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Research Article

PATHOGENICITYOF INDIGENOUS *Beauveria bassiana* (BALSAMO) VUILLEMIN ISOLATES AGAINST DIAMONDBACK MOTH, *Plutella xylostella*

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Abstract- Larval bio-assays were conducted to evaluate the virulence of *B. bassiana* isolates against diamondback moth, *P. xylostella*. A total of eight isolates from different parts of Tamil Nadu and one isolate from Punjab were collected through extensive surveys. Local isolates of *B. bassiana*, BbI8 and BbI3 recorded the highest mean mortality per cent of 86.67 and 83.33, respectively. Virulent isolates thus identified can be further mass produced at larger scale and successfully employed for the biological control of diamondback moth as an innovative tool in the integrated pest management.

Keywords- Entomopathogenic fungi, Diamondback moth, Bioassays.

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Introduction

Incorporation of Biological control is the best alternative tool to chemical pesticides in crop protection and human safety. Among all the insect pests attacking crucifers, the diamondback moth (DBM), *P. xylostella* (L.) (Lepidoptera: Plutellidae) is the most destructive one. Worldwide, this pest generates losses of over 80 per cent (US $4 to US $5 billion) in annual crop production [1-3]. Development of entomopathogens as biological control agents in the management of *P. xylostella* is gaining interest due to continued failure of chemical control measures. Several entomopathogenic fungi including *B. bassiana* [4], *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Paecilomyces fumosoroseus* (Wize) [5] have been reported to infect *P. xylostella*.

B. bassiana is a promising and extensively researched bio-control agent that can suppress a variety of economically important insect pests. It is found naturally on some plants and in soils and is regarded as a safe bio-pesticide [6, 7]. The efficacy of *B. bassiana* against the diamondback moth, *P. xylostella* in greenhouse and the field proved that the fungal spores significantly affected larval survival, indicating the potential for using the entomopathogen against this pest [4]. With this background, present investigations were carried out to find out an effective fungal isolate against DBM.

Materials and Methods

Source of *B. bassiana* isolates

Naturally infested and mycosed insects were collected from different parts of Tamil Nadu and Punjab through panoptic survey. The cadavers were surface sterilized and the fungus was isolated, sub-cultured and maintained on Sabouraud Maltose Agar with Yeast (SMAY) (Maltose – 4%, Peptone–1%, Yeast extract–1% and Agar – 2%) medium. The details on the entomopathogenic fungi, the host insects from which it was isolated and the location of collection were presented in [Table-1].

Test Insects

The diamondback moth (DBM) larvae required for bioassay were obtained from the culture maintained on mustard and cauliflower at Insectary,
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Preparation of fungal spore suspension

For laboratory bioassay, all the nine isolates were cultured in petridishes (9 cm) containing Sabouraud's Maltose Agar enriched with one per cent yeast extract (SMAY) solid medium and incubated at 25±1°C for 10-14 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®. The conidial suspension was vortexed for 5 minutes to produce a homogenous spore suspension. The spore count in the liquid suspension was assessed using a Neubauer haemocytometer. From the stock solution, further dilutions were made to obtain the required concentrations for further studies [8].

Larval bioassays

Larvae of 3rd instar diamondback moth were used for the bioassay. Larvae were treated as a batch of 10 kept in petri dishes with five conidial concentration ranging from 1 x 10^6 to 1 x 10^{10} conidia mL^{-1} in 0.02% aqueous Tween 80 with three replications each. Conidial suspensions of different concentrations were sprayed against the diamondback moth larvae using a hand atomizer. Fresh leaves were provided as a feed everyday and the larval litter was cleaned each day during incubation at 25±2°C. For two consecutive days the larvae were treated with an equal volume of water with 0.02% Tween 80. Three replications were maintained for each treatment. The larvae dead before 24 hours were removed from the experiment. Percentage of larvae infected was recorded up to

seven days of treatment. The dead insects were transferred to moist chambers containing petri dishes autoclaved with moist filter papers to facilitate mycosis. Before transferring, the dead insects were surface sterilized with 0.1% sodium hypochlorite followed by three rinses with sterile distilled water.Time mortality response was carried out at spore concentration of 1 x 10^{10} spores mL^{-1}. Larvae sprayed with 0.01 per cent Tween 80® solution served as control in both cases. The post treatment count was taken at 24 hours interval.

Statistical analysis

The experiments were conducted in a Completely Randomized Design. The data were processed using Probit Analysis [9] for calculating the LC_{50} and LT_{50} values.

Results

Extensive survey was conducted and nine naturally infested local isolates of *B. bassiana* was identified and isolated. These fungal isolates were further subjected to dose mortality response and time mortality response experiments against the larvae of diamondback moth, *P. xylostella*.

