Pak. J. Agri. Sci., Vol. 57(1), 79-86 ;2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/20.8661
http://www.pakjas.com.pk

PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI AGAINST *Sitophilus granarius* (L.) (COLEOPTERA: CURCULIONIDAE) UNDER ABIOTIC FACTORS

Shah Zaman[1,*], Mansoor ul Hasan[1], Farooq Ahmad[1] and Nazir Javed[2]

[1]Department of Entomology, University of Agriculture, Faisalabad, Pakistan;
[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan
[*]Corresponding author's e-mail: shahzaman18@gmail.com

Present bioassays were conducted to determine the effect of temperature and relative humidity (r.h.) on the pathogenicity of two commercially available formulations of entomopathogenic fungi (EPF) i.e. *Metarhizium anisopliae* and *Beauveria bassiana* against *Sitophilus granarius* (L.). Adults of *S. granarius* were exposed to three conidial concentrations (1×10^8, 1.5×10^8 and 2×10^8 CFU kg^{-1} of wheat grains) of each EPF at three temperature ranges (25, 30 and 35°C) and relative humidity levels (45, 60 and75%). Pathogenicity was assessed after 7, 14 and 21days (d) and after last count of 21 d all adults were removed from the vials and vials were maintained at same conditions for additional 60 d to assess the F_1 adult progeny development. Results showed that efficacy of EPF influenced by temperature, relative humidity, conidial concentration and exposure period. In case of *B. bassiana* pathogenicity was increased with reduced temperature 25°C and moderate r.h. 60% and reached up to 72.5% at higher tested conidial concentration after 21 d of exposure period. For *M. anisopliae* optimum conditions for pathogenicity were moderate temperature (30°C) and r.h. (60%) at which maximum pathogenicity of 64.16% was achieved after 21 d with highest concentration. Regarding progeny production, reduced level of r.h. (45%) significantly suppressed the adult emergence with all tested levels of temperature for both EPFs. However, least numbers of adults were emerged on those abiotic conditions where higher pathogenicity was noticed. Overall results illustrated that both EPF have potential to control *S. granarius* but their efficacy highly dependent on abiotic factors and these factors should be seriously considered when planning an IPM program based on EPF.

Keywords: Temperature, relative humidity, concentration, progeny production, *Metarhizium anisopliae* and *Beauveria bassiana*.

INTRODUCTION

Infestation to stored commodities by insect pests is an important concern, as it resulted in dire hazards to the quality and quantity of food products during storage (Phillips and Throne, 2010; Mason and McDonough, 2012). The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), is one of the destructive insect pests of stored grains (Plarre, 2010).

Protection of stored grains from insect pests is currently based on the use of fumigants (phosphine etc.) and residual insecticides that cause serious harms, such as toxicity to no target organisms (Pisa *et al.*, 2017), resistant development in insect population (Opit *et al.*, 2012; Afful *et al.*,2017), residues of pesticides in treated products (Norman, 2000; Lozowicka *et al.*, 2014) and environmental pollution (Van der Sluijs *et al.*, 2015). Therefore, time is needed to evaluate new ways that are more sustainable and eco-friendly for stored grain insects' management especially when consumer demands for organic food and products used for sensitive groups such as infants.

The application of entomopathogenic fungi is a promising alternative to conventional chemicals for stored grains protection (Batta and Kavallieratos, 2018; George *et al.*, 2018). EPF are naturally available enemies of insects with limited impact on environment and have low toxicity toward mammals (Zimmermann, 2007). EPF have been widely evaluated against stored products insect pests (Rumbos and Athanassiou, 2017). In this regard, most broadly tested EPF include *Metarhizium anisopliae* (Metschnikoff) (Batta, 2005; Michalaki *et al.*, 2006) and *Beauveria bassiana* (Balsamo) (Padın *et al.*, 2002; Kaur *et al.*, 2014; Dal Bello *et al.*, 2017; Rehman *et al.*, 2019). The processes of EPF infection starts with the attachment of asexual spores to the insect cuticle then form germ tube and penetrate to the insect body, followed by multiplication of spores and produce toxins that ultimately kills the host (Pedrini *et al.*, 2007). After that mycelium grows out from the cadaver, go through sporulation and repeat the inoculums in fungus-insect environment (Ortiz-Urquiza and Keyhani, 2013; Gabarty *et al.*, 2014).

The pathogenicity of EPF is seriously affected by the certain abiotic features of the environment; among these temperature and humidity are the most important (Sarwar, 2015). For temperature, it is assumed that high levels have negative

impact on germination and viability of conidia (Horaczek and Viernstein, 2004). Regarding the effect of RH on the pathogenicity of entomopathogenic fungi, available literature has often provided contradictory results (Akbar *et al.*, 2004). Keeping in view the above facts and the prevailing situation of climate change, t current bioassays were performed to evaluate the efficacy of commercially available EPF i.e. *M. anisopliae* (Pacer) and *B. bassiana* (Racer ^{TM})under different combinations of temperature and humidity against *S. granarius.*

MATERIALS AND METHODS

***Insects*:** One week old adults of *S. granarius* were used for the experimentation that were reared on sterilized whole wheat grains (var; Millat 2011) in the Grain Research Training and storage Management Cell, Department of Entomology, University of Agriculture Faisalabad at 30±2°C, 65±5% r.h. and under darkness. The population was previously collected from Godowns of Punjab Food Department situated at district Faisalabad, Pakistan.

