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RESEARCH ARTICLE

A biocontrol of stored product pest *Corcyra cephalonica* (Lepidoptera:Pyralidae) by secondary metabolite of *Beauveria bassiana* (Balsamo) Vuillemin (Lepidoptera: Pyralidae)

■ **S. GOWRILAKSHMI, M.S. NALINASUDARI, N. DEEPA AND ARUMUGHAM**

SUMMARY

Entomopathogenic fungus belong to the fungal group which are widely used in the integrated pest management systems. These fungi are insect associated and kills the agricultural pests.So they are used as biopesticides and by replacing the chemical pesticides and do save the human beings and environment from hazardous chemical pollution. These entomopathogenic fungus produce many secondary metabolites which have antimicrobial activities.It also has bioactive compounds that contains larvicidal and insecticidal activities. *Corcyra cephalonica* ,a stored product pest is a major agricultural pest which causes economic loss to our country. Though there may be evidences for the conidial and spore treatments but secondary metabolite usages were very less. So the present study was undertaken to check the secondary metabolite of entomopathogenic fungi *Beauveria bassiana* an effective agent to control the stored product pest *corcyra cephalonica* and can be used in integrated pest management systems.

Key Words : Entomopathogenic fungus, *Corcyra cephalonica*, Insecticide, IPM

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The awarness of replacing chemical pesticide by biopesticide has been increased in recent years due to the interest shown by the researchers and has felt the importance to prevent the chemical pollution and to save the world .The entomopathogens are the group of the fungus which attack the insect pests and does prevent the environment from the pollution.This entomopathogens play a complementary approaches in the integrated pest management systems (IPMS) (Pampathy *et al.*, 2010). Some of the plants and the soil paves a way for the shelter to these entomopathogens other than the micro-organisms (Keller *et al.*, 2003 and Victor *et al.*, 2014). This biopesticide strongly plays a role in decreasing the insect pests (Pedro *et al.*, 2001).

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Now-a-days substituting synthetic insecticide with biocontrol especially with microbes shows greater attention among the farmers to kill the pests. Biocontrol with entomopathogens are the best partners for the pest management (Amutha *et al.*, 2010). The entomopathogenic fungi named *Beauveria bassiana* which belong to hypomycetes is commonly called as white muscardine fungi and the disease caused by it is white muscardine disease. when the spores come in contact with the pest it forms the germination tube and the hyphae penetrates the cuticle which is made up of lipids that protects the insect from any external applications like insecticides (chemical and botanical) (Juarez, 1994 and Crespo *et al.*, 2008). So in order to overcome and to start the penetration the cuticle should be abraded for which the EPF secrete proteases, chitinases and lipases which will digest the cuticle proteins like protein, chitin, lipid (Wang *et al.*, 2005 and Cho *et al.*, 2006). After the entry, it uses the insects body for the growth and brings death to it and the cadavar will be covered by conidia and these conidia are now ready to spread (Bhattachary *et al.*, 2003 and Purwar and Sachan, 2006). Other than the spores, the fungi also produces secondary metabolites which has antimicrobial, cytotoxic, insecticidal, larvicidal activities. The compounds produced by it has toxic effect to the target pests (Demain, 1999 and Narasimha Reddy Parine *et al.*, 2010). These compounds may bring repellency, mounting disruption, growth reduction, developmental abnormalities, oviposition deterrent and mortality (Mitchell *et al.*, 2004 and Islam *et al.*, 2011). So the present study was undertaken to check the toxicity of *Beauveria bassiana* to control the stored product pest *Corcyra cephalonica*.

MATERIAL AND METHODS

Fungi :

B. bassiana was isolated from the soil at Coimbatore, India .The soil was brought to the laboratory for isolation studies .Then serial dilution followed with spread plate were done. The dilutions of 4, 5, 6, 7 were used for the spread plate in PDA media. After 5-7 days of the growth, the colonies were sub-cultured to get the isolate of the desired fungi. Then lacto phenol cotton blue staining method was done to observe spores under microscope for the identification .The fungi were indentified based on the morphological characters .These were then sequenced and DNA barcoding was done to verify that the isolated fungi was *Beauveria bassiana*.

