Mycopathogens Associated with Pests of Chilli and their Pathogenicity Against Thrips (*Scirtothrips dorsalis*) and Mites (*Polyphagotarsonemus latus*)

G. Mikunthan and M. Manjunatha[1]

Department of Agricultural Biology, Faculty of Agriculture
University of Jaffna
Jaffna, Sri Lanka

ABSTRACT. *Mycopathogens are often found to have close associations with pests of crops. An attempt was made to understand the association of mycopathogens with the major pests of chilli in chilli growing fields of Karnataka State of India. Soil samples were collected from the chilli fields representing ten agro-ecological zones of the state. Larvae of Tribolium castaneum and pupae of Scirtothrips dorsalis were used as soil baits to isolate fungal pathogens present in soil. Foliage sampling was also done to isolate diseased cadavers of pests. Fungi associated with the diseased cadavers of insects and mites were isolated on to different culture media. Nineteen fungal species were found associated with the pests of chilli. Thirteen fungal species were isolated from soil using larvae of T. castaneum and G. melonella, respectively as soil baits. Foliage sampling recovered six fungi namely, Fusarium semitectum, Fusarium sp. isolate GM 15, Neozygites floridana from S. dorsalis and Polyphagoratarsonemus latus, Nomuraea rileyi from larvae of S. litura. Among the fungal cultures Fusarium spp. was recovered predominantly from the soil as well as disease cadavers obtained from foliage. Hence, its pathogenicity was studied against S. dorsalis and P. latus in the laboratory. On larvae of S. dorsalis the LC_{50} of F. semitectum and Fusarium spp. isolate GM15 were $2.7x10^7$ spores/ml and $7.6x10^7$ spores/ml, respectively. On active stages of P. latus LC_{50} of F. semitectum and Fusarium spp. isolate GM15 were $7x10^6$ spores/ml and 5.3x107 spores/ml, respectively. Hence, the fungus, Fusarium spp. can be used as a potential biocontrol agent to control thrips and mite pests in an integrated chilli pest management program.*

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the important condiment-cum-vegetable crops, used as a major ingredient in making curry powder and in culinary preparations, mostly in southern states of India and Sri Lanka. Chilli is one of the major remunerative cash crops grown throughout the year in Karnataka State of India. The State ranked third in crop coverage and fifth in production with a measurably poor average of 873 kg/ha, which is 25% less than the all India average of 1162 kg/ha (Anon, 2001). Chilli suffers from characteristic leaf curl symptoms, which affect the pod yield of the chilli crop. The yield loss has been attributed due to the attack of a tarsonemid mite (*Polyphagotarsonemus latus* [Banks]) (Kulkarni, 1922) and thrips (*Scirtothrips dorsalis* Hood) (Ayyar, 1932; Peiris, 1953) or both (Puttarudriah, 1959). The mite is most frequently encountered and widely distributed in tropics and subtropics.

[1] Department of Entomology, College of Agriculture, University of Agricultural Sciences, Shimoga, Karnataka, India.

Besides chilli, it attacks other economic plants like citrus, cotton, jute, potato, tea and grapevine (Jeppson *et al*., 1975). On the other hand, *S. dorsalis* is a polyphagous thrips causing damage to an extremely wide range of hosts (Ananthakrishnan, 1969) and is one of the serious pests of chilli in India (Aiyadurai, 1966) and Sri Lanka (Peiris, 1953). Complete failures of chilli crop due to severe attack of mite (Kulkarni, 1922) and 25-50 % loss in yield due to thrips (Butani, 1976) have been reported. Reddy and Puttaswamy (1983) reported the infestation of these two pests on the chilli plants ranging from 23 to 87 % in Karnataka.

Biological control methods are now receiving more attention, since these alternative methods, compared to chemical control, are energy saving, non-polluting, ecologically sound and sustainable. Microbial insecticides are already used in agricultural systems and knowledge of the biology and ecology of the diseases has greatly increased in recent years. Insect pathogens are safe to humans and other non-target species, hence they can be used harmoniously with other control tactics.

