A
		75	12.50± 0.10bc B	17.50± 0.12bcd B	25.83± 0.15bcd A
	35	45	10.83± 0.12bc B	17.33± 0.11bcd AB	21.83± 0.12cde A
		60	7.50± 0.05c B	12.50± 0.10d B	18.33± 0.15de A
		75	10± 0.10c B	13.33± 0.10cd AB	19.16± 0.10de A
14	25	45	21.66± 0.13cd B	25.83±0.11d B	35± 0.11cd A
		60	40.83± 0.13a C	51.66± 0.16a B	68.33± 0.16a A
		75	27.50± 0.14bc C	39.16± 0.17bc B	56.66± 0.17ab A
	30	45	15.83± 0.12d B	24.33± 0.11d AB	28.83± 0.11d A
		60	34.16± 0.13ab B	44.16± 0.14ab B	57.50± 0.14ab A
		75	22.33± 0.11cd B	33.16± 0.16bcd B	47.50± 0.16bc A
	35	45	20± 0.11cd B	30.16± 0.13cd A	39.16± 0.13cd A
		60	13.33± 0.12d B	22.16± 0.16d B	33.33± 0.16d A
		75	18.50± 0.15cd B	24.83± 0.14d B	35± 0.14cd A
21	25	45	26.83± 0.14cd B	31.66± 0.16d AB	41.66± 0.16d A
		60	49.16± 0.16a B	60.83± 0.17a B	82.50± 0.20a A
		75	33.33± 0.17bc C	47.50± 0.11bc B	66.66± 0.19b A
	30	45	18.33± 0.11d B	29.16± 011d A	35± 0.20d A
		60	41.66± 0.13ab C	53.33± 0.16ab B	69.16± 0.13ab A
		75	27.50± 0.16cd C	38.33± 0.13cd B	56.66± 0.17bc A
	35	45	23.33± 0.13cd C	36.66± 0.16cd B	47.50± 0.14cd A
		60	16.33± 0.14d C	27.50± 0.11d B	40.83± 0.13d A
		75	21.66± 0.13cd B	29.16± 0.13d B	42.50± 0.16d A

For each exposure, means with similar upper case letters within each row and with similar lower case letters within each column are not significantly different (Tukey- Kramer HSD test at $P \leq 0.05$)

Progeny Production

DE Concern®

For progeny development of *R. dominica,* significant effect of all main factors (Temperature, RH, Dose) and associated interactions was noted (table 4).

Significantly fewer numbers of offspring emerged in the jars treated with the various dose rates of DE Concern® as compared to control (0ppm) at all combinations of temperature and RH. Overall results indicated that emergence of offspring was highly suppressed at elevated temperature 35°C as compared to 25° and 30°C and low level of RH (45%) in comparison with 60% and 75% RH levels. Least number of adults (18.66 per jar) emerged at 35˚C and 45% RH with the application of the highest tested dose rate of

800ppm, while maximum numbers of adults were emerged at 0ppm with 30°C and 75% r.h level (Table 5).

Table 4. *ANOVA parameters for progeny production of R. dominica by the application of DE Concern® and B. bassiana*

		DE Concern®		***Beauveriabassiana***	
Source	**Df**	***F***	***P***	***F***	***P***
Temperature	2	73.8	<0.01	770.9	<0.01
RH	2	1971.6	<0.01	1072.5	<0.01
Dose	3	3308.9	<0.01	4011.0	<0.01
Temperatue × RH	4	27.8	<0.01	166.5	<0.01
Temperature × Dose	6	52.3	<0.01	36.5	<0.01
RH × Dose	6	176.6	<0.01	92.7	<0.01
Temperature × RH × Dose	12	5.1	<0.01	19.5	<0.01

Table 5. *Mean number of progeny/treatmentof R. dominica on wheat treated with DE Concern® at different levels of temperature (T) and relative humidity (RH) after 60 days exposure*