***Commodity*:** Bioassy was performed on clean, untreated and infestation free hard wheat, *Triticum aestivum*. (var. Millat 2011). Grains moisture content was ranged between12.7% to 13.3% measured by, Dickey-John moisture meter (Multigrain CACII; Dickey-John Co., Auburn, IL, USA).at the beginning of the trials.

***EPF Formulations*:** The commercial formulations of entomopathogenic fungi namely Racer ^{TM} and Pacer were used during bioassays and were imported from Agri Life, Medak District. Hyderabad, India. The Pacer contains selected strain of *M. anisopliae* (NCIM 1311) while Racer BB have *B. bassiana* (NCIM 1216, ATCC 26851). Both formulations were in powder form with CFU count of 1×10^8/g.

***Bioassays*:** Three concentrations (1×10^8, 1.5×10^8, 2×10^8 CFU/kg of grains) of each entomopathogenic fungi were applied to 1.5kg lots of wheat grains in cylindrical jars. The jars were manually shaken for approximately 10 minutes to achieve equal distribution as both formulations were in powdered form. Three samples of 60 g from each lot were taken and put into separate cylindrical plastic vials with dimension of (12cm height, 7cm diameter). Then, 40 adults of *S. granarius* were released in each vials and ventilation inside the vials was sustained by small holes in the vials closure covered with fine cloth. The control treatment was served with series of vials having untreated gains. These vials were transferred to incubators (Model MIR-254, SANYO) set at desired levels of temperature and relative humidity. The bioassays were performed on all possible combinations of three levels of temperature (25, 30 and 35°C) and r.h. (45, 60 and 75%). In treated and control vials after exposure period of 7, 14 and 21 days adult morality was observed by touching the adults with brush and observe the movement. After last count of pathogenicity, treated individuals were removed from the vials and the vials containing treated and untreated grains were again put in the incubators at same sets of temperature and r.h. for an extra period of 60 d to assess the development of F_1 progeny. Progeny development was recorded by calculating the numbers of adult offspring as immature development of *S. granarius* occurs inside the kernel.

***Statistical Analysis*:** Data for pathogenicity was corrected by Abbott's formula (Abbott, 1925) and subjected to four way ANOVA with temperature, r.h., Exposure and concentrations as main effects. For progeny production data three way ANOVA was performed with Temperature, r.h. and dose as main effects. Means of the treatments were compared by Tukey-HSD test at 5% level of significant (Sokal and Rohlf, 1995) and all analysis were performed using R software 3.5.1.

Table 1. ANOVA parameters for pathogenicity against adults of *S. granarius*

		Metarhizium anisopliae		***Beauveria bassiana***	
Source of Variation	**DF**	***F-value***	***P-value***	***F-value***	***P-value***
Concentration	2	492.4	<0.01	683.0	<0.01
r.h.	2	227.2	<0.01	198.3	<0.01
Temperature	2	574.8	<0.01	297.3	<0.01
Exposure	2	224.2	<0.01	905.2	<0.01
Concentration × r.h.	4	33.0	<0.01	49.2	<0.01
Concentration × Temperature	4	12.4	<0.01	12.0	<0.01
Concentration × Exposure	4	49.7	<0.01	121.8	<0.01
r.h. × Temperature	4	104.9	<0.01	238.6	<0.01
r.h. × Exposure	4	95.1	<0.01	75.0	<0.01
Temperature × Exposure	4	87.4	<0.01	75.1	<0.01
Concentration × r.h.× Temperature	8	5.5	<0.01	19.5	<0.01
Concentration × r.h. × Exposure	8	0.7	0.67	3.9	<0.01
Concentration×Temperature× Exposure	8	0.6	0.76	1.6	0.11
r.h. × Temperature× Exposure	8	12.3	<0.01	33.9	<0.01
Concentration × r.h. × Temperature × Exposure	16	0.4	0.98	1.4	0.14

