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Efficacy of Entomopathogenic Fungi, *Beauveria bassiana* against *Maruca vitrata* (Geyer) under Laboratory Condition

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Pathogenecity of entomopathogenic fungi, *Beauveria bassiana* isolates (Bb111, Bb112, Bb 113 and Bb 114) were assessed against spotted pod borer, *Maruca vitrata* (Geyer). Bioassays were performed in five different pulse hosts *viz.*, lablab, cowpea, green gram, black gram and pigeonpea against third, fourth and fifth instar larvae of spotted pod borer. Efficacy of Bb 112 was higher irrespective of the pulses tested. The median lethal concentrations of Bb 112 for third, fourth and fifth instar larvae on different pulses *viz.*, lablab, cowpea, black gram, green gram and pigeonpea were in range of 0.10 x 10^8 to 2. 04 x 10^8, 0.14 x 10^8 to 2.67 x 10^8 and 0.20 x 10^8 to 4.80 x 10^8 spores ml^{-1}, respectively.

Introduction

The spotted pod borer, commonly known as legume pod borer, *M. vitrata* (Lepidoptera: Crambidae) is a serious pest of grain legumes in the tropics and subtropics due to its extensive host range, distribution and destructiveness. The larvae damage the flower buds, flowers and immature pods by webbing and contaminate with their excreta (Rekha and Mallapur, 2007). The grain yield loss due to legume pod borer was estimated to be 10.0 to 80.0 per cent in various crops (Singh and Allen, 1980; Sharma, 1998). Webbings of flowers and pods during feeding makes the pest hard to reach and hence makes the management difficult (Sharma, 1998). However, the pest is still being managed by means of insecticides only (Jakhar *et al.*, 2016).

Preference of insecticides depends on their easy availability and applicability, but their excessive and indiscriminate use resulted in the development of insecticidal resistance in most of the pests and environmental pollution (Phokela *et al.*, 1990; Sharma *et al.*, 2002). The increasing concern about

pesticide hazards evoked worldwide interest on alternate pest management practices that are ecofriendly in nature. Biologically derived insecticides or microbial insecticides, natural enemies and entomopathogenic fungi provide an alternative, more environmentally friendly option to control this insect pest.

Entomopathogens are being reported as the most important regulating factors of *M. vitrata* under field condition. The usefulness and effectives of *Bacillus thuringiensis* was reported against *M. vitrata* (Karel *et al.,* 1986). Srinivasan *et al.,* (2015) suggested *B. thuringiensis* based biopesticide formulation as the promising component for the integrated management of *M. vitrata*. Sreelakshmi and Paul (2016) reported the efficacy of spinosad and emamectin benzoate (insecticide based on microbial derivative) against *M. vitrata* infesting pulses. The entomopathogenic fungus *Beauveria bassiana* is a promising and extensively researched biological control agent that can suppress a variety of economically important insect pests (Coates *et al.,* 2002; McGuire *et al.,* 2005: Prasad and Syed, 2010; Hussein *et al.,* 2010). Soundararajan and Chitra (2011) reported the potential of *B. bassiana* against *M. vitrata* population under field condition on urdbean. In the present investigation, laboratory efficacy of *B. bassiana* isolates were tested against different life stages of *M. vitrata.*

Materials and Methods

Sources of fungal isolates used for the study

Pure cultures of the different isolates of entomopathogenic fungi, *B. bassiana* maintained at the Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore were utilized for the laboratory bioassay.

Preparation of spore suspensions of fungal isolates for bioassay

For laboratory bioassay, all the four isolates were cultured in Petri dishes (9 cm diameter) containing Sabouraud's Maltose Agar enriched with one per cent yeast extract (SMA+Y) solid medium and incubated at 25 ± 2^{o} C for 10 to 14 days. After complete sporulation, spores were scraped from the surface of SMAY plates and suspended in 20 ml sterile distilled water containing 0.05 per cent Tween 80® (Sisco Research Laboratories Pvt Ltd, Mumbai, India). The conidial suspension was vortexed for 5 minutes to produce a homogenous spore suspension (Saranya *et al.,* 2013). Spore count in each plate was assessed using a Neubauer hemocytometer with a phase contrast microscope (Leica DM750, Leica Microsystems, Heerbrugg, Switzerland) and was estimated using the formula suggested by Lomer and Lomer (1996). The number of spores ml^{-1} was calculated by the following formula.

