Research Article

Age-Specific Response of *Atteva sciodoxa* Meyrick (Lepidoptera: Yponomeutidae) to *Beauveria bassiana* (Bals.)

Ghulam Ali Bajwa[1], Faizah Abood[2], Yusof Bin Ibrahim[3]

[1]Coordinator Sericulture, Pakistan Forest Institute, Peshawar, Pakistan; [2]Faculty of Forestry, University Putra Malaysia, 43400 UPM, Serdang, Malaysia; [3]Faculty of Technical and Vocational Education, Sultan Idris Education University, 35900 Perak, Malaysia.

Abstract | *Beauveria bassiana* (Bals.) was bio-assayed against three larval instars of *Atteva sciodoxa* under laboratory conditions of 27±2°C, 75% RH and 12:12 h photoperiod. Instar 3rd responded with 100% mortality at $5x10^7$ conidia ml^{-1}, while same level of mortality was recorded in 5th instar at $1x10^8$ conidia ml^{-1}. Instar 5 also showed the lowest mortality (33.3%) at $1x10^6$ conidia ml^{-1}. Median effective concentration (EC_{50}) ranged between $9.87x10^5$ and $21.30x10^5$ conidia ml^{-1} for 3rd to 5th instars. Median effective time (ET_{50}) ranged between 3.3 days and 8.2 days for different tested concentrations. A significant ($p<0.05$) 2nd degree correlation between temperature and *B. bassiana* infectivity was analysed. *Beauveria bassiana* infection was in range of 27-30°C. Based on these findings, it is concluded that *B. bassiana* has promising potential as microbial control agent against *A. sciodoxa* and should be exploited under field conditions.

Editor | Tahir Sarwar, The University of Agriculture, Peshawar, Pakistan.
Received | June 25, 2015; **Accepted** | February 23, 2016; **Published** | March 10, 2016
***Correspondence** | Ghulam Ali Bajwa, Pakistan Forest Institute, Peshawar, Pakistan; **Email:** gabajwa64@gmail.com
Citation | Bajwa, G. A., F. Abood and Y. B. Ibrahim. 2016. Age-specific dose-mortality response of *Atteva sciodoxa* Meyrick (Lepidoptera: Yponomeutidae) to *Beauveria bassiana* (Bals.). *Sarhad Journal of Agriculture*, 32(1): 1-8.
DOI | http://dx.doi.org/10.17582/journal.sja/2016/32.1.1.8
Keywords | *Beauveria bassiana*, Infectivity, Age-response, Tiger moth, Temperature

Introduction

Tiger moth, *A. sciodoxa* is a serious pest of economically important medicinal plant species, Tongkat ali, *Eurycoma longifolia*, in South East Asia and pest attacks terminal shoots resulting in stunted plant growth and also results in plant mortality in case of severe infestation (Abood et al., 2011). The common use of *E. longifolia* as herbal medicine and dietary supplement, and legitimate side effects of synthetic pesticides has ushered for searching nonchemical control of *A. sciodoxa*.

The entomopathogenic fungus, *Beauveria bassiana*, has been used against several economically important field and stored grain pests in tropical as well as temperate regions of the world (Ibrahim and Low, 1993; Feng et al., 2004; Faria and Wraight, 2001; Wraight and Ramos, 2002; McGuire et al., 2006; Athanassiou and Steenberg, 2007). Possibility of infection to *A. sciodoxa* by *B. bassiana* under laboratory conditions has also been found (Abood et al., 2010).

All metamorphic insect stages are susceptible to *B. bassiana*, in general. In the presence of a virulent isolate and a susceptible host insect, however, the infectivity depends on the quantity of inoculum, the physiological state of the host and abiotic conditions, especially ambient temperature and humidity (Ferron, 1978). Insect species have been reported to be more susceptible to disease at certain stage(s) in their life cycle (Boucias and Pendland, 1998). This age-specif-

ic susceptibility response is quite noticeable in those species, which have potential of age-maturation immunity (Tanada and Kaya, 1993).

The age-specific dose-mortality response (a set of all possible dose-response relationships) provides an empirical base upon which pathogen can further be evaluated for field application. Hence, these relationships are fundamentals to estimate effectiveness of any insect pathogen. Present study was undertaken for assessing: (i) virulence of the fungus against different larval instars, (ii) estimation of median effective concentrations (EC_{50}) and time (ET_{50}), and (iii) effect of temperature on infection.

Materials and Methods

The study was conducted at the Faculty of Forestry, University Putra Malaysia, Malaysia. An isolate, Bba-Pp, of *B. bassiana,* isolated from the teak bagworm (*Pteroma pendula*), in 2007 was bio-assayed against the 3rd, 4th and 5th larval instars at 27±2°C with 75±5% relative humidity and photophase of 12:12 h. The effect of temperature on the infectivity of *B. bassiana* was assessed using the third instar.