RESULTS

***Adult pathogenicity and progeny development of S. granarius by application of M. anisopliae*:** Significant effect of all main factors (Concentration, r.h., Temperature, Exposure) was noticed after application of *M. anisopliae* against *S. granarius* (Table 1). After 7 d post exposure, pathogenicity of *S. granarius* was low at high temperature 35°C and low r.h. 45% on grains treated with *M. anisopliae* (Table 3). At 30°C maximum adult pathogenicity (33.16%) was noticed with higher dose rate and 60% r.h. followed by 30.5% with r.h. of 75%. Similarly, at 25°C higher Pathogenicity (30%) was observed with 60% r.h. as compared to 45 and 75% r.h. Pathogenicity had further increased after 14 d exposure and the trend of pathogenicity was same to 7 d as the high temperature and lower r.h. among tested levels resulted in lower pathogenicity. Maximum pathogenicity (52.5%) was noted with highest concentration at 30°C and 60% r.h. At 25 and 35°C similarly higher concentration 45.83 and 29.16%, respectively, was recorded with 60% r.h. and maximum tested concentration. After exposure interval of 21 d, concentration was further increased and similarly to 7 and 14 d exposures, maximum adults of *S. granarius* were dead (64.16%) on grains treated with highest tested concentration of *M. anisopliae* at 30°C and 60% r.h. followed by (58.33%) at same temperature and 75% r.h. Generally, at 25°C the

Table 2. ANOVA parameters for adult progeny production of *S. granarius*

		Metarhizium anisopliae		*Beauveria bassiana*	
Source of Variation	**DF**	***F-value***	***P-value***	***F-value***	***P-value***
Concentration	2	3505.4	<0.01	4101.6	<0.01
r.h.	2	2048.2	<0.01	1974.2	<0.01
Temperature	3	1141.4	<0.01	1048.0	<0.01
Concentration × r.h.	4	87.7	<0.01	166.5	<0.01
Concentration × Temperature	6	179.1	<0.01	54.2	<0.01
r.h. × Temperature	6	357.2	<0.01	226.3	<0.01
Concentration×r.h. × Temperature	12	39.8	<0.01	33.5	<0.01

Table 3. Pathogenicity (%) of *S. granrius* on wheat treated with three dose rates of *M. anisopliae* (conidia) at three levels of relative humidity (r.h.) and temperature (T) after 7, 14 and 21 days exposure.

			Pathogenicity (%) ± SE		
Exposure (Days)	**Temperature (°C)**	**Relative Humidity (%)**	**1×10^8**	**1.5×10^8**	**2×10^8**
7	25	45	7.83±0.11ij	8.33±0.11ij	13.33±0.13gh
		60	19.16±0.11ef	22.66±0.12de	30.00±0.16abc
		75	13.00±0.14gh	16.66±0.16fg	22.50±0.14de
	30	45	10.83±0.16hi	13.33±0.13gh	15.83±0.16fg
		60	22.33±0.16de	28.33±0.14bc	33.16±0.21a
		75	20.00±0.14ef	25.83±0.21bc	30.50±0.14ab
	35	45	5.83±0.11j	8.50±0.14ij	12.83±0.16gh
		60	10.00±0.09hij	14.16±0.16gh	19.50±0.14ef
		75	8.33±0.11ij	10.83±0.13hi	15.83±0.13fg
14	25	45	10.83±0.11m	13.33±0.16 lm	20.83±0.13ij
		60	30.00±0.14efg	34.16±0.13de	45.83±0.17bc
		75	19.16±0.13jk	25.83±0.13gh	34.16±0.13de
	30	45	15.83±0.16kl	19.16±0.17jk	23.33±0.16hij
		60	36.66±0.16d	44.33±0.17bc	52.50±0.14a
		75	30.83±0.17ef	41.66±0.16c	47.50±0.16b
	35	45	9.16±0.13m	12.50±0.11lm	19.16±0.13jk
		60	15.66±0.13kl	22.50±0.16hij	29.16±0.21fg
		75	12.50±0.14lm	16.16±0.15kl	24.16±0.17hi
21	25	45	13.33±0.12no	16.16±0.13lmn	25.83±0.19ij
		60	37.66±0.13f	42.66±0.16de	56.66±0.17b
		75	24.16±0.17jk	31.66±0.16gh	42.50±0.16de
	30	45	19.16±0.13lm	24.16±013jk	29.16±0.17hi
		60	45±0.16d	55.00±0.16bc	64.16±0.16a
		75	38.33±0.21ef	50.83±0.21c	58.33±0.21b
	35	45	10.83±0.16o	15.83±0.16mn	24.16±0.19jk
		60	18.33±0.14lm	28.50±0.14hij	35.83±0.13fg
		75	15.00±0.16mno	20.83±0.16kl	30.00±0.19hi

concentration was high as compared to 35°C. At 25°C (56.66%) and (42.5 %) adults were found dead with application of higher concentration at 60 and 75% r.h. respectively. Whereas at 35°C and combination of 45, 60 and 75% r.h. at higher tested concentration resulted in pathogenicity of 24.16, 35.83 and 30% respectively. Overall pathogenicity results indicated that efficacy of *M. anisopliae* was dependent on temperature and r.h. and was high at moderate levels of temperature and r.h. among tested levels.

Regarding progeny development, all main factors (concentration, Temperature, r.h.) were significant (Table 2). The emergence of offspring was increased with increase of temperature and especially r.h. (Table 4). Significantly less numbers of adult were emerged in treated vials as compared to control treatments. At 45% r.h., combination of 25, 30 and 35°C resulted in emergence of 32, 27.33 and 22.66 adults per vial respectively by application of 2×10^8 conidia/ kg of grains. At 60 and 75% r.h., combination of 30°C with higher tested concentration produced less number of adults per vial viz., 23.33 and 53 respectively, in comparison with 25 and 35°C.

