Studies on cross-infectivity with entomofungal islolates of *Aspergillus flavus* Link. against *Stephanitis typica* D. and *Opisina arenosella* W., two pests of coconut

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Aspergillus flavus is a ubiquitous saprophytic mould which inhabits the soil. This fungus infests a number of food crops, most notably corn and peanuts (Diener and Davis, 1966 and 1983) and produces toxic metabolites foremost among them being aflatoxins. It is also well dcoumentd that the fungus is highly entomopathogenic parasitizing a number of insect pests (*Table 1*).

Apart from the references cited, we also have reported field infection of *Stephanitis typica* and *Opisina arenosella* by *Aspergillus flavus* Link. (Gopal *et al.*, in press). Two entomopathogenic fungi were isloated from naturally colonized cadavers of *Opisina* and *Stephanitis* separately. These fungi were able to cause 80-90% mortality in their respective hosts (80% mortality in *Stephanitis typica* nymphs and 80-90% mortality in*Opisina arenosella* larvae) within 3-4 days under laboratory conditions. The mortality was cent per cent when the experiment was continued for 2-3 days more. The ideal spore concentration for achieving 100% mortality in short period (5-6 days) was 10^6 spores/ml. Both the fungi were characterized microscopically and identified as *Aspergillus flavus,* the one from *Stephanitis* designated as AF1 and from *Opisina* as AF2. The conidiophores of these fungi were upright, simple, terminating in a globose or clavate swelling bearing phialides at the apex; conidia were single celled, globose attached in basipetal chains, The identification was later confirmed by Indian Type Culture Collection, I.A.R.I., New Delhi (ITCC 4793).

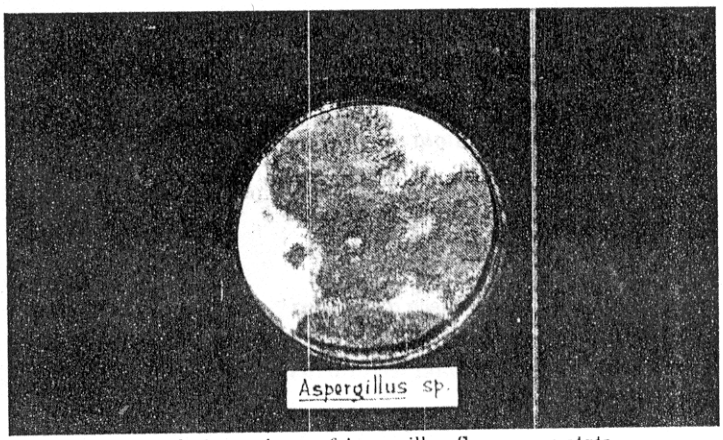
We report here the outcome of a study conducted to find out whether *A. flavus* AF1 originally islolated from *Stephanitis* could parasitize *Opisina* and vice versa.

The pure cultures of the two fungi were grown on potato dextrose agar medium plates at 30^0C for 48-72 hr. till they sporulated heavily. These were then tested for pathogenicity against larvae of *Opisina* and nymphs of *Stephanitis* by two methods *viz.*, crawling and spraying.

In crawl method, healthy *Stephanitis* nymphs and *Opisina* larvae were allowed to crawl over the dense sporulating culture of AF2 and AF1 respectively for 10 minutes and then placed in glass test tubes containing fresh coconut leaflets. In the case of control, healthy larvae and nymphs were allowed to crawl in sterilized petriplates and then transferred to test tubes with coconut leaflets.

In spray method, the fungal inocula were prepared by scraping the spores of AF1 and AF2 into 10 ml of sterile water + 0.1 ml Tween 80. The spore load was adjusted to 10^6 spores/ml. These spore suspensions were sprayed on fresh coconut leaflets after surface sterilizing them with 70% ethanol. By doing so approximately 10^{21} fungal spores were deposited on about 330 sq. cm. leaf lamina. These leaflets were introduced into test tubes and larvae and nymphs were allowed to feed on them separately. Surface-sterilized, unsprayed leaflets were used for control. Experiment was replicated five times with five nymphs and five larvae in each replication. Observations were recorded on the disease development and mortality of the test insects.