To develop economically feasible management strategy and to reduce unwarranted pesticides load in the chilli ecosystem, a sound knowledge of the natural enemy complex of the target pests is very essential. Among the natural enemy complex, the pathogens regulating the population of the pests of chilli have not been extensively studied. An attempt was made to understand the mycopathogens naturally associated with the pests of chilli and to test their pathogenicity against thrips and mite, which are the prime causes of chilli leaf curl.

MATERIALS AND METHODS

A roving survey was conducted in all chilli growing fields of Karnataka State and soil and foliage samples were collected from locations representing all the ten agro-ecological zones of Karnataka.

Soil and foliage sampling

Soil samples were taken from the base of chilli plants up to a depth of 10 cm with a soil auger (7.5 cm diameter) as described by Zimmerman (1986) and Parker *et al*. (1996). Six such samples were collected from each field and placed separately in plastic bags and labeled. Ten such fields were sampled in a location. These samples were brought to the laboratory. All 60 samples collected from ten fields from each location were pooled, mixed thoroughly, sub samples were taken and kept in refrigerator (4^0C) until use. Leaves with large populations of thrips and mites were randomly collected and placed in polythene bags.

Isolation of fungi

The bait method described by Parker *et al.* (1996) was followed to isolate fungi from soils collected from chilli fields. Larvae of the storage beetle (*Tribolium castaneum*, Coleoptera: Tenebrionidae), larvae of wax moth (*Galleria melonella*, Lepidoptera: Galleridae) and pupae of thrips (*Scirtothrips dorsalis,* Thysanoptera: Thripidae) were used as soil baits. Foliage sampling was also done to search for any diseased cadavers of thrips, mite, whitefly, and aphids. Larva exhibiting symptoms of infection was surface-sterilized in 5% sodium hypochlorite (NaOCl) for 3-5 min., rinsed thrice in sterile distilled water, placed on Saboraud medium, and held at 25^0C for at least ten days. After fungal growth on the medium was evident, standard procedures were followed for isolation of each strain.

Culturing and identification of fungi

The fungi isolated from soil baiting and foliage sampling methods were cultured, and their growth characteristics in Sabouraud Dextrose agar supplemented with 0.25% Yeast Extract (SDYE) and Potato Dextrose Agar (PDA) were studied. To enhance sporulation, *Fusarium* cultures were grown in Synthetischer Nährstoffärmer Agar (SNA) medium. Preliminary identification of the isolated entomopathogenic fungi was done by studying their mycelial growth, spore characters and pigmentation on these media. In case of *Neozygites* spp. diseased cadavers were surface sterilized in 3.0% NaOCl followed by subsequent rinsing in sterile distilled water several times and placed on PDA and SDYE media. Dr. R.A. Humber of USDA-ARSEF, who confirmed its identity.

Bioassay to prove fungal pathogenicity

Bioassays were conducted to select the potential fungi pathogenic to thrips and mite following the description of Butt and Goettel (2000). Fungal cultures were grown in SDYE and incubated at 25 ± 2^0C for 10 to 14 days. Conidiospores were harvested from the plates by washing with 0.02% Tween 100. Spore concentration was determined using a double ruled Neubauer's haemocytometer after seven serial dilutions under phase contrast microscope (Hall, 1976).

On thrips

The method used by Parker *et al.* (1996) was followed to the fungal bioassay studies on thrips. Conidial suspension of the fungi was applied to two 7 cm diameter sterile (whatman No1) filter papers (600 µl/paper) and 10 s instar thrips were placed on one of the papers. Second paper was then placed over the top of the thrips and the "paper-thrips-paper sandwich" was kept in a ventilated Petri dish in such a way that the edges of the filter papers were closely attached. Control assays were conducted using sterile distilled water. These petri dishes were then kept at 25 ± 1^0C and 79±6% RH. Three replicates were maintained. Mortality was assessed after 5 days. Dead larvae of thrips were first surface sterilized using 5.0% NaOCl for three minutes and were rinsed in sterile distilled water thrice. The specimens were then placed on glass slides and incubated under conditions of high humidity. Fungal infection on thrips was confirmed after examining them under a phase contrast microscope.