The result of the bioassays showed that all the nine isolates are pathogenic to *P. xylostella* at higher concentrations with considerable variations.The LC_{50} values recorded by the nine isolates ranged from 5.96 x 10^7 to 1.48 x 10^{10} spores mL^{-1}. The lowest LC_{50} value was observed in BbI3 isolate of 4.18 x 10^7 spores mL^{-1} followed by BbI8 isolate with the LC_{50} value of 5.96 x 10^7 spores mL^{-1}. The isolates BbI5, BbI4 and BbI9 were found to be less virulent with the highest LC_{50} values of 2.06 x 10^9 spores mL^{-1}, 5.35 x 10^9 spores mL^{-1} and 1.48 x 10^{10} spores mL^{-1} [Table-2].

Table-1 *Entomopathogenic fungi isolated and identified during the survey*

Isolate Code	Survey areas	Crop	Host	Nature of pathogen
BbI1	Kanyakumari	Rice	Unidentified Bug	Insect pathogen
BbI2	Karaiyur, Dharapuram	Rice	Earhead bug	Insect pathogen
BbI3	PBS, Coimbatore	Rice	Larvae of Stem borer	Insect pathogen
BbI4	PBS, Coimbatore	Rice	Brown plant hopper	Insect pathogen
BbI5	Punjab	Rice	Brown Plant hopper	Insect pathogen
BbI6	PBS, Coimbatore	Daincha	Unidentified larvae	Insect pathogen
BbI7	Tanjore (Dt)	Rice	Coccinellid	Insect pathogen
BbI8	Udumalpet	Mulberry	Silkworm	Insect pathogen
BbI9	Coimbatore	Sugarcane	Unidentified larvae	Insect pathogen

Table-2 *Dose mortality response of entomopathogenic fungal isolatesagainstdiamondback moth, P. xylostella*

Entomopathogenic fungal Isolate	Heterogeneity ($\chi 2$)*	Regression equation	LC_{50} (ConidiamL^{-1})	95% Fiducial Limits		Mortality (%)
				Lower	Upper	
BbI1	0.289	y = 0.445x + 1.325	1.76 x 10^8	5.61 x 10^7	5.54 x 10^8	80.00
BbI2	0.267	y = 0.380x + 1.719	3.98 x 10^8	1.00 x 10^8	1.58 x 10^9	70.00
BbI3	1.750	y = 0.442x + 1.607	4.18 x 10^7	1.32 x 10^7	1.33 x 10^8	83.33
BbI4	3.861	y = 0.400x + 1.151	5.35 x 10^9	5.64 x 10^8	5.08 x 10^{10}	53.33
BbI5	1.234	y = 0.277x + 2.416	2.06 x 10^9	1.92 x 10^8	2.21 x 10^{10}	60.00
BbI6	4.040	y = 0.419x + 1.518	1.85 x 10^8	5.56 x 10^7	6.13 x 10^8	73.33
BbI7	1.837	y = 0.379x + 1.637	7.04 x 10^8	1.54 x 10^8	3.21 x 10^9	63.33
BbI8	0.238	y = 0.491x + 1.161	5.96 x 10^7	2.08 x 10^7	1.17 x 10^8	86.67
BbI9	1.011	y = 0.336x + 1.597	1.48 x 10^{10}	9.34 x 10^8	2.35 x 10^{11}	46.67

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

No.of larvae used per treatment = 30.

The time mortality response test conducted on third instar larvae of *P. xylostella* at higher concentration of 1 x 10^{10} spores mL^{-1} showed variation among the isolates studied. The least LT_{50} value of 117.26 and 124.22 hours was recorded in isolates BbI8 and BbI3, respectively. Other isolates like BbI2, BbI1 and BbI6 had LT_{50} values of 128.06, 130.27 and 133.14 hours, respectively. Very high LT_{50} values of152.54, 156.14 157.61 and 176.75 hours was observed in BbI4, BbI7, BbI5 and BbI9 isolates [Table-3].

Table-3 *Time mortality response of entomopathogenic fungal isolatesagainstdiamondback moth, P. xylostella*

Entomopathogenic fungal Isolate	Heterogeneity ($\chi 2$)*	Regression equation	LT_{50}** (h)	95% Fiducial Limits (h)
BbI1	8.31	y = 2.935x - 1.236	130.27	112.89 - 150.31
BbI2	6.55	y = 3.705x - 2.856	128.06	115.54 - 141.93
BbI3	7.68	y = 3.840x - 3.048	124.22	112.44 - 137.23
BbI4	3.65	y = 3.007x - 1.737	157.61	133.27 - 186.38
BbI5	3.72	y = 3.039x - 1.749	152.54	130.05 - 178.92
BbI6	5.43	y = 3.514x - 2.534	133.14	118.79 - 149.23
BbI7	4.18	y = 3.099x - 1.973	156.14	133.98 - 181.98
BbI8	9.55	y = 4.087x - 3.391	117.26	106.68 - 128.90
BbI9	1.04	y = 2.263x - 0.145	176.75	133.77 - 233.54