Within 24 hours after the treatments were imposed, the larvae and nymphs became lethargic, refused



Sporulating culture of Aspergillus flavus *on potato dextrose agar (PDA) medium*

Table 1 : Record of Aspergillus flavus from some important pests

S. No.	Pest	Common name	Crop	Country	Effect on pest	Reference
	Insects :					
1.	*Solenopsis spp.*	fire ant	--	U.S.A.	Pathogenic	Sikorowski *et al.* (1973)
2.	*Atta texana*	fire ant	--	U.S.A.	Pathogenic	Steinhaus & Marsh (1967)
3.	*Opisina arenosella*	leaf eating caterpillar	Coconut	India	90% mortality	Oblisami *et al.* (1969), Muthukrishnan and Rangarajan (1974)
4.	*Orthaga euadrusalis*	leaf webber	Mango	India	100% mortality	Srivastava and Tandon (1980)
5.	*Calliptamus italicus*	locust	Various crops	U.S.S.R	>60% mortality	Nurzhanov and Lachiniskil (1987)
6.	*Malacosoma indica*	tent caterpillar	Fruit trees	India	Pathogenic	Joshi and Agarwal (1987)
7.	*Scirpophaga excerptalis*	pyralid insect pest	Sugarcane	China	Pathogenic	Fang and Tan (1986)
8.	*Cossus cadambae*	carpenter worm	Teak	India	57% mortality	Mathew and Mohamed Ali (1987)
9.	*Caprophilus* hemipterus	maize insect	Maize	U.S.A.	Toxic	Wicklow *et al.* (1988)
10.	*Bombyx mori*	silkworm	Mulberry	India	Pathogenic	*Patil (1989)*
11.	*Dysdercus cingulatus*	cotton stainer	Cotton	India	100% mortality	Kshemkalyani *et al.* (1989)
12.	*Epilachna vigintioctopunctata*	Epilachna beetle	Potato	India	Toxic	Rajagopal and Trivedi (1989)
13.	*Heliothis zea*	Corn earworm	Maize	U.S.A.	Toxic	Wicklow and Dowd (1989)
14.	*Spodoptera frugiperda*	Fall army worm	Maize	U.S.A.	Toxic	Wicklow and Dowd (1989)
15.	*Saccharicoccus sacchari*	Mealybug	Sugarcane	Cuba	Toxic	Martinez and Bravo (1989)
16.	*Plancococcus citri*	Mealybug	Sugarcane	Cuba	Pathogenic	Martinez and Bravo (1989)
17.	*Pogonomyrmex rugosus*	Queen harvester ant	-	Arizona	Pathogenic	Gilliam *et al.* (1990)
18.	*Helopeltis antonii*	Tea mosquito bug	Cashew	India	22.5-47.5% mortality	Sathiamma and Saraswathy (1990)
19.	*Latoia viridissima*	Nettle caterpillar	Oil palm	Nigeria	60-80% mortality	Igbinosa (1992)
20.	*Oxya nitidula*	Grasshopper	Rice	India	23-94% mortality	Ambethgar and Kumaran (1998)
21.	*Proutista moesta*	Plant hopper	Coconut	India	62.5% mortality	Ponnamma *et al.* (Pers. Comm.)
22.	*Musca domestica*	Housefly	-	India	100% mortality in 21 days	Amonkar and Nair (1965)
23.	*Cimex lectularius*	bed bug	-	England	Pathogenic	Cockbain and Hastie (1961)
24.	*Noorda blitealis*	leaf caterpillar	Drumstick	India	70-80% mortality	Kalia *et al.* (1996)
	Nematode :					
25.	*Meloidgyne incognita*	Root knot nematode	Chickpea	India	Toxic	Siddiqui and Husain (1991 a & b)
	Fungus					
26.	*Macrophomina phaseolina*	fungus	Chickpea	India	Pathogenic	Siddiqui and Husain (1991 b)

MAYR by microorganisms. Labdev. *J. Sci. Tech.* 12 B : 106-108.

