A FUNGAL PATHOGEN OF LACE BUG AND LEAF EATING CATERPILLAR, TWO INSECT PESTS OF COCONUT PALM

By

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ABSTRACT

Lace bug, Stephanitis typica (D) and the leaf eating caterpillar, Opisina arenosella W. are two common pests in the coconut ecosystem. Lace bug is the vector of root (wilt) disease of coconut as it harbours and transmits the pathogenic "phytoplasma". Leaf eating caterpillar is an outbreak defoliator pest. During field collection of these insects, it was observed that in some samples there was green colour sporolation on the cadavers of these insects. From such specimens a fungus was isolated and purified. It was identified as Aspergillus and then confirmed as A. flavus Link. In the laboratory, this fungus was tested for pathogenecity on hosts by different methods of spore application. It was observed that 80% S. typica nymphs died within 3 days and 80-90% of the O. arenosella larvae were mycosed within 3-4 days. In this paper, we discuss the above aspects in detail.

INTRODUCTION

Coconut palm is infested by many pests, common among them being the lace bug, *Stephanitis typica* D. (Tingide: Heteropera) and the leaf eating caterpillar, *Opisina arenosella* W. (Crytophasidae: Lepidoptera). Though lace bug inflicts only minor damage by sucking the sap of the leaves, its roles as a vector of the root (wilt disease caused by phytoplasma makes it prominent among the insects associated with coconut palm (Shanta *et al*., 1964; Mathen *et al*., 1990). This debilitating disease results in an estimated loss of approximately 968 million nuts annually (Anon., 1985). Leaf eating caterpillar, on the other hand, is a major outbreak pest. The larvae feed voraciously on the chlorophyllous tissue by remaining hidden on the underside of the leaf in silken galleries, studded with leaf bits and its own excreta.

Control of the lace bug by chemical means is not a feasible proposal for the amelioration of the disease (Anon., 1997) but recent research findings indicate the possibility of using predators for the biosuppression of this pest (Sathiamma *et al.,* 1996). In the case of leaf eating caterpillar, management by releasing indigenous parasitoids is well established (Cock and Perera, 19878; Sathiamma *et al.,* 1996). There are also record of microbial pathogens like *Serratia marcescens*, *Bacillus thuringiencsis, Aspergillus flavus, Plaecilomyces farinousus* and a Nuclear Polyhedrosis Virus (Nirula 1956 a, b. Oblisamy *et al.,* 1969, Muthukrishnan and Rangarajan, 1974; Philip *et al*., (1982). However, information on the use of microbial control agents against these two pests is scarce.

While collecting the nymph and adults of *Stephanitis* and larval stages of *Opisina* from the farmers' fields in Quilon District of Kerala, we came across some fungal infected species of these two pests. On plating them on Potato Dextrose Agar (PDA) three fungal diseases from *Opisina* and two from *Stephanitis* were isolated. Pathogenicity trials with these fungi on the respective hosts were undertaken in the laboratory. As an outcome of the studies we report *Aspergillus flavus* Link as a potential biocontrol agent of *S. typica* and *O. arenosella.*

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MATERIALS AND METHODS

Isolation of fungi: the fungi infected cadavers of both the pests collected from field were plated on PDA plates. The mycelium and spores of different fungal species were isolated, purified and maintained on sterile PDA slants.

Inoculum preparation: the fungal spores were incubated on PDA plates at 30°C for 48-72 hours which resulted in production of fungal lawn with heavy sporulation. Spore suspension was prepared by scraping off the spores into 10 ml of sterile water + 0.1 ml Tween 80. This was serially diluted 10 fold to give varying spore concentrations (10^2 to 10^8 spores/ml).

Bioassay: Lace bugs and leaf eating caterpillars of comparable size and age (III Instar nymphs of *Stephanitis* and III Instar of *Opisina*) were selected at random from the field collected-laboratory maintained cultures to keep the variation caused by differences in their life stage constant. Inoculation of the fungus was done by the following methods.

a) Crawl method: known number of nymphs of *Stephanitis* and larvae of *Opisina* were gently transferred from the coconut leaflets with fine camel hair brush (sterilized) to PDA plate with 3-days old sporulating culture of the test fungus and were left on the surface of the culture for 10 min. The hosts were then transferred individually to glass bioassay cells of 24 x 180 mm and 54 x 135 mm dimensions for *Stephanitis* and *Opisina* respectively. Appropriate controls were maintained by transferring the hosts to fresh leaf cuttings after allowing them to remain in sterilized petri plates for 10 min.

b) Spray method I: Different conidial concentrations were sprayed on fresh coconut leaf surface using a glass bottle atomizer after surface sterilizing the leaf with 70% ethanol. Approximately 3-4 ml of spore suspension was required to completely cover the standard leaf lamina surface (330 cm^2) used in this experiment. In control treatment, sterile water + Tween 80 mixture (100:1) was sprayed. The test insects were then transferred on to the sprayed leaflets.

c) Spray Method II : in this method the hosts were transferred to the fresh sterilized leaflets. After 24 hours they were sprayed with different fungal spore concentrations. This treatment stimulated the field situation as the pests had colonized the leaflets. Appropriate controls were also maintained.