Adult pathogenicity and progeny development of S. granarius by application of B. bassiana: Adult pathogenicity of *S. granarius* by application of *B. bassiana* was significantly affected by all main factors (Concentration, r.h., Temperature,

Table 4. Mean numbers of emerged adults/vial of *S. granarius* on wheat treated with *M. anisopliae* (Conidia) at three levels of relative humidity (r.h.) and temperature (T) after 60 days exposure.

		Progeny ± SE			
T (°C)	**Relative Humidity (%)**	**0**	**1×10^8**	**1.5×10^8**	**2×10^8**
25	45	182.00±0.69ef	56.66±0.33mno	53.33±0.22no	32.00±0.19pq
	60	294.66±0.61c	115.33±0.47hij	97.66±0.49jk	89.66±0.48kl
	75	314.33±0.84b	128.33±0.43h	127.66±0.64hi	108.33±0.69ijk
30	45	187.33±0.82ef	42.66±0.20op	40.00±0.19opq	27.33±0.14pq
	60	331.33±0.91ab	41.33±0.20opq	33.33±0.14pq	23.33±0.14pq
	75	338.33±0.93a	74.66±0.45lm	62.66±0.29mn	53.00±0.19no
35	45	176.00±0.60f	40.33±0.24opq	33.66±0.20pq	22.66±0.14q
	60	322.33±0.92ab	200.33±0.84e	174.66±0.77f	151.33±0.64g
	75	327.33±0.73ab	229.33±0.72d	196.33±0.61e	185.66±0.94ef

Table 5. Pathogenicity (%) of *S. granrius* on wheat treated with three dose rates of *B. bassiana* (conidia) at three levels of relative humidity (r.h.) and temperature (T) after 7, 14 and 21 days exposure.

			Pathogenicity % ± SE		
Exposure (days)	**Temperature (°C)**	**Relative Humidity (%)**	**1×10^8**	**1.5×10^8**	**2×10^8**
7	25	45	9.16±0.08hij	12.33±0.11fgh	15.16±0.13ef
		60	18.00±0.11de	21.66±0.11bcd	29.16±0.21a
		75	12.50±0.14fgh	18.33±0.13de	24.16±0.13bc
	30	45	6.66±0.06ij	10.33±0.12ghi	12.50±0.16fgh
		60	14.16±0.11f	19.16±0.11d	25.00±0.11b
		75	10.33±0.12ghi	14.16±0.13f	20.83±0.13cd
	35	45	9.16±0.08hij	13.33±0.13fg	18.33±0.11de
		60	5.83±0.10j	9.16±0.08hij	14.16±0.16f
		75	8.33±0.08ij	10.16±0.10ghi	15.00±0.14ef
14	25	45	16.83±0.12jkl	19.16±0.13ij	26.00±0.24fg
		60	30.83±0.21e	38.33±0.13c	51.66±0.21a
		75	20.16±0.13hij	29.16±0.17ef	43.33±0.16b
	30	45	11.66±0.16m	18.33±0.12ijk	22.66±0.13ghi
		60	25.83±0.19fg	33.00±0.19de	43.33±0.17b
		75	17.50±0.14jkl	24.16±0.16gh	35.83±0.16cd
	35	45	14.33±0.11klm	22.33±0.13ghi	29.16±0.13ef
		60	10.83±0.13m	16.66±0.12jkl	25.83±0.16fg
		75	13.33±0.13lm	18.33±0.17ijk	26.00±0.14fg
21	25	45	23.83±0.13mn	27.83±0.16klm	37.50±0.14gh
		60	43.33±0.14ef	54.16±0.19c	72.50±0.14a
		75	29.16±0.16jkl	41.83±0.17fg	60.00±0.20b
	30	45	16.66±0.16op	25.83±017lm	30.83±0.16jk
		60	36.66±0.17hi	46.66±0.16de	60.83±0.16b
		75	24.16±0.13mn	35.83±0.16hi	50.83±0.17cd
	35	45	20.83±0.13no	32.50±0.14ij	41.66±0.15fg
		60	14.16±0.11p	24.16±0.11mn	35.83±0.19hi
		75	19.16±0.16o	25.83±0.17lm	37.50±0.14gh

Table 6. Mean number of emerged adults/vial of *S. granarius* on wheat treated with *B. bassiana* (conidia) at three levels of relative humidity (r.h.) and temperature (T) after 60 days exposure.