Fungal culture

The fungal isolate was passed through *A. sciodoxa* and cultured on potato dextrose agar 3.9% (PDA) (potato starch 4.0 g/L, dextrose 20.0 g/L, and agar 15 g/L) supplemented with 0.5% yeast extract (YE) that was sterilised at 121°C at a pressure of 1.05 kg cm^{-2} for 20 minutes. The sterilised medium was poured aseptically in sterilised Petri dishes at 40°C. A conidial suspension of $1x10^7$ conidia ml^{-1} concentration was prepared from 2-week old culture and the suspension was pipetted in each Petri dish (0.1 ml per Petri dish), and spread evenly using a cell spreader. The culture was incubated at 27°C for 2-week in darkness.

The 2-week old culture was harvested using 20 ml of 0.02% aqueous Tween 80 with a glass rod. Aliquot was transferred to test tubes and vortexed for 5 minutes using MS 3-Digital Vortex at a speed of 3000 I per minute. The homogenized suspension was filtered twice using cheesecloth and conidial concentration was determined using Neubauer improved haemocytometer.

Viability of conidia was assessed simultaneously. Petri dishes with PDA+YE were inoculated evenly with 10^6 conidia per plate using cell spreader on turning table. The inoculated plates were sealed with parafilm and incubated at 27°C for 24 h in darkness. Four drops of lactophenol cotton blue were poured onto the medium to stain and fix the germinating conidia. A rectangular piece of medium (1.5x2cm) was cut and mounted on microscopic slide. A total of 3x100 conidia were counted using light microscope (400x). The method was followed as described by Bajwa and Zimmermann (1996). Ninety nine percent conidia were germinated after incubation of 24 h at 27°C in darkness.

Insect culture

Different larval instars of *A. sciodoxa* were collected from the field and reared in the laboratory at 27±2°C, 75±5% relative humidity and 12:12 h photoperiod. The larvae were collected from the same plantation and on the same day. The larvae were reared in a single colony for one generation before bioassay. The insect culture was fed on *E. longifolia* leaves in cylindrical containers (28cm diameter x 30cm) covered with double layer of plastic mesh (2x2 mm). A fresh *E. longifolia* shoot with 4-5 leaflets was affixed upright through the centre.

Bioassays

Five concentrations of Bba-Pp including: $1x10^6$, $5x10^6$, $1x10^7$, $5x10^7$ and $1x10^8$ conidia ml^{-1} were used for bioassays against the three larval instars. The concentrations were adjusted by adding 0.02% aqueous Tween 80 in stock suspension. Fifty larvae of 24-36 h old each of the 3rd, 4th and 5th instar per concentration were randomly selected from the stock culture. The larvae and leaves both were sprayed using TLC sprayer (Preval® TLC sprayer (Precision Valve Corporation, NY, USA), till running off using 7-8 ml suspension. In the control treatments, 0.02% aqueous Tween was used. The inoculated larvae and leaves were air dried at room temperature and transferred to cylindrical containers (11cm diameter x 8cm) lined with moist filter paper. The larvae were fed on the inoculated leaves for 24-h then fresh untreated leaves were fed (Treated leaves are also source of inoculation through contact and ingestion of spores). The survival of the larvae was monitored twice daily. The larvae were considered to be dead when there was no visible movement by pricking.

The effect of temperature on the infectivity of *B. bassiana* was tested at five constant levels of 21, 24, 27, 30 and 33(±1) °C at a concentration of $5x10^7$ conidia

Smith & Franklin Academic Publishing Corporation

ml^{-1}. Fifty larvae per temperature level were inoculated as described previously. The inoculated larvae and leaves were transferred to cylindrical containers (11cm diameter x 8cm) lined with moist filter paper. The containers were placed in multi-chambered incubators with 24 h prior calibrated temperature. The larvae were fed on the inoculated leaves for 24-h then fresh untreated leaves were fed. The survival of larvae was monitored daily. The larval death was validated as described earlier. There were separate control treatment for each temperature level where 0.02% aqueous Tween 80 was used.

The dead larvae were surface sterilised with 1% sodium hypochlorite and rinsed thrice with sterile distilled water. The surface sterilised cadavers were transferred into humidity chambers (9 cm Petri dish lined with two moist filter papers, almost 100% RH) and sealed with parafilm. The humidity chambers were incubated at 27±2°C except for study of temperature effect. The moribund larvae were incubated in the respective temperature levels. The mortality was enumerated after mycelial appearance on cadavers. The cadavers without mycelia were excluded from the final mortality data.