		Mean Number of Progeny/Treatment ± SE			
T (˚C)	**RH (%)**	**0ppm**	**200ppm**	**400ppm**	**800ppm**
25	**45**	154.33± 0.90d A	37.66± 0.32d B	34.66± 0.28c B	31.33± 0.23c B
	60	265.66± 1.65bc A	77± 0.28c B	73.66± 0.32b B	64.66±0.35b B
	75	250.33± 0.84c A	109.66± 0.51a B	96.66± 0.37a BC	90.33± 0.30a C
30	**45**	165.66± 0.66d A	25.66± 0.23ef B	23.33± 0.19de B	20.33± 0.19de B
	60	294.66± 0.93ab A	35± 0.24de B	32± 0.18cd B	28.66± 0.23cd B
	75	311.33± 0.89a A	99.33± 0.37b B	90.33± 0.32a B	83.33± 0.28a C
35	**45**	153.33± 0.70d A	22± 0.18f B	20.66± 0.14e B	18.66± 0.19e B
	60	272.33± 0.70bc A	28.66± 0.23def B	26.33± 0.23cde B	23.66± 0.19cde B
	75	289.66± 1.03ab A	83.66± 0.44c B	77.33± 0.32b B	70± 0.40b B

Means with same upper case letters within each row and same lower case letters within each column are not significantly different (Tukey- Kramer HSD test at $P \leq 0.05$)

B. bassiana

In case of *B. bassiana* all main factors and their related interactions significantly affect the progeny development of *R. dominica* (Table 4). Considerably, less number of offspring was developed in *B. bassiana* treated jars as compared to the control treatment at all combinations of temperature and RH. It was revealed that the low temperature regime of 25°C along with low level of RH (45%) has strongly suppressed the emergence of offspring as compared to other tested levels of temperature and RH. The minimum number of adults was 19.33 per jar that was noticed at 25°C and 60% RH level with maximum concentration of $2x10^8$ spores/kg of grain (table 6).

Table 6. *Mean number of progeny/treatment of R. dominica on wheat treated with B. bassiana at different levels of temperature (T) and relative humidity (RH) after 60 days exposure*

T (˚C)	RH (%)	Mean Number of Progeny/Treatment ± SE			
		0	1×10^{8}	1.5×10^{8}	2×10^{8}
25	45	157.66± 0.92d A	34.66± 0.14f B	30.66± 0.11f B	20.33± 0.14f B
	60	272.66± 1.47bc A	34.33± 0.14f B	29.66± 0.14f B	19.33±0.16f B
	75	252.33± 1.08c A	61.33± 0.24e B	55± 0.20e B	45.66± 0.14e B
30	45	168.33± 0.73d A	46.66± 0.14ef B	39.66± 0.16ef BC	28± 0.16f C
	60	300.66± 1.34ab A	98.66± 0.20d B	83.66± 0.22d BC	74.66± 0.22d C
	75	321.66± 0.87a A	125.66± 0.24c B	106.33± 0.33c C	95± 0.27c C
35	45	169.66± 0.96d A	33.66± 0.14f B	28.33± 0.21f B	20± 0.09f B
	60	280.33± 1.01bc A	148.66± 0.36b B	128.66± 0.29b C	105.33± 0.29ab C
	75	296.66± 0.96ab A	161.66± 0.30a B	144.33± 0.34a C	118± 0.34a C

Means with same upper case letters within each row and same lower case letters within each column are not significantly different (Tukey- Kramer HSD test at $P \leq 0.05$)

Abiotic factors, specifically temperature and relative humidity are key aspects which affect the success of entomopathogenic fungi and diatomaceous earth [22,43]. In current research effect of these factors was evaluated on bioactivity of DE and *B. bassiana* against *R. dominica*. The influence of RH and temperature on the toxicity of DEs has been evaluated extensively earlier and generally, it is considered that elevated temperature increased the efficiency of diatomaceous earth against stored grain beetles [39,41,53,54,55,56]. Results of the current study support this hypothesis, as the effectiveness of DE showed progressive increase when temperature rose from 25° to 30° and 35°C. The rise of temperature increases the insect movement and assimilation of DE elements on the body surface. Elevated temperature, additionally lead to increased respiration and water loss [22,39]. Similar to present results, Athanassiou *et al.* [42], testified that toxicity of different DEs against *O. surinamensis* and *T. castaneum* increased with the rise of temperature from 20 to 25 and 30°C. Riasat *et al.*[29] and Athanassiou *et al.*[55]also reported increased toxicity of various DE formulations against *R. dominica* at higher temperature among tested levels of 20, 25 and 30°C. The effectiveness of DE reduces with the increase of RH that has been reported in many previous studies [41,57,58]. In moist environments, loss of water can be moderated by insects due to reduced transpiration through cuticle [59] whereas the particles of DE can also absorb more moistness from humid sorrundings [60]. In a previous work, Vayias and Athanassiou [41] noticed that DE was considerably more toxic at 55% RH against *T. confusum* rather than 65% RH. Athanassiou *et al.* [58] reported tolerant behavior of adults of *T. confusum, R. dominica*and *S. oryzae* on wheat grains, admixed with various formulations of DE under RH of 55% as compared to 65%. Higher susceptibility of *O. surinamensis* and *T. castaneum* registered by Athanassiou *et al.* [42] at low RH of 55% with various DE formulations. These outcomes propose that even a marginal change in RH can alter DE efficiency. For the applied DE, 1present findings show that survival of *R. dominica*is directly proportional to RH level as mortality was considerably high on 45 and 60% RH in comparison of 75%. Similarly, progeny was highly suppressed when RH decreased from 75, 60 and 45%. The interaction of RH with temperature is also important as decrease of RH with low temperature of 25°C did not show increase efficacy of DE.