		Mean of progeny/Treatment ± SE			
T (°C)	**Relative Humidity (%)**	**0**	**1×10^8**	**1.5×10^8**	**2×10^8**
25	45	171.66±0.56ef	42.00±0.14pqr	34.33±0.20rs	22.00±0.14s
	60	297.33±0.77c	38.66±0.30qr	32.33±0.20rs	20.66±0.22s
	75	268.66±0.70d	69.33±0.30no	56.66±0.20op	50.66±0.24pq
30	45	185.66±0.76e	53.33±0.14pq	43.66±0.14pqr	30.33±0.09rs
	60	319.33±0.82b	108.33±0.16kl	83.33±0.14mn	70.00±0.1no
	75	337.66±0.86a	137.66±0.20hi	98.00±0.25lm	85.66±0.20m
35	45	186.33±0.80e	38.33±0.14qr	31.00±0.14rs	21.33±0.11s
	60	301.33±0.77c	151.00±0.19gh	125.33±0.15ij	104.33±0.14kl
	75	310.66±1.60c	164.66±0.24fg	143.33±0.14h	118.66±0.16jk

Exposure period) (Table 1). After post exposure of 7 d, adult pathogenicity of *S. granarius* on grains treated with *B. bassiana* decreased with the increase of temperature and decrease of r.h. (Table 5). The maximum pathogenicity (29.16%) was observed at 25°C and 60% r.h. among all tested combinations with highest tested concentration of 2×10^8 Conidia/kg of grains. At 30°C, pathogenicity was also high with 60% r.h. and ranged between 14.16 to 25% with significant difference among concentration. Whereas at 35°C, combination of 60% r.h. resulted in lowest pathogenicity compared to 45 and 75%. At 35°C higher pathogenicity (18.33%) was noticed with 45% r.h. at higher tested conidial concentration. Similar results to 7 d exposure were observed after 14 d, as the increase of temperature responsible for decreased efficacy of *B. bassiana*. At 25 and 30°C maximum pathogenicity 51.66 and 43.33% respectively was recorded with 60% r.h. while at 35°C pathogenicity (29.16%) was high with 45% r.h. in comparison to 60 and 75% at maximum concentration. The lowest pathogenicity at this exposure was (10.83%) and noted at 35°C and 60% r.h. among all tested combinations of temperature and r.h. with lower applied concentration. Exposure interval of 21 d showed similar trend of *S. granarius* adult pathogenicity as noticed after 7 and 14 d exposure. At 25 and 30°C pathogenicity was low with 45% r.h. as compared to the combinations of 60 and 75%. Maximum numbers of exposed adult (72.5%) were found dead at 25°C and 60% r.h. followed by 60.83% at 30°C and 60% r.h. with higher tested concentration. At 35°C, pathogenicity did not reach up to 50% in any combination of r.h. and concentration and maximum pathogenicity (41.66%) was observed with 45% r.h. Overall pathogenicity results showed that lower temperature 25°C was most favourable for *B. bassiana* efficacy among tested levels and in case of r.h. trend was same at 25 and 30°C as maximum results were obtained with 60% but in case of 35°C trend was changed and combination of 45% contributed to prominent results.

Results of *S. granarius* progeny development showed significant effect of all main factors (Table 2). Generally, progeny was low in treated grains as compared to control treatment (Table 6). At 25°C, least mean numbers of adult per vial (20.66) were emerged with 60% r.h. among all tested combinations of temperature and r.h. at higher tested concentration. Progeny emergence was high at 75% r.h. as compared to 45 and 60% r.h. in most of the cases. At 30 and 35°C progeny was greatly reduced with 45% r.h. as compared to 60 and 75% r.h.

DISCUSSION

The success and virulence of EPF for the management of storage insect pests, strongly affected by abiotic factors of the environment such as relative humidity and temperature (Sarwar, 2015; Rumbos and Athanassiou, 2017). Temperature is assumed as a key factor for efficacy of EPF against stored grain insect pests. Many studies have reported that the germination and conidial viability of fungi decreases under conditions with a higher level of temperature (Moore *et al.*, 2000; Horaczek and Viernstein, 2004). Vassilakos *et al.* (2006) reported increased pathogenicity of *B. bassiana* at 26°C rather than 30°C against *R. dominica* and *S. oryzae*. Similar results has been observed by Athanassiou and Steenberg (2007) who noticed that increase of temperature from 25 to 30°C significantly reduced the pathogenicity of *B. bassiana* against *S. granarius* adults. Moreover, Thompson and Reddy (2016) described that efficacy of *B. bassiana* significantly affected by temperature against *T. castaneum* and was high at 25°C among all tested levels. Latifian *et al.* (2018) also illustrated that pathogenicity of *B. bassiana* against *O. surinamensis* was high at 25°C compared to 15, 20, 30 and 35°C. Present results are in agreements with these findings as the toxicity of *B. bassiana* against adults of *S. granarius* was increased with the decrease of temperature regime. The maximum results regarding pathogenicity and F1 progeny inhibition were noted at 25°C as compared to 30 and 35°C. However, a contradictory result has been stated by Shaheen *et al.* (2016) and reported that effectiveness of *B. bassiana* against *Callosobruchus chinensis* decreased with the decrease of temperature level from 30 to 25°C. This difference of results may be due to varied response of different strains of *B. bassiana* to temperature as described by