Number of spores ml^{-1} of suspension = DX/NK, where
D = Dilution factor
X= Total number of spores
N= Number of small squares counted
K=Volume above one small square in cm^3 (2.5 x 10^{-7} cm^3)

From the stock solution, dilutions were made to obtain the required concentrations for further studies.

Method of bioassay

Larvae of *M. vitrata* from the laboratory cultures maintained at Insectary in Tamil Nadu Agricultural University were used for the bioassays.

For each isolate, five different spore concentrations (1x10^8 to 1x10^4 spores ml^{-1}) were prepared from the stock suspension for the assay of concentration mortality response. The whole fresh pods of different pulses *viz.,*

lablab, cowpea, green gram, black gram and pigeonpea were placed separately in a plastic disposable container (10 cm dia and 3.5 cm ht.) lined with a cotton wad (8 cm dia.) and water-soaked filter paper to ensure high relative humidity. For each treatment forty prestarved third instar larvae were released at the rate of 10 per container. Four replications were used for each isolate and each concentration.

After 6 hrs of release (i.e. after larvae entered into the pod), ten ml of respective concentrations were sprayed on the pods infested with third instar larvae using glass atomizer. Pods sprayed with 0.05 per cent Tween 80® served as control.

The most preferred host was used as a positive control for comparing the pathogenicity. After spraying, post treatment counts were taken at 24 hours interval upto 7 days and the median lethal concentration (LC_{50}) was worked out according to the probit analysis methodology (Finney, 1971).

Similar experimental setup was used for the time mortality response studies. The time mortality response was carried out at higher spore concentration of 1×10^8 spores ml^{-1}. Pods sprayed with 0.05 per cent Tween 80® served as control.

The post treatment counts were taken at 12 hours interval upto 7 days and the median lethal time (LT_{50}) was worked out according to the probit analysis methodology (Finney, 1971).

In both bioassays, dead larvae were collected daily and kept in humid chamber. Dead larvae which produced mycelial growth were considered for the mortality count (IRAC, 2007). Similar procedure was adopted for 4th instar and 5th instar larvae using different pulse crops.

Results and Discussion

Median lethal concentration (LC_{50}) against *M. vitrata* larvae

The results of the bioassay showed that all the tested fungal isolates were effective against all the instars tested on different pulses. Among all the isolates, Bb 112 had higher virulence to *M. vitrata* larvae, irrespective of the pulses tested. The median lethal concentrations of Bb 112 for third, fourth and fifth instar larvae on different pulses *viz.*, lablab, cowpea, black gram, green gram and pigeonpea were in range of 0.10×10^8 to 2.04×10^8, 0.14×10^8 to 2.67×10^8 and 0.20×10^8 to 4.80×10^8 spores ml^{-1}, respectively (Table 1, 2 and 3). The efficacy of Bb 112 against third instar on different pulses were in the order of lablab > cowpea > black gram > green gram > pigeonpea with the LC_{50} values of 0.10, 0.13, 0.15, 0.33 and 0.52×10^8 spores ml^{-1}, respectively. This was followed by the isolates Bb 111, Bb 113 and Bb 114. Similar trend was also observed against fourth and fifth instar, with the LC_{50} values of 0.14, 0.41, 0.46, 0. 53 and 0.79×10^8 spores ml^{-1} and 0.20, 0.48, 0.60, 0.92 and 1.73×10^8 spores ml^{-1}, respectively on lablab, cowpea, black gram, green gram and pigeonpea.

Median lethal time (LT_{50}) against *M. vitrata* larvae

The results of the bioassay revealed distinct variation in time response of all the fungal pathogens (at higher concentration 1×10^8 spores ml^{-1}) tested against different instars of *M. vitrata* larvae.

The isolate Bb 112 had faster lethal effect against third, fourth and fifth instar larvae followed by Bb 113, Bb 114 and Bb 111. The median lethal time for Bb 112 against third, fourth and fifth instar larvae of different pulses were found to be in range of 110.48 to

125.93 h, 114.01 to 131.76 h and 120.69 to 147.97 h, respectively (Table 4, 5 and 6). The lowest LT_{50} of 110.48, 114.01 and 120.69 h was recorded against third, fourth and fifth instar, respectively on lablab treated with Bb 112.