On mites

The "Detached-leaf bioassay method" used by Yokomi and Gottwald (1988) was followed to conduct bioassay on mites. Fourth tender leaf of chilli infested heavily with mite was detached with the petiole and kept in a plastic vial. The active stages of mites present on both sides of the leaf was counted using stereobinocular microscope (10x). Spore suspensions were sprayed on the leaves using an atomizer and leaves were air dried for few seconds in a laminar flow hood. The set up was incubated at 26 ± 2^0C and mortality was recorded daily. Three replicates were maintained. Since *Fusarium semitectum* and *Fusarium* spp. isolate GM15 had caused higher mortality on thrips in the initial studies, these isolates were selected and their efficacy was tested on thrips at varying spore concentrations as suggested by Parker *et al*. (1996). Serial dilution was done to prepare spore concentrations of *F. semitectum* at 1×10^4 to 10^9 spores/ml and *Fusarium* spp. isolate GM15 at 1×10^4 to 10^9 spores/ml. Three replications were used. Mortality of the thrips was

recorded daily. Mortality of different treatments was adjusted with that of control using Abbott's formula. The LC_{50} values of the *Fusarium* isolates were calculated using Log-probit analysis.

Small scale efficacy trials

Based on the results obtained from the pathogenicity tests the *F. semitectum* was found more virulent than the isolate GM15 against thrips and mites. Following the method described by Parker *et al.* (1996) *F. semitectum* was selected to study its efficacy against these two organisms in a small-scale trial under laboratory conditions.

The data were analyzed using probit-log dose transformation and the results were interpreted. The LC_{50} and LC_{90} values were computed from the Log-probit analysis. LT_{50} values for the different spore concentrations 3.4×10^6 to 0^9 spores/ml were also computed using log-time transformation.

Efficacy trial on *Scirtothrips dorsalis*

Chilli plants (var. *Byadgi*) were raised in the nursery in a confined and isolated place to avoid infestation of any other insect or mite pests and one month old seedlings were transplanted in to plastic cups filled with equal proportion of sand and vermicompost mixture. Seedlings were left to stabilize in the cups and the cups with seedlings were confined in a rearing cage. About 150 newly emerged adult thrips were collected in an aspirator from the stock cultures and were released into the cage. The setup was left for three days to enable the adults to lay eggs. Then the plants were removed from the cages and adult thrips remaining on the plants were removed using a camel hair brush (0 number series).

The plants were left for 4-5 days to allow the eggs to hatch, and four days later adequate number of second instar larvae were found on the foliage of the chilli plants. The number of second instar larvae present on the leaves was counted. These plants were removed from the cages and tagged. Different spore concentrations *viz*; 3.4×10^4 to 10^9 spores/ml of the *F. semitectum* were prepared using serial dilution and sprayed. A control was kept by spraying equal amount of water. The treatments were replicated thrice. Mortality was recorded with the help of a hand lens (10x). Infection on mummified thrips was confirmed using stereo binocular microscope.

Efficacy trial on *Polyphagotarsonemus latus*

Mite infested chilli plants were obtained from the rearing unit. The plastic cups with chilli seedlings were kept in the mite rearing cages for ten days to enable build up of enough number of mites on the leaves. These chilli plants were removed from the cages and number of active stages of mites present in the leaves was recorded. Different spore concentrations *viz*; 3.4×10^4 to 10^9 spores/ml of the *F. semitectum* used against thrips were tested against mites. Equal volume of water was sprayed in one set and kept as control. Three replications were maintained. Mortality of mites was recorded daily by examining the mites using stereo binocular microscope.