Klingen and Haukeland (2006) or due to vulnerable response at high temperature by *C. chinensis* as Thomas and Blanford (2003) suggested that susceptibility of host can have considerable effect of temperature. Regarding the affect of temperature on the pathogenicity of *M. anisopliae* present results shown that overall efficacy was high at 30°C in comparision with 25 and 35°C against *S. granarius*. Similarly, Athanassiou *et al.* (2017) reported that the highest pathogenicity of *E. kuehniella* larvae was noted by the application of *M. anisopliae* at 30°C as compared to 25°C. Michalaki *et al.* (2006) also described that with an increase of temperature level from 20 to 25 and 30°C, the efficacy of *M. anisopliae* against larvae of *T. confusum* was increased. The results of our study also proposed the same trend of *M. anisopliae* toxicity as increase of temperature from 25 to 30°C resulted in high pathogenicity but opposed was noticed when temperature increased from 30 to 35°C. This indicated that there is specific range of temperature at which *M. anisopliae* is more effective and this could be between 28-32°C. The actual reasons for increased efficacy of *M. anisopliae* at high temperature against insect pests of stored grains are not completely understood; anyhow the increased metabolic activity and more insect movement at higher temperatures (Fields and Korunic, 2000) might be associated with the increased conidial attachment and consequently results in higher fungal infections or the slightly heat tolerant behaviour of *M. anisopliae* as Horaczek and Viernstein (2004) described that *M. anisopliae* is more tolerant to heat than *B. brongniartii*. When temperature increases from 30°C to 35°C may be it becomes unfavorable for the germination of *M. anisopliae* conidia that could be the reason for lower pathogenicity rate at 35°C.

As far as concerning the effect of r.h. on the pathogenicity of EPF against insects, it was considered that EPF requires very moist conditions for their effectiveness and dry environments are not favorable for their pathogenicity. This hypothesis has been supported by many previously reported studies. Like, Searle and Doberski (1984) illustrated that efficacy of *B. bassiana* decreased against *O. surinamensis* when r.h. decreased from 100 to 90% and suggested that r.h. is an important factor that determines fungal spores would germinate and infect or not. The commercialization and exploitation of the fungal formulation for the management of stored grain insect pests have been impacted negatively by this perception as in case of stored grains high humidity level is not considered desirable (Navarro *et al.*, 2012). Other studies have submitted that the process of EPF infection to stored grain insects could not be dependent on humidity (Adane *et al.*, 1996; Akbar *et al.*, 2005). Like, Akbar *et al.* (2005) stated that toxicity of *B. bassiana* against *T. castaneum* larvae at two different r.h. levels (55 and 75%) remained similar. Athanassiou *et al.* (2017) reported no significant difference to the infection of *M. anisopliae* between 55 and 75% r.h. against *E. kuehniella* larvae and suggested that tested strain could be effective at ambient level of 50% r.h. However, some reports revealed improvement in the efficacy of EPF with reduced levels of r.h. (Lord, 2005; Michalaki *et al.*, 2006; Athanassiou and Steenberg, 2007; Lord, 2007b). At reduced r.h. levels, the insecticidal effect of *B. bassiana* was increased against *R. dominica* (Lord, 2005) and *T. castaneum* (Lord, 2007a). Likewise pathogenicity of *S. granarius* in wheat treated with *B. bassiana,* was high at 55% of r.h. as compared to 75%. (Athanassiou and Steenberg, 2007). Similarly, *M. anisopliae* was found to be more effective against larvae of *T. confusum* at 55% then at 75% r.h. level (Michalaki *et al.*, 2006). Wakil *et al.* (2011) also reported improved pathogenicity of *B. bassiana* at r.h. level of 55% than 75% against *R. domonica.* Present results had revealed that efficacy of both EPF, *B. bassiana* and *M. anisopliae* was high at moderate r.h. of 60% as compared to 45 and 75% against *S. granarius*. Findings of our research proposed that there is a specific range of r.h. that is optimum for fungal growth at least for the current tested strains of EPF and this could be between 55% and 65% r.h. levels which are also in accordance with the previous studies as described above. The actual reason behind these results has been not completely understood. According to Lord (2005), the stress due to desiccation might cause an alteration in cuticular chemistry which then affects the conidial ability of attachment, germination and penetration or even changes the insect response in such a way that favors infection of the fungus. Although these findings have great practical value as it supports the hypothesis that, EPF could be very effective in places where mostly dry conditions prevails such as storage structures. Furthermore, higher levels of r.h. might decrease the persistence and stability of the fungal conidia as it has been found for *Rozsypal* (Deuteromycotina: Hyphomycetes) and *Metarhizium flavoviride* Gams (Hedgecock *et al.*, 1995; Moore *et al.*, 2000). Beside the temperature and relative humidity, conidia application rates and exposure time are also play important role in efficacy of EPFs (Khashaveh *et al*., 2011; Rehman *et al*., 2019)

***Conclusion*:** Abiotic factors especially relative humidity (R.H) and temperature are of prime importance regarding effectiveness of entomopathogenic fungi (EPFs) like *Beauveria bassiana* and *Metarhizium anisopliae*. So, these two factors should be adjusted while applying the EPF in the management of stored grains insect pests.