Microbial insecticides such as entomopathogenic fungi can provide an alternative, more environmentally friendly option to control insect pest. The entomopathogenic fungus, *B. bassiana* is a promising and extensively researched biological control agent that can suppress a variety of economically important insect pests (Coates *et al.,* 2002; McGuire *et al.,* 2005; Prasad and Syed, 2010; Hussein *et al.,* 2010). Hence, in the present investigation four fungal isolates of *B. bassiana viz.,* Bb 111, Bb 112, Bb 113 and Bb 114 were assayed for its relative pathogenicity against *M. vitrata*. The results of the laboratory study showed that the isolate, Bb 112 had higher virulence to *M. vitrata* larvae with a LC_{50} values ranged from 0.10 x 10^8 to 2. 04 x 10^8, 0.14 x 10^8 to 2.67 x 10^8 and 0.20 x 10^8 to 4.80 x 10^8 spores ml^{-1}, respectively against third, fourth and fifth instar larvae on different pulse hosts *viz.,* lablab, cowpea, black gram, green gram and pigeonpea. Several studies have confirmed the susceptibility of *M. vitrata* to entomopathogenic fungi such as *B. bassiana* and *M. anisopliae* isolates and/or their formulations (Ekesi *et al.,* 2002; Sunitha *et al.,* 2008). Yule and Srinivasan (2013) reported 16 to 22 per cent mortality of *M. vitrata* by *B. bassiana* formulation at a concentration of 5,000 to 50,000 ppm. Similar results were also documented by Sreekanth and Seshamahalakshmi (2012). According to them, pigeonpea treated with highest dose (300 mg L^{-1}) of *B. bassiana* SC formulation recorded reduced pod damage by *M. vitrata*.

Mehinto *et al.,* (2014) reported a larval mortality of 65.8 ± 3.5 to 79.0 ± 3.0 per cent when treated with *B. bassiana* isolate Bb 115. Similarly, Soundararajan and Chitra (2011) also reported that the foliar application of *B. bassiana* reduced the spotted pod borer damage in urd bean.

Present investigation also revealed that irrespective of the isolates tested, younger larvae (third instar) are more vulnerable to fungal infection than older ones (fourth and fifth instar). This is in accordance with Bateman *et al.,* (1996) who reported that the infection of insects by fungi depends on their weight. Also, the higher mortality caused by *B. bassiana* may be attributed to its stronger ability to produce enzymes and other toxic metabolites (Ferron, 1981).

The details of the fungal isolates used for the study were as follows:

Isolate	Isolate source	Dosage tested
***B. bassiana* (Bb 111)**	*Tetranychus urticae* Koch	10^4 to 10^8 spores ml^{-1}
***B. bassiana* (Bb 112)**	Unknown larva	10^4 to 10^8 spores ml^{-1}
***B. bassiana* (Bb 113)**	Rice black bug	10^4 to 10^8 spores ml^{-1}
***B. bassiana* (Bb 114)**	*Bombyx mori* L.	10^4 to 10^8 spores ml^{-1}

Table.1 Dose mortality response of *B. bassiana* against third instar larvae of *M. vitrata* on different pulses