RESULTS AND DISCUSSION

Soil baiting with larvae of both *T. cataneum* and *G. melonella* yielded 13 fungal isolates representing six genera from ten agro-ecological zones of Karnataka (Table 1). The fungi recovered were unevenly distributed among the agro-ecological zones. No fungus was recovered from Northeast and Northern dry zones and North-east transitional and coastal zones whereas 85% of fungal isolates were obtained from Central, Eastern, Southern dry zones and Southern and Northern transitional zones. Equal proportion of *Fusarium* spp. (35%) and *Aspergillus* spp. (35%) were obtained. Among the four isolates of *Fusarium*, three were *F. oxysporum* Schlecht, and one was *F. sporotrichioides* Sherb. Many workers supported the erratic presence of entomopathogenic fungi in soil. Low moisture and erratic presence of fungi in the soil samples might be the cause for the low recovery of the fungi. Draganova (1999) also able to collect 46 isolates of *B. bassiana* from 60 isolates collected and reported that *B. bassiana* was the most frequently occurring (73%) entomopathogenic fungus in dead insects and mites in Bulgaria.

Foliage sampling

Nomuraea relayii was isolated from *Spodoptera litura. F. semitectum* (ARSEF 7233) was recovered from *S. dorsalis* (Plate 1)*, P. latus,* and *Aphis gossypi. Fusarium* spp. isolate GM15 (ARSEF 7381) (Plate 2), *Penicillium* spp. and *Neozygetes floridana* were isolated from *S. dorsalis* (Table 2). Among the fungal cultures *Fusarium* spp. was recovered predominantly from the soil as well as foliage. Existence of entomopathogenic *Fusarium* spp. has been reported by many workers during their exploration (Ganassi *et al*., 2000; Cozzi *et al*., 2002).

Table 1. Fungi isolated using soil bait method.

Agro-ecological zones of Karnataka	Fungi isolated
Northeast transitional zone	Nil
Northeast dry zone	Nil
Nothern dry zone	*Aspergillus niger*
Central dry zone	*A. niger* *Cunninghamella elegans*
Eastern dry zone	*Aspergillus niger* *Fusarium oxysporum*
Southern dry zone	*F. oxysporum*
Southern transitional zone	*A. niger* *F. sporotrichoides* *F. oxysporum* *A. nidulens*
Northern transitional zone	*Trichoderma harzianum*
Hilly zone	*F. oxsporum*
Coastal zone	*A. niger*

Parker *et al*. (1996) reported that *Metarhizium anisopliae* var. *anisopliae* and *Fusarium* spp. were recovered most frequently and accounted for 32.4 and 31.4% of the isolates collected, respectively. Draganova (1999) also reported the natural occurrence of entomopathogenic *Fusarium* spp. in Bulgaria. In another instant, *Fusarium* spp. was isolated from unsterilized live eriophyid mite, *Aceria guerreronis* damaging coconut in Kerala, India (Gopal *et al*., 2002). Parker *et al*. (1996) opined that the *Fusarium* isolate, though classified as weak entomopathogen, should be bio assayed for pathogenicity against thrips. This has given an insight in the present investigations to explore the efficacy of *Fusarium* isolates against thrips and mite.

Plate 1. Larvae of *S. dorsalis infected by Fusarium semitectum.*

Plate 2. Mycosis of *Fusarium* isolate GM15 on adult *S. dorsalis.*

Parker *et al*. (1996) reported that *M. anisopliae* var. *anisopliae* and *Fusarium* spp. were recovered most frequently and accounted for 32.4 and 31.4% of the isolates collected, respectively. Draganova (1999) also reported the natural occurrence of entomopathogenic *Fusarium* spp. in Bulgaria. In another instant, *Fusarium* spp. was isolated from unsterilized live eriophyid mite, *A. guerreronis* damaging coconut in Kerala, India (Gopal *et al*., 2002). Parker *et al*. (1996) opined that the *Fusarium* isolate, though classified as weak entomopathogen, should be bio assayed for pathogenicity against thrips. This has given an insight in the present investigations to explore the efficacy of *Fusarium* isolates against thrips and mite.