***Acknowledgment*:** This research has been financed by Higher Education Commission, Islamabad, Pakistan under 5000 Ph.D. Indigenous Fellowship Program.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265-267.

Adane, K., D. Moore and S. Archer. 1996. Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. J. Stored Prod. Res. 32:105-113.

Afful, E., B. Elliott, M.K. Nayak and T.W. Phillips. 2017. Phosphine resistance in North American field populations of the Lesser Grain Borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Econ. Entomol. 111:463-469.

Akbar, W., J.C. Lord, J.R. Nechols and R.W. Howard. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. J. Econ. Entomol. 97:273-280.

Akbar, W., J.C. Lord, J.R. Nechols and T.M. Loughin. 2005. Efficacy of *Beauveria bassiana* for red flour beetle when applied with plant essential oils or in mineral oil and organosilicone carriers. J. Econ. Entomol. 98:683-688.

Athanassiou, C.G. and T. Steenberg. 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biol. Control. 40:411-416.

Athanassiou, C.G., N.G. Kavallieratos, C.I. Rumbos and D.C. Kontodimas. 2017. Influence of temperature and relative humidity on the insecticidal efficacy of *Metarhizium anisopliae* against larvae of *Ephestia kuehniella* (Lepidoptera: Pyralidae) on wheat. J. Insect Sci. 17: 1-7.

Batta, Y. 2005. Control of the lesser grain borer (*Rhyzopertha dominica* (F.), Coleoptera: Bostrichidae) by treatments with residual formulations of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes). J. Stored Prod. Res. 41: 221-229.

Batta, Y.A. and N.G. Kavallieratos. 2018. The use of entomopathogenic fungi for the control of stored-grain insects. Int. J. Pest Manage. 64:77-87.

Dal Bello, G.M., C.B. Fuse, N. Pedrini and S.B. Padín. 2017. Insecticidal efficacy of *Beauveria bassiana*, diatomaceous earth and fenitrothion against *Rhyzopertha dominica* and *Tribolium castaneum* on stored wheat. Int. J. Pest Manage. 63:1-8.

Fields, P. and Z. Korunic. 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. J. Stored Prod. Res. 36:1-13.

Gabarty, A., H. Salem, M. Fouda, A. Abas and A. Ibrahim. 2014. Pathogencity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotis ipsilon* (Hufn.). J. Radiat. Res. Appl. Sci. 7:95-100.

George, C.G., S.K. Maria and A.G. Christos. 2018. Efficacy of *Beauveria bassiana* in combination with an electrostatically charged dust for the control of major stored-product beetle species on concrete. J. Stored Prod. Res. 79:139-143.

Hedgecock, S., D. Moore, P. Higgins and C. Prior. 1995. Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. Biocontrol Sci. Technol. 5:371-378.

Horaczek, A. and H. Viernstein. 2004. Comparison of three commonly used drying technologies with respect to activity and longevity of aerial conidia of *Beauveria brongniartii* and *Metarhizium anisopliae*. Biol. Control. 31:65-71.

Kaur, S., A. Thakur and M. Rajput. 2014. A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). J. Stored Prod. Res. 59:185-189.

Khashaveh, A., Y. Ghosta, M.H. Safarlizadeh and M. Ziaee. 2011. The use of entomopathogenic fungus, Beauveria bassiana (Bals.) Vuill. in assays with storage grain beetles. J. Agric. Sci. Tech. 13:35-43.

Klingen, I. and S. Haukeland. 2006. The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes. In An ecological and societal approach to biological control, Springer, Dordrecht: pp.145-211.

Latifian, M., M. Ghazavi and E. Soleimannejadian. 2018. The role of temperature on the pathogenicity of *Beauveria bassiana* in populations of sawtoothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) fed on stored date fruits. J. Crop Prot. 7:395-402.

Lord, J.C. 2005. Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). Biol. Control. 34:180-186.

Lord, J.C. 2007a. Enhanced efficacy of *Beauveria bassiana* for red flour beetle with reduced moisture. J. Econ. Entomol. 100:1071-1074.

Lord, J.C. 2007b. Desiccation increases the efficacy of *Beauveria bassiana* for stored-grain pest insect control. J. Stored Prod. Res. 43:535-539.

Lozowicka, B., P. Kaczynski, A. Paritova, G. Kuzembekova, A. Abzhalieva, N. Sarsembayeva and K. Alihan. 2014. Pesticide residues in grain from Kazakhstan and potential health risks associated with exposure to detected pesticides. Food Chem. Toxicol. 64:238-248.

Mason, L.J. and M.M. Donough. 2012. Biology, behavior, and ecology of stored grain and legume insects. Stored Prod. Prot. 1:7-20.