Pulses	Fungal isolate	Heterogeneity ($\chi 2$)	Regression equation	LC_{50} (10^8 spores ml^{-1})	95% Fiducial Limits (10^8 spores ml^{-1})
Lablab	Bb 111	1.75	y = 0.573x + 1.735	0.47	0.21-1.06
	Bb 112	1.62	y = 0.658x + 1.320	0.10	0.16-0.73
	Bb 113	1.34	y = 0.587x + 1.413	1.16	0.52-2.58
	Bb 114	1.29	y = 0.574x + 1.596	0.79	0.35-1.78
Cowpea	Bb 111	1.09	y = 0.465x + 2.318	0.62	0.23-1.62
	Bb 112	1.69	y = 0.456x + 2.716	0.13	0.04-0.39
	Bb 113	1.22	y = 0.375x + 2.825	0.65	0.20-2.05
	Bb 114	1.79	y = 0.495x + 1.986	1.09	0.44-2.72
Green gram	Bb 111	1.08	y = 0.688x + 0.959	0.67	0.34-1.33
	Bb 112	1.91	y = 0.655x + 1.410	0.33	0.15-0.72
	Bb113	1.01	y = 0.502x + 2.071	0.70	0.29-1.72
	Bb 114	1.67	y = 0.653x + 0.963	1.40	0.67-2.94
Black gram	Bb 111	1.20	y = 0.552x + 1.580	0.19	0.06- 3.32
	Bb 112	1.11	y = 0.489x + 2.530	0.15	0.03-0.31
	Bb 113	1.71	y = 0.665x + 1.195	0.48	0.24-0.97
	Bb 114	1.16	y = 0.516x + 1.809	1.47	0.61-3.55
Pigeonpea	Bb 111	1.39	y = 0.719x + 0.422	1.04	1.01-4.12
	Bb 112	1.33	y = 0.616x + 1.454	0.52	0.24-1.12
	Bb 113	1.15	y = 0.564x + 1.517	1.37	0.60-3.11
	Bb 114	1.32	y = 0.544x + 1.597	1.68	0.72-3.95

* All lines are significantly good fit @ $P \leq 0.05$

Table.2 Dose mortality response of *B. bassiana* against fourth instar larvae of *M. vitrata* on different pulses

Pulses	Fungal isolate	Heterogeneity ($\chi 2$)	Regression equation	LC_{50} (10^8 spores ml^{-1})	95% Fiducial Limits (10^8 spores ml^{-1})
Lablab	Bb 111	1.21	y = 0.539x + 1.719	1.16	0.50-2.71
	Bb 112	1.44	y = 0.621x + 1.802	0.14	0.05-0.34
	Bb 113	1.32	y = 0.544x + 1.597	1.68	0.72-3.95
	Bb 114	1.03	y = 0.509x + 1.715	2.67	1.07-6.66
Cowpea	Bb 111	1.37	y = 0.505x + 2.011	0.79	0.32-1.91
	Bb 112	1.34	y = 0.614x + 1.497	0.41	0.21-0.99
	Bb 113	1.14	y = 0.627x + 1.269	0.78	0.37-1.65
	Bb 114	1.68	y = 0.442x + 2.331	1.18	0.43-3.19
Green gram	Bb 111	1.62	y = 0.745x + 0.487	1.17	0.60-2.32
	Bb 112	1.18	y = 0.612x + 1.474	0.53	0.25-1.15
	Bb113	1.39	y = 0.645x + 1.009	1.35	0.64-2.85
	Bb 114	1.67	y = 0.725x + 0.395	2.09	1.03-4.21
Black gram	Bb 111	1.89	y = 0.692x + 0.871	0.98	0.48-1.99
	Bb 112	1.01	y = 0.528x + 2.034	0.46	0.17-0.99
	Bb 113	1.25	y = 0.599x + 1.356	1.15	0.53- 2.52
	Bb 114	1.08	y = 0.620x + 1.094	1.84	0.85-3.97
Pigeonpea	Bb 111	1.45	y = 0.643x + 0.725	2.10	1.83-9.17
	Bb 112	1.28	y = 0.574x + 1.596	0.79	0.35-1.78
	Bb 113	1.83	y = 0.548x + 1.595	1.48	0.63-3.45
	Bb 114	1.09	y = 0.611x + 1.135	1.93	0.88-4.23

* All lines are significantly good fit @ $P \leq 0.05$

Table.3 Dose mortality response of *B. bassiana* against fifth instar larvae of *M. vitrata* on different pulses