For all the experiments, five replication sets were maintained. Each replication received 10 nymphs in case of *Stephanitis* and 5 larvae in case of *Opisina*. After introduction of insects in all the treatments in their respective bioassay cells, their mouths were plugged with loose cotton and the projecting end of the coconut leaflet was dipped into a beaker containing sterile water. This helped in maintaining the freshness of the leaflet provided as food and shelter for the test insects. The whole set up was placed in ambient temperature and mortality was recorded everyday over a 7 day bioassay period for *Stephanitis* and 10 day for *Opisina.* The dead insects resulting from various treatments were immediately plated on PDA plates to confirm the Koch postulates.

RESULTS AND DISCUSSION

The initial studies showed that one of the three fungi from *Opisina* and two from *Stephanitis* gave positive pathogenic result. These fungi grew luxuriantly on PDA plates. Covering the whole plate within 48 to 72 hours at ± 2°C. Both the fungi produced cream yellow mycelium with parrot green spores. Under light microscope, their lactophenol wet mounts showed typical characteristics of *Aspergillus* sp., with conidiophore, being upright, simple, terminating in a globose or clavate bearing phaliades at the apex, conidia single celled globose and attached in basipetal chains. They were later confirmed as *Apergillus flavus* Link (Indian Type Culture Collection ITCC Number 4793). The isolates from *Stephanitis* and *Opisina* were designated AF1 and AF2, respectively.

Table 1 presents the period of occurrence of the natural infection by AF1 and AF2 in the field on the two pests. *Stephanitis* was mycosed when the temperature was around 25-27°C and relative humidity more than 80%. In the case of *Opisina,* the galleries served as trapping nets for the AF2 spores and the detritus enmeshed in it provided nutrient for the fungus survival. Moreover, continuous and vigorous feeding the cholorophyllous tissue by the larvae kept the gallery area sufficiently moist to give proper environment for the fungal growth. The *Opisina* larvae being proteinecious and larger in size as compared to *Stephanitis* helped the fungus to pathogenize more easily. Hence, natural infection of the *Opisina* by AF2 was observed almost during most of the months.

The nymphs and adult stages of *Stephanitis* were susceptible to this fungus; whereas, all the developmental stages of *Opisina* were infected. Fungal presence was detected in various body parts of both the pests. (Table 2). Only the head of *Opisina* larvae remained resistant because of its hard skeletal nature.

In the laboratory bioassay where nymphs of lace bug were allowed to crawl on the lawn of AF1 spores, initially the insects became sluggish and showed no response to external stimulus. Later they died anchored to the spot on the leaf. 100% mortality was observed by the 7th day of fungal infection by which time the insect body was completely covered with fungal mycelia and spores (Table 3.) The spray method 1 also resulted in 100% mortality with three concentrations (10^4, 10^5 and 10^6 spores/ml). The optimum lethal dose was found to be approximately 10^6 spores/ml, which killed 80% nymphs by 3rd day and 100% by the 5th day. In the other two spore concentrations (10^4 and 10^5), mycosis of all the test insects occurred within one week time (Table 3). In the spray method II, mortality was quicker because spores were deposited directly on the host body as well as the leaf surface. 100% mortality was achieved in all the three spore concentrations, again the optimum lethal dose proved to be 106 spores/ml (Table 3). Higher spore concentrations did not enhance the killing efficiency. In the case of adult lace bug 10^7 spores /ml was needed to produce cent percent killing by the 8th day. Almost 75% of the adults died by 5th day (data not shown). These results are in agreement with the findings of Sathiamma and Saraswathi (1990) who reported that *A. flavus* causes mortality of 22.5-47.5% within 24-48 hours of appearance of inactivity in cashew tea mosquito bug, another heteropteran pest. *Proutista moesta,* another confirmed vector of root (wilt) disease of coconut is also pathogenised by the same fungus. 62.5% death was observed by the 4th day after application of *A. flavus* (Anon., 1997).