Michalaki, M.P., C.G. Athanassiou, N.G. Kavallieratos, Y.A. Batta and G.N. Balotis. 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) Sorokin applied alone or in combination with diatomaceous earth against *Tribolium confusum* Du Val larvae: Influence of

temperature, relative humidity and type of commodity. Crop Prot. 25:418-425.

Moore, D., J.C. Lord and S.M. Smith. 2000. Pathogens, In Bh. Subramanyam and D. W. Hagstrum (eds.), Alternatives to pesticides in stored product IPM. Kluwer Academic Publishers, Dordrecht. pp.193-227.

Navarro, S., R.T. Noyes, M. Casada and F.H. Arthur. 2012. Grain aeration, In D. W. Hagstrum, T. W. Phillips and G. Cuperus (eds.),Stored product protection. Kansas State University Kansas, USA: pp.121-134.

Norman, K.N.T. 2000. The persistence of methyl bromide residues in rice, dried fruit, seeds and nuts following laboratory fumigation. Pest Manage. Sci. 56:154-158.

Opit, G., T.W. Phillips, M.J. Aikins and M. Hasan. 2012. Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. J. Econ. Entomol. 105:1107-1114.

Ortiz-Urquiza, A. and N.O. Keyhani. 2013. Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects. 4:357-374.

Padın, S., G.D. Bello and M. Fabrizio. 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. J. Stored Prod. Res. 38:69-74.

Pedrini, N., R. Crespo and M.P. Juarez. 2007. Biochemistry of insect epicuticle degradation by entomopathogenic fungi. Comp. Biochem. Physiol. Part C. Toxicol. Pharmacol. 146:124-137.

Phillips, T.W. and J.E. Throne. 2010. Biorational approaches to managing stored-product insects. Annu. Rev. Entomol. 55:375-397.

Pisa, L., D. Goulson, E.C. Yang, D. Gibbons, F. Sanchez-Bayo, E. Mitchell, A. Aebi, J. van der Sluijs, C.J.M. Quarrie and C. Giorio. 2017. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems. Environ. Sci. Pollut. Res. :1-49.

Plarre, R. 2010. An attempt to reconstruct the natural and cultural history of the granary weevil,*Sitophilus granarius* (Coleoptera: Curculionidae). Eur. J. Entomol. 107: 1-11.

Rehman, H., M. Hasan, M. Sagheer and S.T. Sahi. 2019. Bio-efficacy of entomopathogenic fungus *Beauveria bassiana* (Bals.) against *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst). Pak. J. Agri. Sci. DOI: 10.21162/PAKJAS/18.6746.

Rumbos, C.I. and C.G. Athanassiou. 2017. Use of entomopathogenic fungi for the control of stored-product insects: can fungi protect durable commodities? J. Pest Sci. 90:839-854.

Sarwar, M. 2015. Microbial insecticides-an ecofriendly effective line of attack for insect pests management. Int. J. Eng. Adv. Res. Technol. 1:4-9.

Searle, T. and J. Doberski. 1984. An investigation of the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. as a potential biological control agent for *Oryzaephilus surinamensis* (L.). J. Stored Prod. Res. 20:17-23.

Shaheen, F.A., M.W. Akram, M.A. Rashid, M. Nadeem, M. Saeed, M. Husain and K. Mehmood. 2016. Biological control of pulse beetle *Callosobruchus chinensis* L. (Bruchidae: Coleoptera) in stored chickpea grains using entomopathogenic fungus *Beauveria bassiana* Balsamo. J. Entomol. Zool. Stud. 4:1076-1083.

Sokal, R. and F. Rohlf. 1995. Biometry, 3rd edn New York. NY: WH Freeman and Company.

Thomas, M.B. and S. Blanford. 2003. Thermal biology in insect-parasite interactions. Trends Ecol. Evol. 18:344-350.

Thompson, B.M. and G.V.P. Reddy. 2016. Effect of temperature on two Bio-Insecticides for the control of confused flour beetle (Coleoptera: Tenebrionidae). Fla. Entomol. 99:67-71.

Van der Sluijs, J.P., V. Amaral-Rogers, L. Belzunces, M.B. Van Lexmond, J.M. Bonmatin, M. Chagnon, C. Downs, L. Furlan, D. Gibbons and C. Giorio. 2015. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environ. Sci. Pollut. Res. 22:148-154.

Vassilakos, T.N., C.G. Athanassiou, N.G. Kavallieratos and B.J. Vayias. 2006. Infuence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. Biol. Control. 38:270-281.

Wakil, W., T. Riasat, M.U. Ghazanfar, Y.J. Kwon and F.A. Shaheen. 2011. Aptness of *Beauveria bassiana* and enhanced diatomaceous earth (DEBBM) for control of *Rhyzopertha dominica* (F.). Entomol. Res. 41:233-241.

Zimmermann, G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci. Technol. 17:553-596.

[Received 13 Feb 2019; Accepted 14 July- 2019; Published (online) 16 Nov 2019]