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

** LT_{50} values recorded at the highest concentration of 10^{10}spores ml^{-1}

Discussion

Development of mycoinsecticides begins with the collection of fungal isolates and screening them for virulence against the target pest [10]. The present study revealed that the isolates BbI8 and BbI3 isolates have higher virulence against *P. xylostella* under laboratory conditions. These two isolates have recorded least LC_{50} and LT_{50} values which make it suitable for further mass production and formulation development studies. The effectiveness of a fungal isolate is measured in terms of its pathogenicity (LC_{50}) and the speed (LT_{50}) with which it kills the target pest [11]. The present study is in agreement with the result that the concentrations of $1x10^5$ conidia cm^{-2} were low to cause a mortality percentage exceeding 60 per cent in *P. xylostella* [12-14]. The results obtained from laboratory and greenhouse revealed that of the four selected isolates of *M. Anisopliae* and *B. bassiana* offered very high efficacy in against DBM at the dose of 6×10^{12} conidia ha^{-1} [15]. Similarly, the differences in virulence were more pronounced for *B. bassiana* strains than for *M. anisopliae* [16].The comparative virulence of local isolates and standard strain of *B. bassiana* tested against few lepidopteran pests showed that all the test insects were susceptible to *B. bassiana* isolates [17].

Conclusion

The current study fulfilled the preliminary objective of identifying a virulent isolate for the management of diamondback moth, *P. xylostella*. These virulent isolates should be further used for studies such as cost-effective mass production, conidial production, development of formulation, shelf-life studies and safety of selected isolates to natural enemies and pollinators under both laboratory and field conditions for the successful biological control of DBM.

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References

[1] Castanon-Cervantes O., Lugo C., Aguilar M., Gonzalez-Moran G. & Fanjul-Moles M.L. (1995) *Comp. Biochem. Phys. A.,* 110, 139-146.
[2] Ogle J.T. (1992) *Invertebr. Reprod. Devel.,* 22, 267-274.
[3] Verkerk R. and Wright D.J. (1996) *Bulletin of Entomological Research*, 86, 205-216.
[4] Sarfraz M., Keddie A. and Dosdall LI. (2005) *Biocontrol Science and Technology*, 15 (8), 763-789.
[5] Zalucki M.P., Shabbir A., Silva R., Adamson D., Shu-Sheng L. and Furlong M. (2012) *Journal of Economic Entomology,* 105(4), 1115–1129.
[6] Vandenberg J.D., Shelton A.M., Wilsey W.T. and Ramos M. (1998) *Journal of Economic Entomology,* 91(3), 624-630.
[7] Altre J.A., Vandenberg J.D. and Cantone F.A. (1999) *Journal of Invertebrate Pathology,* 73, 332–338.
[8] Coates B.S., Hellmich R.L. and Lewis L.C. (2002) *Genome,* 45(1), 125-132
[9] McGuire M. R., Leland J. E., Dara S., Park Y. H. and Ulloa M. (2006) *Biological Control,* 38, 390-396.
[10] Saranya S., Ramaraju K. and Jeyarani S. (2013) *Biopestic. Inc.,* 9(2), 127-131.
[11] Finney D.J. (1971) *Probit analysis*, 3rd edn. Cambridge University Press, Cambridge, UK. 333p.
[12] Jenkins N. E., Heriefo G., Longewald T., Cherry A. J. and Lomer C. J. (1998) *Biocontrol News and Information,* 19, 21-31.
[13] Negasi A., Parker B. L. and Brown Bridge M. (1998) *Insect Sci. Appl.,* 18(1), 37-44.
[14] Quesada-Moraga E. and Vey A. (2004) *Mycological Research*, 108(4), 441–452
[15] Hui Wu J., Ali S. and Xiang Ren S. (2010) *Pakistan Journal of Zoology,* 42 (5), 521-528.
[16] Anaisie P., Eziah V. and Owusu E. (2011) *Journal of Biochemistry and Bioinformatics,* 1(10), 275-281.
[17] Nguyen T.L. and Vo T.B.C. (2007) *Omonrice,* 15, 86-93
[18] Ekesi S., Maniania N.K., Onu I. and Lohr B. (1998) *Journal of Applied Entomology,* 122, 629-634.
[19] Wraight S.P., Ramos M.E., Avery P.B., Jaronski S.T. and Vandenberg J.D. (2010) *Journal of Invertebrate Pathology*, 103(2010), 186–199.