16. Nurzhanov A.A. and Lachiniski A. V. 1987. Entomopathogenic micro organisms of locusts in Uzbekistan. *In :* Saranchovye - ekologiya i mery bord'by (ed. M.S. Nikitina). All - Union Sci. - Res. Inst. Plant Protection, Leningrad, U.S.S.R pp. 62-69.

17. Oblisamy, G. Ramamoorthi, K. and Rangaswamy, G. 1969. Studies on the pathology of some crop pests of South India. Mysore. *J. Agric. Sci.* 3 : 86-98.

18. Patil, C. S. 1989. New record of fungal pathogen *Aspergillus flavus* Link on mulberry silkworm *Bombyx mori* L. from India. *Curr. Sci.* 58 (12) 638.

19. Rajagopal, D. and Trivedi, T. P. 1989. Status, bioecology and management of epilachna beetle, *Epilachna viginitoctopuntata* (Fab.) (Coleoptera : Coccinellidae) on potato in India : a review. Tropical Pest Management. 35 (4) : 410-413.

20. Sathiamma, B. and Saraswathy. N. 1990. Mycosis on tea mosquito *Helopeltis antonii,* S. *Indian J. Entomol.* 52 (3) : 516.

21. Siddiqui, Z. A and Husain, S. I. 1991. Studies on the bilogical control of root-knot nematode. *Curr. Nematol.* 2 (1) : 5-6.

22. Siddiqui, Z. A. and Husain, S. I. 1991. Control of *Meloidogyne incognita* and *Macrophomina phaseolina* on chickpea by fungal filtrates. *Pak. J. Nematol.* 9 (2) : 131-137.

23. Sikorowski, P. P. Norment, B. R. and Broome, J. R. 1973. *Miss. Agric. Forest. Exp. Sta. Inf. Sheet* 1212.

24. Srivastava, R. P., Tandon, P. L. 1980. Studies of insect pathogens on mango leaf webber, *Orthaga euadrusalis* Walker (Lepidoptera : Pyralidae). *Entomon.* 5 (3) : 219-221.

25. Steinhaus, E.A. and Marsh, G.A. 1967. Previously unreported accessions for diagnosis and new record. *J. Invertebrate Pathol.* 9 : 436-438.

26. Wicklow, D.T. and Dowd, P.F. 1989. Entomotoxigenic potential of wild and domesticated yellow-green aspergilli : toxicity of corn earworm and fall armyworm larvae. *Mycologia.* 81 (4) : 561-566.

27. Wicklow, D.T. Dowd, P.F. Tepaske, M. R. and Glober, J. B. 1988. Sclerotial metabolites of *Aspergillus flavus* toxic to a detritivorous maize insect (*Carpophilus hemipterus,* Nitidulidae). *Trans. British Mycol. Soc.* 91 (3) : 433 - 438.

Table 2 : Effect of A-flavus isolates AF1 and AF2 on Opisina larvae and Stephanitis nymphs, respectively

Treatments	% mortality*	
	after 2 days	after 5 days
Opisina arenosella		
1. Control	0	0
2. Spray method	44	100
	32	100
Stephanitis typica		
1. Control	0	0
2. Crawl method	40	100
3. Spray method	28	100

**Mean of five replications for a total of 25 insects*

food indicating the loss of appetite and did not respond to external stimuli. By 48 hr, a few larvae and nymphs died of fungal parasitism. The dead larvae and nymphs became mummified and brittle. The fungus grew over the body of the pests and microscopic examination revealed the presence of spores and mycelia in abundance. 100% mortality of *Stephanitis* and *Opisina* was achieved within 5 days in the case of both the methods. The fungus was reisolated from all the infected larvae and nymphs.

