05-16

AN INVESTIGATION INTO THE CAUSE OF NON DEVELOPMENT OF MYCOSIS ON ROOT GRUB CADAVERS TREATED WITH ENTOMOPATHOGENIC FUNGI

Sreekumar K. M, Bindu T.N, Govindan M and Joy M

College of Agriculture, Kerala Agricultural University, Padannakkad, Kasaragod

Introduction

The coconut root grub, *Leucopholis coneopora* is an endemic pest in sandy soil which attack coconut, arecanut, vegetables, pulses, fruits, tuber crops, spices, medicinal plants, fodder, etc. The present recommendation for the management is two applications of Phorate 10 G @ 10g ai per palm in the coconut basin or drenching with Chlorpyriphos @ 0.04% ai. (Chandrika *et al*, 1999) which is hazardous. *Metarrhizium anisopliae, Beauveria bassiana* and *B. brongniartii* are entomopathogenic fungi (EPF) reported to cause mortality in white grubs. The present study is an attempt to find out whether there is any inhibition by bacteria on the EPF growth since many of the EPF treated grubs did not develop mycosis after death.

Materials and Methods

EPF cultures obtained from Project Directorate of Biological Control, Bangalore was maintained in Potato Dextrose Agar (PDA) medium and used for experiment. 100 ml of the media was dispensed into the 250 ml conical flasks and inoculated with 7 day old EPF culture. Three replications of each were made. All the conical flasks were kept at room temperature. After 7-10 days of incubation, the fungal mat formed in each conical flask along with the spore suspension was crushed using a mixer grinder**.** Two drops of Tween – 80 was added to get a homogenous suspension and its spores were counted using haemocytometer. The grubs were inoculated by

dipping in the spore suspension for 1-2 minutes. After drying the spore suspension, they were transferred to plastic containers containing approximately 250 g of sterilized sandy soil and 3 ml of respective spore suspension was added in the soil. Grubs treated with distilled water were kept as control. All of them were provided with sweet potato pieces as food. Dead larvae were removed from the container, surface sterilized using 70% alcohol and kept in petridishes with wet cotton to maintain humidity.

Results and Conclusions

Table 1 Treatment of larval instars of root grubs with EPF grown in PDA medium

Fungus	Spores/ml	No. of grubs	Mortality days after treatment						Total
			5	10	15	20	25	30	
I instar									
B.bassiana	$2.8X10^9$	10	1	1	3	1	-	-	6
B.brongniarti	$4.0X10^8$	10	-	2	4	2	-	-	8
Control	-	10	-	-	-	-	-	-	0
II instar									
B.bassiana	$9.69X10^8$	10	1	2	-	-	-	-	3
B.brongniarti	$3.84X10^8$	10	1	-	4	-	1	3	9
Control	-	10	-	1	-	-	1	-	2
III instar									
B.bassiana	$9.69X10^8$	10	-	-	-	-	-	1	1
B.brongniarti	$3.84X10^8$	10	-	-	-	-	-	-	0
Control	-	10	-	-	-	-	-	1	1

The table shows that as the larval instars advance, there is a reduction in mortality. Within the same instar, there is no significant difference between EPF in inflicting mortality. Though there was larval mortality ranging from 10% to 90%, there was no fungal growth on the cadavers. The cadavers become putrefied, emitted foul smell and disintegrated, instead of becoming stony hard

and exhibiting fungal development. When the haemolymph of some of the cadavers were plated in PDA medium, there was only bacterial development. But Koch's postulate could not be proved with this bacterium.

Another experimented was conducted to study the interaction between the bacterial flora associated with the grubs and the EPF. For this, bacterial floras are collected from the haemolymph of the dead grubs treated with all the above EPF. In addition to this, the bacteria present on the body surface, in the haemolymph and gut of the healthy grubs were also extracted and cultured in PDA and Nutrient Agar (NA) media. The bacteria obtained are given below:

Table 2 Bacteria obtained from healthy and dead grubs

Material used for culturing the bacteria	Types of bacteria obtained from		
	PDA plates	NA plates	Total
Surface wash of healthy grub	-	2	2
Haemolymph of healthy grub	2	1	3
Gut of healthy grub	1	1	2
Haemolymph of dead, *B. bassiana* treated grub	-	1	1
Haemolymph of dead, *B. brongniartii* treated grub	1	2	3

In the dual culture experiment was done to know the interaction between bacteria and EPF, 1 ml of the nutrient broth cultures of the above bacteria were made upto 10^{-1} dilution and 50 µl of these was plated over PDA medium taken in 9 cm diameter Petri dishes. In the centre of each pertri plate, a 0.5 cm diameter disc of EPF was inoculated and a bacterial free control was maintained. 3 replications were made in each case. The diameter of EPF was measured after 5 and 8 days of inoculation and the details are given in table 3.

Table 3: Inhibition by bacteria on EPF growth

Source of bacteria	Mean diameter	Mean diameter of

	of *B. bassiana* disc (in cm)	*B. brongniartii* disc (in cm)
Haemolymph of dead grub treated with *B. brogniartii* and cultured in NA	0.5	0.5
Haemolymph of dead grub treated with *B. brogniartii* and cultured in NA	0.6	0.5
Haemolymph of dead grub treated with *B. brogniartii* and cultured in PDA	0.5	0.5
Haemolymph of dead grub treated with *B. bassiana* and cultured in NA	1.68	0.5
Gut of healthy grub	0.5	0.5
Gut of healthy grub	0.5	0.5
Surface wash of healthy grub	0.8	1.17
Surface wash of healthy grub	0.5	0.5
Haemolymph of healthy grub	1.1	1.17
Haemolymph of healthy grub	0.88	1.57
Haemolymph of healthy grub	0.93	1.22
Control	2.73	2.63
CD value	0.29	0.33

From the above table it is clear that there is inhibition by bacteria on the growth of EPF. Therefore it is inferred that the grubs become dead due to the action of the fungal toxin called beauvericin and subsequently the bacteria multiply faster than the EPF in the haemolymph of the grubs resulting in putrefaction of cadavers rather than developing mycosis. Further studies in this line are in progress.

References

Chandrika Mohan, Vidyasagar,P.S.P.V. , Mohammed Basheer, B. M. and Sujatha, A. (1999). *Pestology Vol. XXIII No.8*. 5 – 8.