Table 2. Characteristics of fungi isolated from pests of chilli.

Fungi isolated	Characteristics of mycelia	Characters of spores/ conidia
Nomureae rileyi	White	Conidia dark green, round
Neozygites floridana	Grey colored	Conidia elongate, pear shape
Fusarium semitectum	White without pinkish pigmentation	Macro conidia with 5 septate, single cell micro conidia and many chlamydospores
Fusarium spp. isolate GM 15	White with pinkish pigmentation	Macro conidia 5 septate, micro conidia single celled and many chlamydospores
Aspergillus spp.	White	Conidia initially yellowish and later light green, round
Penicillium spp.	White	Dark green spores, round shape
Trichoderma spp.	White	Olive green conidia

Pathogenicity of mycopathogens on thrips and mite

On *Scirtothrips dorsalis*

The bioassay conducted to screen the fungal isolates against *S. dorsalis* revealed that the two *Fusarium* isolates caused 86 and 76% mortality, respectively while *N. floridana* caused 82% mortality. *A. niger* and *M. anisopliae* caused 33 and 13% mortality, respectively. *F. semitectum* and *Fusarium* spp. isolate GM15 at 2.1 x 10^8 and 2.1 x 10^9 spores/ml caused 63 and 76% mortality of larvae, respectively and were superior to the remaining spore concentrations of the mycopathogen. The LC_{50} and LC_{90} values were 2.7 x 10^7 and 2.96 x 10^{12} spores/ml. A maximum of 66% mortality of *S. dorsalis* was achieved with the *Fusarium* spp. isolate GM15 at 2.67 x 10^9 spores/ml. The spore concentration at 2.67 x 10^8 spores/ml ranked second (53%). The LC_{50} and LC_{90} values of the isolate GM15 were 7.66 x 10^7 spores/ml and 2.96 x 10^{12} spores/ml, respectively.

On *Polyphagotarsonemus latus*

Three fungal isolates were found infected on *P. latus* of which, the two *F. semitectum* and *Fusarium* spp. isolate GM15 caused 94 and 89% mortality, respectively while *Aspergillus niger* caused 9.7% mortality. Commonly known mycopathogens were not able to infect *P. latus.*

Spore concentrations of *F. semitectum* differed significantly to cause mortality of mites. The concentrations at 2.1 x 10^9 and 2.1 x 10^8 spores/ml of *F. semitectum* were found superior to cause mortality on *P. latus* than other concentrations. The concentration at 2.1 x 10^7 spores/ml recorded next best (48.21%) over the rest of the spore load. LC_{50} and LC_{90} values of *F. semitectum* were 8.68 x 10^6 and 1.757 x 10^{10} spores/ml, respectively.

The concentrations at 2.6 x 10^9 and 2.6 x 10^8 spores/ml of the isolate GM15 caused 73.09 and 60.32 % mortality of *P. latus*, respectively, however, these were statistically on par with each other. The LC_{50} and LC_{90} values of the isolate GM15 were 5.27 x 10^7 spore/ml and 3.89 x 10^{11} spore/ml, respectively.

Small-scale efficacy trial on *Scirtothrips dorsalis*

Small-scale efficacy trial has shown promising results. Mortality of *S. dorsalis* was first noticed three days after spraying from the spore concentrations at 2.6 x 10^7, 10^8 and 10^9 spores/ml of *F. semitectum* while it was after four days at 2.6 x 10^4, 10^5 and 10^6 spores/ml (Table 3).

The mean percentage mortality recorded at four days after spraying did not differ significantly while it was significant on the next day among the spore concentrations of *F. semitectum*. The LC_{50} and LC_{90} values of *F. semitectum* were 5.75 x 10^7 and 2.39 x 10^{10} spores/ml, respectively.