Insect rearing :

The eggs were collected in a commercial rearing place and then were mixed with diet (broken millet and enriched with groundnut) and antibiotic were added to it to avoid any infection. These were then transferred into tubs and covered with muslin cloth and kept for a week for hatching. The third in star larvae were selected for the bioassay. The remaining larvae were left for the moth stage. The moths were transferred to the egg laying cage where the eggs were collected and the culture was then maintained for further works.

Production of secondary metabolite :

B. bassiana was inoculated in the complete media (CMA) (KH_2PO_4, $NaNO_3$, NH_4NO_2, $MgCl_2$, yeast extract, glucose) broth. Three flasks were maintained for 3rd, 6th and 9th day. The culture filtrate was then filtered and centrifuged and the supernatant were collected for the bioassay. The secondary metabolite of day 3, 6 and 9 were tested on *Corcyra cephalonica*. The broken millet was used as feed for it. 1 g of feed was mixed with the secondary metabolite of 25, 50 and 75 per cent and air dried and the control was mixed with sterile water. Then 10 larvae were introduced into each Petriplates (9cm × 12cm). Triplicates were maintained for each concentration. Results were observed for 7 days.

Statistical analysis :

All data were subjected to analysis of variance; the means were separated using Duncan multiple range tests. Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The result of the bioassay of secondary metabolite is shown in the Table 1. The results were significant and are dose dependant. Among the 3 days tested, day 6 showed maximum mortality rate. The highest mortality rate was found to be 86.66 per cent in 75 per cent concentration ($f=96.250$, $df=3,8$, $p<0.01$) (Table 1) and followed by day 9 metabolites which gave the mortality rate as 56.66 per cent ($f=17.788$, $df=3,8$, $p<0.01$) and then by day 3 as 40.00 per cent ($f=12.458$, $df=3,8$, $p<0.01$). The treatments of day 3, 6 and 9 were significant as shown in the Table 2, 3 and 4.

In the day 3 secondary metabolite induced mortality ranging from 13 to 40 per cent (Fig. 1). The day 6 showed the range as 23 to 86 per cent (Fig. 2) and for day 9, it

was 23 to 56 per cent (Fig. 3). As far as the dose-mortality relationship is concerned, it showed maximum mortality in higher dose.

come in to contact with the insect and starts to germinate. Enzymes like protease, lipase, amylase, chitinase are produced during germination as these enzymes are cell degrading enzymes. So it disrupts the insect cells and brings

Table 1 : Mortality (%) induced by secondary metabolite of *Beauveria bassiana* on *Corcyra cephalonica*

	Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
3rd day	25	3.33±5.77a	6.66±5.77b	10.00±0.00 b	10.00±0.00 b	13.33±5.77 b	13.33±5.77 ab
	50	3.33±5.77a	10.00±0.00 b	13.33±5.77 b	16.66±11.54 bc	16.66±11.54 b	16.66±11.54 b
	75	6.66±5.77a	10.00±0.00 b	16.66±5.77b	26.66±5.77c	33.33±5.77c	40.00±10.00 c
6th day	25	3.33±5.77a	6.66±5.77 ab	13.33±5.77b	16.66±11.54b	20.00±10.00b	23.33±11.54b
	50	6.66±5.77ab	13.33±5.77b	20.00±0.00b	30.00±0.00c	40.00±0.00c	46.66±5.77c
	75	13.33±5.77b	26.66±5.77c	43.33±5.77c	60.00±0.00d	76.66±5.77d	86.66±5.77d
9th day	25	3.33±5.77a	6.66±5.77a	13.33±5.77ab	20.00±10.00b	23.33±5.77b	23.33±5.77b
	50	3.33±5.77a	10.00±0.00ab	16.66±5.77ab	23.33±5.77b	26.66±5.77b	30.00±10.00b
	75	6.66±5.77a	20.00±10.00b	26.66±15.27b	40.00±10.00c	50.00±17.32c	56.66±15.27b
	Control	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 b

Different superscripts in the same column are significantly different at p<0.05 level.