The fungal infection by AF2 on *Opisina* larvae caused inactivity and reduced feeding rate which was obvious from the feeding marks on the coconut leaves in comparison with the control treatments. Nearly 90% *Opisina* larvae died in 3-4 days in the crawl experiment. Approximately 10^5 spores/ml proved to be optimum lethal dose for achieving 72% mortality of *Opisina* by 3rd day in the spray method I, whereas the next higher spore concentration (10^6 spores/ml) gave similar mortality rate by the 4th day in the spray method II (Table 4). This could possibly be due to the protection offered by the galleries made by the larvae.

In the control also, mortality was observed in case of both the insects. This could have been possibly due to improper handling of these delicate pests while transforming or due to desiccation. This is supported by the fact that when these dead test insects were plated on PDA, growth of the fungal pathogen was absent. On the other hand, we could isolate *Aspergillus flavus (*AF1 and 2) in case of other treatments thus confirming Koch postulates.

The probable mode of killing by *A. flavus* is by invasion of the host through respiratory orifices, wound and by ingestion, which results in damage through mycotoxin production, histolysis, physical damage and blockage of the alimentary canal from mycelial growth (Shamila Kalia *et al*., 1996).

CONCLUSION

This is the first report of the fungal pathogen *Aspergillus flavus* on *S. typica* from Kerala, India. The only report of *A. flavus* on *Opisina* is from Tamil Nadu, India (Oblisamy *et al.,* 1969); Muthurishnan and Rangarajan, 1974) mentioning 90% mortality in the laboratory trials. There is no previous report of mycosis on *Opisina* by the same mold from Kerala. Experiments on the cross infectivity of AF1 and AF2 on the given pests and their aflatoxigenic nature is being undertaken. If these fungal strains prove to be non-toxigenic to human beings and non-target organisms as many are reported (Wicklow *et al*., 1988), the experiments will be extended to field trials.

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Table 1. Record of natural infection by Apergillus flavus in farmers' fields on Stephanitis typica and Opisina arenosella during different months.

Year 1997	*S. typica*	*O. arenosella*
January	-	+
February	-	-
March	-	-
April	-	+
May	-	+
June	+	+
July	+	+
August	+	+
September	+	+
October	+	+
November	-	+
December	-	+

+ - infection observed - no infection

Table 2. Presence of Aspergillus flavus infection from various body parts of the pests

PEST	Body Surface	Head		Thorax		Abdomen	
		Surface	Inside	Surface	In-side	Surface	In-side
S. typica							
a)Nymph	+	+	+	+	+	+	+
b) Adult	+	+	+	+	+	+	+
O.arenosella							
a) karvae	+	-	-	+	+	+	+
b) adult	+	+	+	+	+	+	+

+ = Mycelia present
- = Mycelia absent

Table 3: Mortality observed in Stephanitis typica nymphs after Aspergillus flavus AF1 inoculation

Treatments	Spore concⁿ (spores/ml)	Mortality (in days)							Mortality	
		1	2	3	4	5	6	7	%	Total days
Crawl method										
a) on AF1 spore lawn	∞	-	-	5	25	5	-	15	100	7
b) Control	0	-	1	-	1	-	1	-	6	6
Spray method–1										
a) On leaf surface	10^4	3	7	10	8	3	8	11	100	7
	10^5	-	10	9	13	7	6	5	100	7
	10^6	11	8	22	5	4	-	-	100	5
b) Control	0	-	-	-	-	-	-	-	0	-
Spray method 2										
a) on leaf surface	10^4	7	14	14	2	8	5	-	100	6
	10^5	10	10	8	6	16	-	-	100	5
	10^6	16	15	10	8	1	-	-	100	5
b) Control	0	-	2	-	2	-	-	-	8	4

Table 4. Mortality observed in *Opisina arenosella* larvae after *Aspergillus flavus* AF2 inoculation

Treatments	Spore concⁿ (spores/ml)	Mortality (in days)								Mortality	
		1	2	3	4	5	6	7	8	%	Total days
Crawl method											
c) On AF1 spore lawn	∞	4	4	8	3	6	-	-	-	100	5
d) Control	0	-	-	-	-	2	1	-		8	5
Spray method – 1											
b) On leaf surface	10^4	-	3	5	5	3	3	4	2-	100	8
	10^5	5	7	6	4	3	-	-	-	100	5
	10^6	7	3	6	4	5	-	-	-	100	5
b) Control	0	-	1	1	-	1	-	-		12	5
Spray method 2											
a) on leaf surface	10^4	-	-	-	2	4	2	6	-	56	7
	10^5	-	3	2	6	9	3	2	-	100	7
	10^6	-	4	6	8	5	2	-	-	100	6
b) Control	0	1	-	-	2	-	-	-	-	8	4