Statistical analyses

The effect and dose-larval instar response were conducted in factorial design (2x6 levels; 3x6 levels) with five times, while temperature effect was assessed in completely randomized design. The percent mortality over time was corrected according to Abbott's correction formula (1925). The dose-larval instar response was analysed using General Linear Model (GLM), and effect of temperature by 1-Way Analysis of Variance. The means were compared by pairwise contrast applying Tukey's HSD test using Minitab 15.1 Statistical Software. The median effective concentration (EC_{50}) and median effective time (ET_{50}) were estimated on the basis of seven day after inoculation (DAI) using Probit Programme Version 1.5, U.S. Environmental Protection Agency (EPA). The Intercept and slope values were calculated with Linear Regression analysis and further subjected to χ^2 test. The effect of temperature on infectivity of *B. bassiana* was regressed for $\log_{10}$ transformed mortality applying second order regression. The linear and quadratic function was analysed using Sequential Analysis of Variance.

Results

The results revealed that tested larval instars were susceptible to *B. bassiana* isolate Bba-Pp. The morality response, however, was highly age-specific as well as dose dependent. There was a highly significant main effect of larval age ($F_{2, 60}$= 43.29; p<0.01), *B. bassiana* concentration ($F_{4,60}$= 595.15; p<0.01) and their interactions ($F_{8,60}$= 6.43, p<0.01) on the mortality response. At 7 day after inoculation (DAI), larval mortality differed significantly among the 3rd, 4th and 5th instars (p= 0.05; CV= 2.61). The 3rd instar stage was the most susceptible with 87.2% pooled mortality across five concentrations, while 5th instar was least susceptible. The mortality was 7.8% and 13.0% greater in the 3rd instar as compared to 4th and 5th instars, respectively (Figure 1). The difference between 4th and 5th instars was 4.8%.

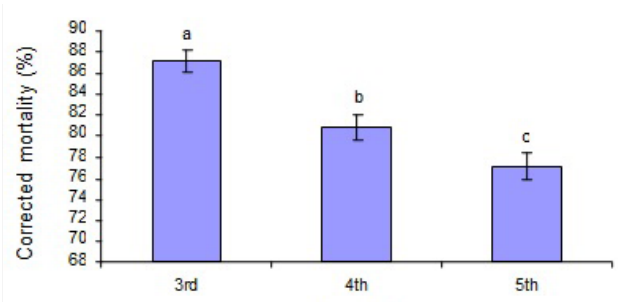

Figure 1: *Pooled larval mortality of A. sciodoxa caused by B. bassiana*

The concentration effect differed significantly (p= 0.05, CV= 3.95). The highest mortality, pooled across the three larval instars, was 100% obtained using $1x10^8$ conidia ml^{-1} and the lowest mortality was 41.7% at concentration of $1x10^6$ conidia ml^{-1} (Figure 2). The mortality was 2.4, 1.3, 1.1 times higher at concentration of $1x10^8$ conidia ml^{-1} as compared to $1x10^6$, $5x10^6$ and $1x10^7$ conidia ml^{-1}, respectively. There was a non-significant difference of 0.7% between concentration of $1x10^8$ and $5x10^7$ conidia ml^{-1}.

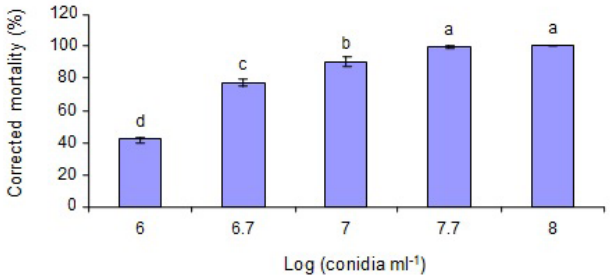

Figure 2: *Pooled concentration effect of B. bassiana on larvae of A. sciodoxa*

The effect of combinations between larval instars and concentrations on mortality was significant (p= 0.05, CV= 8.59). The combination of 3rd instar with

Table 1: *Median effective concentrations of three larval instars of A. sciodoxa*

Stage (Instar)	EC_{50}	(95% fiducial limits)		EC_{99}	(95% fiducial limits)		χ^2-value
		Lower	Upper		Lower	Upper	
3rd	9.87*	5.2*	14.7*	210.1*	1.13*	662.4*	1.24
4th	16.15	9.6	23.32	447.3	2.37	1321.1	0.99
5th	21.30	12.89	30.87	788.5	4.04	2373.3	0.52