Table 2 : Analysis of variance for the mortality caused by secondary metabolite of *Beauveria bassiana* on *Corcyra cephalonica* for the day 3

		Sum of squares	df	Mean square	F	Sig.
Day1	Between groups	66.667	3	22.22	0.889	0.487*
	Within groups	200	8	25		
Day2	Between groups	200	3	66.667	8	0.009*
	Within groups	66.667	8	8.333		
Day3	Between groups	466.667	3	155.556	9.333	0.005*
	Within groups	133.333	8	16.667		
Day4	Between groups	1133.333	3	377.778	9.067	0.006*
	Within groups	333.333	8	41.667		
Day5	Between groups	1691.667	3	563.889	11.278	0.003*
	Within groups	400	8	50		
Day6	Between groups	2491.667	3	830.556	12.458	0.002*
	Within groups	533.333	8	66.667		

* indicate significance of values at P<0.05 NS = Non-significant

Table 3 : Analysis of variance for the mortality caused by secondary metabolite of *Beauveria bassiana* on *Corcyra cephalonica* for the day 6

		Sum of squares	df	Mean square	F	Sig.
Day1	Between groups	291.667	3	97.222	3.889	0.055*
	Within groups	200	8	25		
Day2	Between groups	1166.667	3	388.889	15.556	0.001*
	Within groups	200	8	25		
Day3	Between groups	2958.333	3	986.111	59.167	0*
	Within groups	133.333	8	16.667		
Day4	Between groups	5800	3	1933.333	58	0*
	Within groups	266.667	8	33.333		
Day5	Between groups	9625	3	3208.333	96.25	0*
	Within groups	266.667	8	33.333		
Day6	Between groups	12291.667	3	4097.222	81.944	0*
	Within groups	400	8	50		

* indicate significance of values at P<0.05 NS = Non-significant

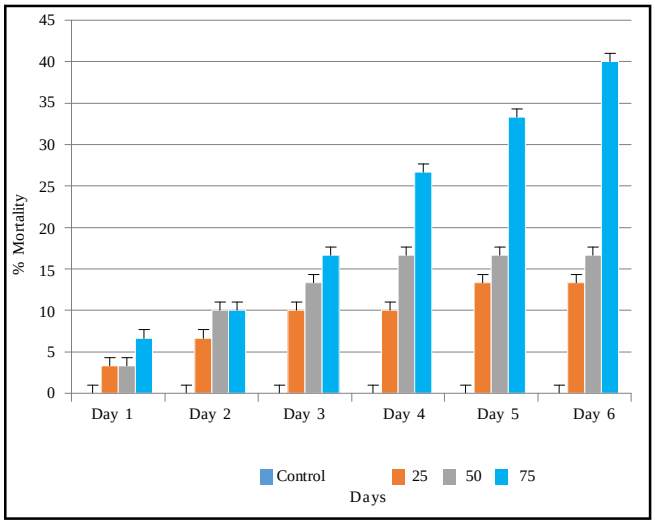

Fig. 1 : Mortality rate caused by the 3rd day secondary metabolite of *beauveria bassiana* on *Corcyracephalonica*