Temperature plays a vital role for the success of entomopathogenic fungi. The germination and conidial viability of fungi reduces under conditions with high temperature [24,44]. Vassilakos *et al.*[61] observed that *B. bassiana* was less effective towards *R. dominica*and *S. oryzae* at 26°C as compared to 30°C. Similarly [62] reported that with rise of temperature level from 25 to 30°C, efficacy of *B. bassiana* against the adults of *S. granarius* (L.) was significantly decreased. Thompson and Reddy [63] also described that temperature significantly affect the efficacy of EPF and demonstrated that pathogenicity of *B. bassiana* was high at 25°C as compared to all high tested temperature regimes against *T. castaneum*. In current study, the pathogenicity of *B. bassiana* against *R. dominica*, declined with the increase of temperature levels from 25 to 30 and 35°C, that is totally in accordance with the above-mentioned findings of the earlier studies. Whereas, Shaheen *et al.*[64] reported a contradictory result and described that efficacy of *B. bassiana* was high at 30°C as compared to 25°C against *Callosobruchus chinensis*. The variation of results may be due to diverse response to temperature by various isolates of *B. bassiana* as implied by Klingen and Haukeland [65] or due to the susceptible response of *C. chinensis* at high temperature such as Thomas and Blanford [66] described that temperature can have significant effect on the host vulnerability.Regarding the response of RH on the viability of EPF, contradictory outcomes have given by available literature [45,67]. Such as, Searle and Doberski [46] stated that *B. bassiana* was infectious at 100% RH to *O. surinamensis* but not at 90%, while for the same fungus Akbar *et al.*[45] found no statistically remarkable difference for the larval mortality of *T. castaneum* at 75% and 54% RH. Whereas Wakil *et al.*[68] reported enhance pathogenicity of *B. bassiana* against *R. domonica* at reduced RH of 55% compared to 75% but Lord [47] found no significant difference between RH of 43%, 56%, 75% and 85% on the efficacy of *B. bassiana* against *R. dominica*. Recently, Athanassiou *et al.*[69] described no significant difference between 55% and 75% RH on the effectiveness of *M. anisopliae* to the larvae of *E. kuehniella.* Whereas, Michalaki *et al.* [70] stated that *M. anisopliae* was less infectious at 75% than 55% RH against *T. confusum.* Current results indicated that *B. bassiana* was more effective at 60% RH as any decrease or increase of RH from 60, to 45 and 75% result in decreased pathogenicity, which proposed that there is a specific range of RH which is optimum for the growth of *B. bassiana* conidia. The findings of our research concludes that range is between 55 and 65% RH.

Our results summarized that the tested biopesticide, *B. basssiana* and DE Concern® are effective against *R. dominica* but their efficacy is highly dependent on abiotic factors of the environment i.e. temperature and RH.Hence,steps should be taken for practical application of these eco-friendly insecticides for the control of stored grain insect pests at commercial as well as domestic levels with keeping in mind the influence of abiotic factors of the environment in order to get maximum results. This study furthermore recommends that with the changing climate, attention is required to be paid to collaboration of the abiotic conditions of the environment with effectiveness of insecticides against insect pests of stored grains.

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