**10^5 conidia ml^{-1}*

$1x10^8$; 3rd instar with $5x10^7$; 4th instar with $1x10^8$, 4th instar with $5x10^7$ and 5th instar with $1x10^8$ conidia ml^{-1} caused 100% mortality. The treatment combination of 3rd instar with $1x10^6$, 3rd instar with $5x10^6$ and 3rd instar with $1x10^7$ conidia ml^{-1} resulted in significantly higher mortality as compared to combinations of 4th and 5th instars with corresponding concentrations (Figure 3). The mortality caused in 3rd instar by inoculating with $5x10^6$ conidia ml^{-1} was greater compared to the mortality of 5th instar with $1x10^7$ conidia ml^{-1} (indicated an increase in age-maturation immunity between the two larval stages). The combinations of 4th instar with the five tested concentrations did not differ significantly with 5th instar. The lowest mortality was observed in combination of the 5th instar with 10^6 conidia ml^{-1}.

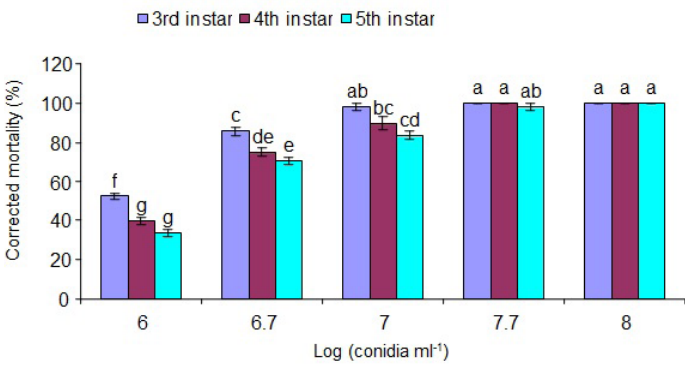

Figure 3: *Effect of larval instar and inoculum concentration on larval mortality*

Median effective concentrations

Median effective concentration (EC_{50}) estimated for the 3rd, 4th and 5th larval instars showed considerable difference in EC_{50} values among three larval instars. At 7 DAI, the lowest EC_{50} was $9.87x10^5$ conidia ml^{-1} for 3rd instar. This was 0.6 and 0.5 times of the 4th and 5th instar (Table 1). The 5th instar larva required 1.3 times more inoculum as compared to 4th instar for causing 50% mortality in larval population. The EC_{99} also differed greatly among larval instars. The EC_{99} of the 3rd instar was 0.47 and 0.27 times of the 4th and 5th instars, while EC_{99} of the 4th instar was 0.57 times of the 5th instar. The slope values for the 3rd, 4th and 5th instars stages were 1.75±0.3, 1.61±0.24 and 1.48±0.20, respectively. This signified relatively greater inoculum requirement for each mortality increase unit in successive larval stage. The effective concentrations values, both at 50 and 99% mortality levels, also substantiated this trend.

Median effective time

Overall median effective time (ET_{50}) varied significantly across the larval instars ($F_{2, 14}$= 42.47; p<0.01), and concentrations ($F_{4, 14}$= 468.88, p<0.01). The shortest mean survival time of 50% larval population, pooled across concentrations, was 4.7±0.68 days for the 3rd larval instar, while the longest ET_{50} was 5.5±0.78 days for the 5th larval instar (Table 2). ET_{50} of larval 3rd instar was 0.92 times of the 4th instar (5.1±0.72 days). The survival time varied significantly among the larval instars (p= 0.05; CV= 0.24). The shortest pooled ET_{50}, across the three larval instars, was 3.6±0.17 days at concentration of $1x10^8$ conidia ml^{-1}, while the longest ET_{50} was 7.7±0.32 days at $1x10^6$ conidia ml^{-1}. The difference in ET_{50} values among $1x10^6$, $5x10^6$ and $1x10^7$ conidia ml^{-1} concentrations was significant, while this was non-significant between $5x10^7$ and $1x10^8$ conidia ml^{-1} (p= 0.05, CV= 0.24).

The correlation coefficient (r^2) value was -0.968, -0.965 and -0.792 for the 3rd, 4th and 5th instar, respectively, thus indicating an inverse correlation between concentration and median effective survival time within larval instars. The slope and intercept values showed positive trend with concentration (Table 2). The longest mean survival time of 50% population was 8.2 days in combination of 5th instar x 10^6 conidia ml^{-1} while survival time was the shortest (3.3 days) in 3rd instar x 10^8 conidia ml^{-1}. The maximum shortening in ET_{50} was 1.9 days (3rd instar), 2.3 days (4th instar), and 2.3 days (5th instar) between concentrations $1x10^6$ and $5x10^6$ conidia ml^{-1}, while the minimum shortening was 0.3 days, and 0.2 days between $5x10^7$ and $1x10^8$ conidia ml^{-1}.