A. flavus kills the host either by invasion through respiratory orifices or wounds or by ingestion. Inside the host body it produces mycotoxins and results in hydrolysis and physical damage and probably blockage of the alimentary canal from mycelial growth (Kalia *et. al.*, 1996).

The results obtained in the present study are comparable to our earlier findings where AF1 was tested on *Stephanitis* and AF2 was tested on *Opisina*. It is possible that the same strain of *Aspergillus flavus* is parasitizing these pests in natural field conditions. Hence, we conclude that either of the *Aspergillus flavus* isolate could be effectively used for field control of *Stephanitis typica* and *Opisina arenosella,* two important pests of coconut palm.

References

1. Ambethgar V. and Kumaran K. 1998. Biological suppression of grasshoppers. Science Express Supplement of The Indian Express, Tuesday, 17 November, pp. 4.

2. Amonkar S. V. and Nair K. K. 1965. Pathogenicity of *Aspergillus flavus* link to *Musca domestica nebulo* fabricius. *J. Invertebrate Pathol.* 7 : 513-514.

3. Cockbain A. J. and Hastie A.C. 1961. Susceptibility of the bed bug, *Cimex lectularius* Linnaeus, to *Aspergillus flavus* Link. *J. Insect Pathol.* 3 : 93-102.

4. Diener U. L. and Davis N. D. 1966. Aflatoxin production by isolates of *Aspergillus flavus*. Phytopathology. 56 : 1390-1399.

5. Diener U. L. and Davis N. D. 1983. Aflatoxins in corn. *In* : Xenobiotics in foods and feeds. Symposium Series 234. (eds. J. W. Finley and D. E. Schwass). American Chem. Soc., Washington D. C., pp. 242-269.

6. Fang H. M. and Tan S. M. 1986. Pathogenic fungi of several insect pests on sugarcane. *Microbiology* 13 (3) : 97-100.

7. Gilliam, M., Spangler, H. G., Prest D. B. and Lorenz B. J. 1990. Mycosis of a queen harvester ant, *Pogonomyrmex rugosus. J. Invertebrate Pathol.* **55** (3) : 437-438.

8. Gopal, Murali, Gupta, Alka, Sathiamma B., Mohan, Chandrika, Nair, K. R. Chandramohanan, Soniya V. P. 2000. A fungal pathogen of lace bug and leaf eating caterpillar, two insect pests of coconut palm. *CORD* (in press).

9. Igbinosa I. B. 1992. Field and laboratory techniques for assessing infestations of the nettle caterpillar, *Latoia viridissima* Holland (Lepidoptera : Limacodidae). Insect Sci. and its application 13 (3) : 389-398.

10. Joshi K.C. and Agarwal S. B. 1987. Bionomics of the tent caterpillar, *Malacosoma indica* Wlk. *Bulletin of Entomol.* 28 (1) 1-11.

11. Kalia Shamila, Harsh N.S.K. and Joshi K. C. 1996. Pathogenic fungus of *Noorda blitealis* Walker (Lepidoptera-Pyralidae), a major pest of *Moringa oleifera* Tanss. *Indian J. Forestry.* 19 (1) : 94-96.

12. Kshemkalyani S.B., Enliley M.R., Hakim S.S.Y. and Prabhakar J.D. 1990. Pathogenic fungus of *Dysdercus cingulatus. Indian J. Entomol.* 51 (3) 322-324.

13. Martinez A. M. and Bravo N. 1989. *Aspergillus flavus Dirk.*, control natural de diferentes especies de chinches harinosas. Revista de Proteccion Vegetal. 4 (1) : 83-84.

14. Mathew G. and Mohamed Ali M. I. 1987. Microbial pathogens causing mortality in the carpenter worm, *Cossus cadambae* Moore (Lepidoptera, Cossidae), a pest of teak (*Tectona grandis* Lin. f.) in Kerala (India). *J. Tropical Forestry.* 3 (4) : 349-351.

15. Muthukrishnan P. and Rangarajan M. 1974,. Laboratory studies on the control of black headed caterpillar, *Nephantis serinopa*