Pulses	Fungal isolate	Heterogeneity ($\chi 2$)	Regression equation	LC_{50} (10^8 spores ml^{-1})	95% Fiducial Limits (10^8 spores ml^{-1})
Lablab	Bb 111	1.20	y = 0.574x + 1.273	2.90	1.22-6.73
	Bb 112	1.08	y = 0.417x + 3.189	0.20	0.04- 1.00
	Bb 113	1.98	y = 0.652x + 0.705	3.43	1.57-7.72
	Bb 114	1.45	y = 0.643x + 0.725	2.10	1.83-9.17
Cowpea	Bb 111	1.50	y = 0.422x + 2.473	0.90	0.32-2.55
	Bb 112	1.00	y = 0.599x + 1.610	0.48	0.22-1.06
	Bb 113	1.30	y = 0.611x + 1.322	0.96	0.44-2.08
	Bb 114	1.51	y = 0.591x + 1.242	2.08	0.92-4.65
Green gram	Bb 111	1.30	y = 0.611x + 1.322	0.96	0.44-2.08
	Bb 112	1.83	y = 0.497x + 2.027	0.92	0.37-2.27
	Bb113	1.31	y = 0.708x + 0.408	2.73	1.34-5.58
	Bb 114	1.62	y = 0.679x + 0.577	2.94	1.30-6.29
Black gram	Bb 111	1.04	y = 0.518x + 1.719	1.99	0.82-4.84
	Bb 112	1.97	y = 0.745x + 0.644	0.60	0.31-1.18
	Bb 113	1.23	y = 0.602x + 1.246	2.13	1.53-4.83
	Bb 114	1.24	y = 0.600x + 1.176	2.31	1.04-5.12
Pigeonpea	Bb 111	1.94	y = 0.573x + 1.342	2.16	0.93-4.98
	Bb 112	1.02	y = 0.690x + 0.493	1.73	1.50-6.60
	Bb 113	1.67	y = 0.638x + 0.768	3.83	1.71-8.59
	Bb 114	1.31	y = 0.636x + 0.738	4.80	2.12-10.90

* All lines are significantly good fit @ $P \leq 0.05$

Table.4 Time mortality response of *B. bassiana* against third instar larvae of *M. vitrata* on different pulses

Pulses	Fungal isolate	Heterogeneity ($\chi 2$)	Regression equation	LT_{50} (h)	95% Fiducial Limits (h)
Lablab	Bb 111	1.67	y = 4.561x - 4.528	122.61	110.43- 136.13
	Bb 112	1.52	y = 5.208x - 5.569	110.48	101.04 - 120.79
	Bb 113	2.64	y = 5.712x - 6.749	115.48	106.60- 125.11
	Bb 114	2.18	y = 4.209x - 3.744	119.77	106.37- 134.86
Cowpea	Bb 111	1.22	y = 4.315x - 4.082	125.93	112.25 - 141.28
	Bb 112	2.71	y = 5.014x - 5.217	111.72	101.82 - 122.59
	Bb 113	1.30	y = 5.426x - 6.229	117.84	108.13 - 128.42
	Bb 114	1.74	y = 4.250x - 3.873	122.16	108.58 - 137.43
Green gram	Bb 111	1.55	y = 5.162x - 5.700	118.20	107.69 - 129.73
	Bb 112	1.46	y = 5.866x - 6.953	112.23	103.56 - 121.63
	Bb113	1.55	y = 5.486x - 6.279	116.39	106.60 - 127.07
	Bb 114	2.03	y = 5.334x - 6.030	117.46	107.61 - 128.21
Black gram	Bb 111	1.84	y = 4.396x - 4.208	125.22	109.83 - 142.76
	Bb 112	1.21	y = 5.658x - 6.590	113.46	104.54 - 123.14
	Bb 113	1.98	y = 3.389x - 1.985	114.95	100.05 - 132.06
	Bb 114	1.71	y = 4.928x - 5.246	120.69	108.92 - 133.73
Pigeonpea	Bb 111	1.80	y = 4.970x - 5.371	121.81	110.18 - 134.66
	Bb 112	2.57	y = 5.672x - 6.602	115.44	104.42 - 123.23
	Bb 113	1.55	y = 5.166x - 5.699	119.08	108.27 - 130.98
	Bb 114	1.64	y = 4.938x - 5.293	120.25	109.45 - 134.32

* All lines are significantly good fit @ $P \leq 0.05$

Table.5 Time mortality response of *B. bassiana* against fourth instar larvae of *M. vitrata* on different pulses