Smith & Franklin Academic Publishing Corporation

Table 2: *Median effective time (ET_{50}) for three larval instars of A. sciodoxa at different concentrations*

Larval Instar	Conc.	ET_{50} (days)	95% fiducial limits		Slope± SE	Intercept± SE	χ^2-value
			Lower	Upper			
3rd	$1x10^6$	7.1	6.4	8.9	4.99±1.23	-6.15±2.61	1.24
	$5x10^6$	5.2	4.7	5.6	6.45±1.02	-8.50±2.13	3.63
	$1x10^7$	4.3	4.0	4.6	7.05±0.91	-9.21±1.87	3.32
	$5x10^7$	3.6	3.2	3.8	7.34±0.95	-9.18±1.90	1.79
	$1x10^8$	3.3	3.1	3.6	9.19 ±1.33	-12.49±2.60	1.77
4th	$1x10^6$	7.7	6.8	10.9	5.10±1.46	-6.56±3.13	0.24
	$5x10^6$	5.4	5.0	5.9	6.07±0.98	-7.83±2.07	0.59
	$1x10^7$	4.8	4.4	5.2	6.97±0.98	-9.39±2.03	0.67
	$5x10^7$	3.9	3.6	4.1	8.02±0.98	-10.76±1.98	1.66
	$1x10^8$	3.6	3.3	3.8	9.59±1.29	-13.51±2.56	1.34
5th	$1x10^6$	8.2	7.1	13.1	5.70±1.80	-8.08±3.87	0.61
	$5x10^6$	5.9	5.4	6.4	6.43±1.15	-8.82±2.44	0.06
	$1x10^7$	5.2	4.8	5.6	7.45±1.13	-10.63±2.38	0.24
	$5x10^7$	4.1	3.8	4.4	8.28±1.00	-11.54±2.06	0.46
	$1x10^8$	3.9	3.6	4.1	8.49±1.03	-11.7±2.08	1.58

Table 3: *Effect of temperature on infectivity of B. bassiana in A. sciodoxa*

Temp. (°C)	Mortality ± SE (%)	ET_{50}(days)	95% fiducial limits		Slope± SE	χ^2-value
			Lower	Upper		
21	62.0±1.99 c	6.5	6.2	7.0	10.42±2.15	0.34
24	89.8±0.22 b	4.8	4.5	5.1	8.26± 1.21	0.58
27	100.0±0.0 a	3.5	3.2	3.8	7.30± 0.90	1.77
30	100.0±0.0 a	3.8	3.4	4.1	6.70± 0.90	3.60
33	60.9±2.48 c	4.9	4.3	5.5	4.10± 0.69	3.85

Means followed by the same letter (s) are not significant at p=0.05 according to Tukey's HSD

Effect of temperature on infectivity

A highly significant ($F_{4, 20}$= 188.46; p<0.01) effect of temperature was found on the infectivity of *B. bassiana*. At 7 DAI, there was 100% mortality at 27 °C and 30°C, while the lowest mortality was 60.9% at 33°C. The mortality at 24°C was significantly (p<0.05) less as compared to 27°C but it was greater, both than 21°C and 33°C (Table 3). The difference between 21°C and 33°C was not significant (p= 0.05, CV= 6.07). The median survival time was the longest at 21°C. The ET_{50} decreased gradually between 21°C and 27°C, and increased between 27°C at 33°C. There was a marginal difference in ET_{50} between 27°C and 30°C.

There was a curvilinear correlation between temperature and mortality (Figure 4). The regression analysis indicated a significant quadratic function of temperature ($F_{2, 22}$= 132.71; p<0.01) with mathematical expression: $Log_{10}(y)$= -2.658+0.346t-0.006t^2. The R^2 indicated 92.4% change in mortality due to temperature.

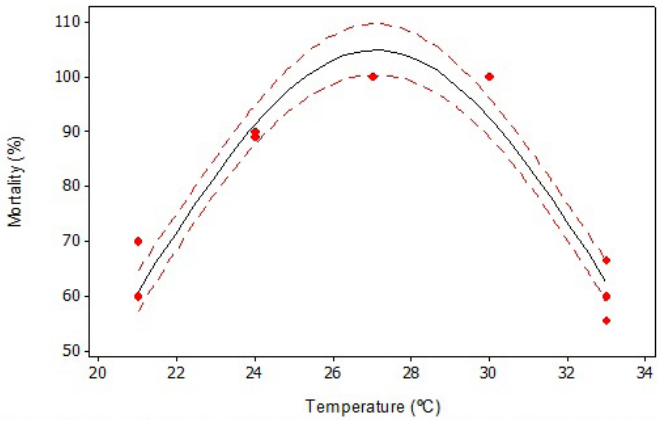

Figure 4: *Correlation between temperature and B. bassiana infectivity in A. sciodoxa*

Discussion

A standardised bioassay method was developed in

Smith & Franklin Academic Publishing Corporation

which an attempt was made to remain closer to field exposure method with least disturbance of treatment handling. The leaves were kept fresh in the treatment preparation as described earlier. Secondly, the larvae were sprayed topically on leaves instead of immersing in conidial suspension as has been reported previously against other pest insects (Marannino et al., 2006). The method ensured the target stage be inoculated as natural as possible.