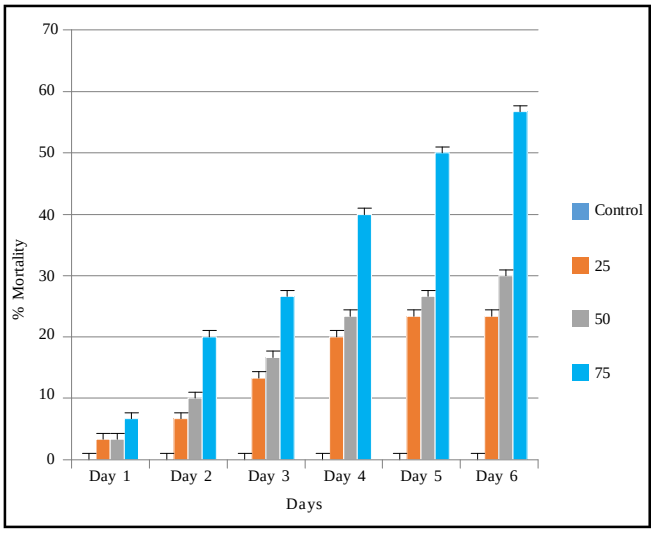

Fig. 3 : Mortality rate caused by the 9th day secondary metabolite of *beauveria bassiana* on *Corcyracephalonica*

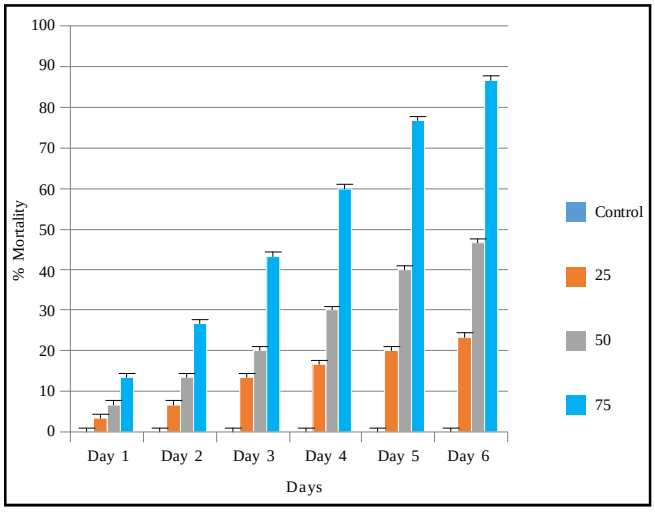

Fig. 2 : Mortality rate caused by the 6th day secondary metabolite of *beauveria bassiana* on *Corcyracephalonica*

death. But in the secondary metabolite treatment the enzymes are produced in the broth and applied on the pests as it takes less time and the process of killing the pest can be simplified. It also had adverse effect in adult longevity and the pupal stage also delayed and the adults were malformed. Many research works have gone in the field level to control agricultural pests like rose sawfly, *Arge rosea* (Roya Khosravi, *et al.*, 2015) and tobacco caterpillar, *Spodoptera litura* fabricius (Malarvannan *et al.*, 2010). But when it comes to post harvest storage level not much works were done. Even though there were few works based on spores, conidial concentration on stored pest (Kaur *et al.*, 2014), work on secondary metabolite was sparse. The present study will help to use secondary metabolite to control *Corcyra cephalonica* in the ware houses which can be used as one of the methods in IPMS.

Table 4 : Analysis of variance for the mortality caused by secondary metabolite of *Beauveria bassiana* on *Corcyra cephalonica* for the day 9

		Sum of squares	df	Mean square	F	Sig.
Day1	Between groups	66.667	3	22.222	0.889	0.487*
	Within groups	200	8	25		
Day2	Between groups	625	3	208.333	6.25	0.017*
	Within groups	266.667	8	33.333		
Day3	Between groups	1091.667	3	363.889	4.852	0.033*
	Within groups	600	8	75		
Day4	Between groups	2425	3	808.333	13.857	0.002*
	Within groups	466.667	8	58.333		
Day5	Between groups	3766.667	3	1255.556	13.697	0.002*
	Within groups	733.333	8	91.667		
Day6	Between groups	4891.667	3	1630.556	17.788	0.001*
	Within groups	733.333	8	91.667		

* indicate significance of values at P<0.05 NS = Non-significant

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