Pulses	Fungal isolate	Heterogeneity ($\chi 2$)	Regression equation	LT_{50} (h)	95% Fiducial Limits (h)
Lablab	Bb 111	2.72	y = 4.286x - 4.042	126.95	113.22 - 142.35
	Bb 112	1.79	y = 5.838x - 6.940	114.01	105.35- 123.38
	Bb 113	2.37	y = 4.358x - 4.069	120.48	107.69 - 134.80
	Bb 114	5.18	y = 4.446x - 4.322	123.70	110.96 - 137.89
Cowpea	Bb 111	1.89	y = 3.637x - 2.717	129.76	113.00 - 149.02
	Bb 112	1.03	y = 5.320x - 6.000	117.80	107.84 - 128.69
	Bb 113	1.70	y = 3.890x - 3.166	125.63	109.31 - 144.39
	Bb 114	3.44	y = 4.222x - 3.915	127.48	113.01 - 143.82
Green gram	Bb 111	2.48	y = 4.766x - 5.054	127.60	114.31 - 142.44
	Bb 112	1.39	y = 4.301x - 3.920	110.31	105.61 - 132.47
	Bb113	3.44	y = 4.012x - 3.454	127.30	111.58 - 145.24
	Bb 114	1.34	y = 4.542x - 4.558	127.35	112.33 - 144.37
Black gram	Bb 111	1.47	y = 4.609x - 4.707	127.36	113.03 - 143.50
	Bb 112	2.10	y = 5.789x - 6.762	118.28	101.73 - 119.62
	Bb 113	1.03	y = 2.846x - 0.946	122.67	102.12 - 147.37
	Bb 114	1.87	y = 4.396x - 4.230	126.64	110.76 - 144.78
Pigeonpea	Bb 111	1.35	y = 4.346x - 4.203	131.76	114.53 - 151.58
	Bb 112	1.41	y = 4.097x - 3.551	121.78	107.48 - 137.99
	Bb 113	1.11	y = 4.685x - 4.774	122.33	109.00 - 137.28
	Bb 114	2.57	y = 3.906x - 3.261	129.57	112.63 - 149.04

* All lines are significantly good fit @ $P \leq 0.05$

Table.6 Time mortality response of *B. bassiana* against fifth instar larvae of *M. vitrata* on different hosts

Pulses	Fungal isolate	Heterogeneity ($\chi 2$)	Regression equation	LT_{50} (h)	95% Fiducial Limits (h)
Lablab	Bb 111	1.10	y = 4.003x - 3.460	128.64	112.55 - 147.02
	Bb 112	1.49	y = 4.198x - 3.735	120.69	106.99 - 136.15
	Bb 113	1.11	y = 3.767x - 2.924	127.18	109.56 - 147.62
	Bb 114	1.69	y = 4.515x - 4.506	127.45	112.61 - 144.25
Cowpea	Bb 111	1.93	y = 3.381x - 2.224	138.25	113.79 - 167.95
	Bb 112	1.33	y = 3.893x - 3.183	126.40	109.87 - 145.42
	Bb 113	1.91	y = 4.308x - 4.130	131.97	114.87 - 151.61
	Bb 114	1.46	y = 3.804x - 3.099	133.32	114.69 - 154.99
Green gram	Bb 111	1.88	y = 3.751x - 2.937	135.47	112.09 - 151.85
	Bb 112	3.38	y = 3.549x - 2.499	128.96	110.86 - 150.00
	Bb113	1.39	y = 4.894x - 5.153	129.84	107.01 - 131.98
	Bb 114	5.26	y = 4.728x - 5.011	132.58	115.99 - 144.76
Black gram	Bb 111	1.15	y = 3.985x - 3.658	147.97	123.93 - 176.67
	Bb 112	3.33	y = 3.843x - 3.151	131.36	113.62 - 151.88
	Bb 113	1.99	y = 4.555x - 4.661	133.06	116.03 - 148.05
	Bb 114	1.87	y = 4.349x - 4.247	133.83	116.28 - 154.03
Pigeonpea	Bb 111	1.80	y = 4.660x - 4.862	139.59	115.39 - 145.54
	Bb 112	2.64	y = 3.448x - 2.315	134.42	111.92 - 154.32
	Bb 113	3.47	y = 4.512x - 4.620	133.46	117.93 - 151.04
	Bb 114	1.97	y = 3.552x - 2.575	135.70	114.24 - 161.18

* All lines are significantly good fit @ $P \leq 0.05$

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