The precise and accurate comparison of fungal infectivity among different insect species is difficult. The same fungal isolate may get variable response against different insect species and vice versa. The present results also confirm age-specific dose-mortality response. Previously, decreasing susceptibility to *B. bassiana* and increasing average survival time with maturation of the host stage was demonstrated in the European corn borer, *Ostrinia nubilalis* (Carruthers et al., 1985; Feng et al., 1985), the diamondback moth, *Plutella xylostella* (Vandenberg et al., 1998), and the western flower thrips, *Frankliniella occidentalis* (Ugine et al., 2005).

The present median effective concentration (EC_{50}) ranging from 9.87 to 21.30x10^5 conidia ml^{-1} are mixed (either slightly lower or greater) with other insect species. These values are, nevertheless, comparable with that of 1.9x10^6 and 2.2x10^6 conidia ml^{-1} against *Lygus hesperus* and *L. lineolaris* adults (Steinkraus and Tugwell, 1997; Noma and Strickler, 1999) 1.5x10^5 conidia ml^{-1} against 2nd instar *Helicoverpa armigera* (Nguyen et al., 2007); 4.8×10^5 conidia ml^{-1} against last larval instar of *O. nubilalis* (Demir et al., 2012).

Median effective time (ET_{50}) significantly varied across the developmental stages as well as concentrations. There was an inverse proportion between ET_{50} values and dose rate, as well as larval instars. Such concentration dependent ET_{50} values have also been estimated for the maize weevil, *Sitophilus zeamais* (Adane et al., 1996) and the Russian wheat aphid, *Diuraphis noxia* (Vandenberg, 1996).

Present ET_{50} values of 3.3 days to 3.9 days at 1x10^8 conidia ml^{-1} of the 3rd and 5th instars, respectively, are in conformity with Bidochka et al. (1993) and Kouassi et al. (2003) who obtained ET_{50} values of 3.0 days and 4.9 days at a concentration of 1x10^8 conidia ml^{-1} of the adult *Lygus lineolaris* and *L. borealis*, respectively, using other different *B. bassiana* isolates. The slight variation in present EC_{50} and ET_{50} values and previously reported may be due to isolate and host specific infectivity. Besides, there may be different degree of maturation immunity in the hosts as had been reported by Khachatourians (1992) and Gindin et al. (2000).

Effect of temperature on infectivity of *B. bassiana* revealed non-linear temperature function. The mortality level and mean survival time decreased, both at low and high temperature levels. This temperature function on the *B. bassiana* infectivity is in agreement with previous findings reported by Vandenberg et al. (1998); Fargues and Luz (2000); Tsay et al. (2001); Tefera and Pringle (2003); Kreutz et al. (2004) and Vassilakos et al. (2006). In general, *B. bassiana* has high efficacy with low mean survival time between 25°C to 30°C. The present results also indicated optimum temperature range between 27°C to 30°C with 100% mortality and survival time of 3.5 days to 3.8 days. The optimum temperature range, however, depends on the fungal isolate and insect host. Isolate Bba-Pp shows slightly higher optimum temperature range which may be due to its tropical origin. Effect of origin of isolate on vegetative growth and infectivity of *B. bassiana* has been observed earlier (Ekesi et al., 1999).

Conclusion

Based on present findings it is concluded that *B. bassiana* is infective to different larval instars of *A. sciodoxa* (field temperature was 26±3°C). The infectivity of *B. bassiana* depends on the larval stage and inoculum concentration. The 3rd instar is found to be the most susceptible, while the 5th instar the least. The temperature shows significant effect on the infectivity of *B. bassiana*, with optimum range between 27°C and 30°C. The optimum temperature range shows promising signs of the isolate as microbial control agent against *A. sciodoxa*.

Authors Contributions

This paper is a part of PHD thesis of Ghulam Ali Bajwa (GAB) who developed the concept, and designed and executed research project. Assoc. Prof. Dr. Faizah Abood and Prof. Dr. Yusof Bin Ibrahim supervised the thesis research as supervisor and co-supervisor, respectively. They also helped to draft manuscript.

References

- Abbott, W.S. 1925. A method of computing the

effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267. http://dx.doi.org/10.1093/jee/18.2.265a

- Abood, F., Bajwa, G.A., Ibrahim, Y.B. and Sajap, A.S. 2010. Pathogenicity of *Beauveria bassiana* against the tiger moth, *Atteva sciodoxa* (Lepidoptera: Yponomeutidae). Journal of Entomology 8(1): 19-32. http://dx.doi.org/10.3923/je.2010.19.32
- Abood, F., Bajwa, G.A., Ibrahim, Y.B., Rasip, Ab. A.G. and Sajap, A. S. 2011. Feeding behaviour, food consumption indices and infestation of the tiger moth, *Atteva sciodoxa*, on *Eurycoma Longifolia* Jack (Tongkat Ali). The Malaysian Forester 74(1): 9-22.
- Adane, K., Moore, D. and Archer, S.A. 1996. Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. Journal of Stored Products Research 32: 105-113. http://dx.doi.org/10.1016/0022-474X(96)00009-4
- Athanassiou, C.G. and Steenberg, T. 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological Control 40: 411-416. http://dx.doi.org/10.1016/j.biocontrol.2006.12.001
- Bajwa, G.A. and Zimmermann, G. 1996. Effects of Decis 2.5EC (deltamethrin) on growth, sporulation and germination of conidia of some entomopathogenic fungi. The Nucleus 33:125-128.
- Bidochka, M.J., Miranpuri, G.P. and Khachatourians, G.G. 1993. Pathogenicity of *Beauveria bassiana* (Balsamo) Vuill. toward *Lygus* bug (Hemiptera: Miridae). Journal of Applied Entomology 115: 313-317. http://dx.doi.org/10.1111/j.1439-0418.1993.tb00396.x
- Boucias, D.G. and Pendland, J.C. 1998: Principles of Insect Pathology. Boston: Kluwer Academic Publishers http://dx.doi.org/10.1007/978-1-4615-4915-4
- Carruthers, R.I., Feng, Z., Robson, D.S. and Roberts, D.W. 1985. *In-vivo* temperature dependent development of *Beauveria Bassiana* (Deuteromycotina: Hyphomycetes) mycosis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Journal of Invertebrate Pathology 46: 305-311. http://dx.doi.org/10.1016/0022-2011(85)90073-4
- Demir, I., Seçil, E.S., Demirbağ, Z. and Sevim, A. 2012. Molecular characterization and virulence of the entomopathogenic fungus *Beauveria bassiana* from *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae). Turkish Entomology Bulletin 2(1): 23-30.
- Ekesi, S., Maniania, N.K. and Ampong-Nyarko, K. 1999. Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti*. Biocontrol Science and Technology 9: 177-185 http://dx.doi.org/10.1080/09583159929767
- Fargues, J. and Luz, C. 2000. Effects of fluctuating moisture and temperature regimes on the infection potential of *Beauveria bassiana* for *Rhodnius prolixus*. Journal of Invertebrate Pathology 75: 202-211. http://dx.doi.org/10.1006/jipa.1999.4923
- Faria, M. and Wraight, S.P. 2001. Biological control of *Bemisia tabaci* with fungi. Crop Protection 20: 767-778. http://dx.doi.org/10.1016/S0261-2194(01)00110-7
- Feng, M.G., Up, X.Y., Ying, S.H. and Wang, Y.G. 2004. Field trials of an oil-based mollifiable formulation of *Beauveria bassiana* conidia and low application rates of imidacloprid for control of false-eye leafhopper *Empoasca vitis* on tea in southern China. Crop Protection 23: 489-496. http://dx.doi.org/10.1016/j.cropro.2003.10.004
- Feng, Z., Carruthers, R.I., Roberts, D.W. and Robson, D.S 1985. Age-specific dose-mortality effects of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) on the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Journal of Invertebrate Pathology 46: 259-264. http://dx.doi.org/10.1016/0022-2011(85)90067-9
- Ferron, P. 1978. Biological control of insect pests by entomopathogenic fungi. Annual Review of Entomology 23: 409-442. http://dx.doi.org/10.1146/annurev.en.23.010178.002205
- Gindin, G., Geschtovt, N.U., Raccah, B. and Barash, I. 2000. Pathogenicity of *Verticillium lecanii* to different developmental stages of the silverleaf whitefly, *Bemisia argentifolia*. Phytoparasitica 28: 229-239. http://dx.doi.org/10.1007/BF02981801
- Ibrahim, Y.B and Low, W. 1993. Potential of mass-production and field efficacy of isolates of the Entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against *Plutella xylostella*. International Journal of Pest Management 39: 288-292. http://dx.doi.org/10.1080/09670879309371807

- Khachatourians, G.G. 1992. Virulence of five *Beauveria bassiana* strains, *Paecilomyces farinosus* and *Verticillium lecanii* against the migratory grasshopper, *Melanoplus sanguinipes*. Journal of Invertebrate Pathology 59: 212-214. http://dx.doi.org/10.1016/0022-2011(92)90038-6
- Kouassi, M., Coderre, D. and Todorova, S. 2003. Effect of plant type on the persistence of *Beauveria bassiana*. Biocontrol Science and Technology 13: 415-427. http://dx.doi.org/10.1080/0958315031000104532
- Kreutz, J., Vaupel, O. and Zimmermann, G. 2004. Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the spruce bark beetle, *Ips typographus* L., in the laboratory under various conditions. Journal of Applied Entomology 128(6): 384-389. http://dx-.doi.org/10.1111/j.1439-0418.2004.00813.x
- Nguyen, T.H., Christian, B., Poehling, H.M. and Zimmermann, G. 2007. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. Biocontrol Science and Technology 17: 853-864. http://dx.doi.org/10.1080/09583150701546375
- Marannino, P., Álvarez, C.S., Lillo, E. and Moraga, E.Q. 2006. A new bioassay method reveals Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* against early stages of *Capnodis tenebrionis* (Coleoptera: Buprestidae). Journal of Invertebrate Pathology 93: 210-213. http://dx.doi.org/10.1016/j.jip.2006.08.002
- McGuire, M.R., Leland, J.E., Dara, S., Park, Y.H. and Ulloa, M. 2006. Effect of different isolates of *Beauveria bassiana* on field populations of *Lygus hesperus*. Biological Control 38: 390-396. http://dx.doi.org/10.1016/j.biocontrol.2005.12.010
- Noma, T. and Strickler, K. 1999. Efficacy of *Beauveria bassiana* for control of *Lygus* bug (Hemiptera: Miridae) in alfalfa seed fields. Journal of Agriculture and Urban Entomology 16: 215-233.
- Steinkraus, D.C. and Tugwell, N.P. 1997. *Beauveria bassiana* (Deuteromycotina: Monilliales) effects on *Lygus lineolaris* (Hemiptera: Miridae). Journal of Entomological Science 32: 79-90.
- Tanada, Y. and Kaya, H.K. 1993: Insect Pathology. New York: Academic Press Inc.
- Tefera, T. and Pringle, K. 2003. Germination, radial growth and sporulation of *Beauveria bassiana* and *Metarhizium anisopliae* isolates and their virulence to *Chilo partellus* (Lepidoptera: Pyralidae) at different temperatures. Biocontrol Science and Technology 13: 699-704. http://dx.doi.org/10.1080/0958315031000151756
- Tsay, J.G., Lee, M.J. and Chen, R.S. 2001. Evaluation of *Beauveria bassiana* for controlling casuarinas tussock moth (*Lymantria xylina* Swinhoe) in casuarinas plantations. Taiwan Journal of Forest Science 16: 201-207.
- Ugine, T.A., Wraight, S.P., Brownbridge, M. and Sanderson, J.P. 2005. Development of a novel bioassay for estimation of median lethal concentrations (LC_{50}) and doses (LD_{50}) of the entomopathogenic fungus *Beauveria bassiana*, against western flower thrips, *Frankliniella occidentalis*. Journal of Invertebrate Pathology 89: 210-218. http://dx.doi.org/10.1016/j.jip.2005.05.010
- Vandenberg, J. D. 1996. Standardized bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against the Russian wheat aphid (Homoptera: Aphididae). Journal of Economic Entomology 89:1418-1423. http://dx.doi.org/10.1093/jee/89.6.1418
- Vandenberg, J.D., Ramos, M. and Altre, J.A. 1998. Dose–response and age-and temperature related susceptibility of the diamondback moth (Lepidoptera: Plutellidae) to two isolates of *Beauveria bassiana* (Hyphomycetes: Moniliaceae). Environmental Entomology 27: 1017-1021. http://dx.doi.org/10.1093/ee/27.4.1017
- Vassilakos, T.N., Athanassiou, C.G., Kavallieratos, N.G. and Vayias, B.J. 2006. Influence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. Biological Control 38: 270-281. http://dx.doi.org/10.1016/j.biocontrol.2006.03.009
- Wraight, S.P. and Ramos, M.E. 2002. Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. Biological Control 23: 164-178. http://dx.doi.org/10.1006/bcon.2001.1004

Smith & Franklin Academic Publishing Corporation