Time required to *F. semitectum* to cause 50% mortality of larvae of *S. dorsalis* varied greatly among the spore concentrations. The spore concentration at 3.4 x 10^9 spores/ml of *F. semitectum* required 4.9 days to cause 50% mortality of larvae of thrips while it was 5.3, 5.6 and 7.3 days for the spore concentrations at 10^8 10^7, and 10^6 spores/ml, respectively.

Table 3. Mortality of *Scirtothrips dorsalis* due to *Fusarium semitectum* using small scale efficacy trial method.

Spores/ml	No. of thrips released	Mean mortality of thrips (%)		
		4 DAS	5 DAS	6 DAS
$3.4x10^4$	10.8	09.3 (17.76)	11.9 (17.82)^b	14.6 (19.44)^{bc}
$3.4x10^5$	11.3	15.6 (23.22)	20.0 (25.10)^b	22.7 (26.41)^{bc}
$3.4x10^6$	10.0	17.5 (24.73)	27.5 (30.44)^b	34.2 (34.40)^b
$3.4x10^7$	11.3	24.4 (29.63)	46.5 (43.59)^a	65.1 (55.50)^a
$3.4x10^8$	11.8	26.7 (31.09)	55.6 (47.86)^a	68.9 (55.73)^a
$3.4x10^9$	11.8	32.6 (34.82)	67.4 (55.70)^a	71.7 (58.12)^a
F test		NS	*	*
CD at p=0.01			(10.247)	(12.314)

Note: * Significant; NS - Not significant; DAS - Days After Spraying; figures in parenthesis are Arc sine transformed values; Means in the same column for a given property with same superscript are not significantly different at p=0.01.

Efficacy trial on *Polyphagotarsonemus latus*

The mortality of *P. latus* was noticed three days after spraying in all spore concentrations except at 3.4×10^4 spores/ml, but a maximum of 35% mortality of mites was recorded. Hence, the mortality of mites recorded four days after spraying was used to calculate LC_{50} and LC_{90} values and were 9.78×10^7 spores/ml and 6.54×10^{11} spores/ml, respectively (Table 4). The time required to *F. semitectum* to cause 50% mortality of *P. latus* was 3.7, 3.9 and 4.5 days to the spore concentrations of 3.4×10^9, 10^8 and 10^7 spores/ml, respectively.

Table 4. Mortality of *Polyphagotarsonemus latus* due to *Fusarium semitectum* using small scale efficacy trial method.

Spores/ml	No. of mites released	Mean mortality of mites (%)		
		3 DAS	4 DAS	5 DAS
$3.4x10^4$	71.7	00.0 (4.05)^e	09.4 (17.65)^e	12.6 (19.91)^c
$3.4x10^5$	72.0	13.0 (21.16)^d	31.1 (33.73)^d	42.6 (40.73)^b
$3.4x10^6$	75.7	26.8 (30.77)^c	42.7 (39.49)^{cd}	49.4 (44.53)^b
$3.4x10^7$	77.3	29.7 (33.10)^{bc}	46.6 (42.87)^{bc}	52.2 (46.17)^b
$3.4x10^8$	92.0	33.7 (35.49)^{ab}	51.2 (45.80)^{ab}	78.5 (60.68)^a
$3.4x10^9$	86.7	35.7 (36.79)^a	60.8 (50.99)^a	83.1 (65.59)^a
F test		*	*	*
CD at p=0.01		(3.601)	(6.201)	(7.184)

Note: * Significant; NS - Not significant; DAS – Days after spraying; figures in parenthesis are Arc sine transformed values; Means in the same column for a given property with same superscript are not significantly different at p=0.01.

CONCLUSIONS

Of the fungal isolates recovered from soil using baits with larvae of *T. castaneum,* none were under the groups of common entomopathogens. Four fungi *viz*. *Fusarium* spp. isolate GM15, *N. floridana* and *Penicillium* spp. were isolated from diseased cadavers of *S. dorsalis,* and *F. semitectum* from *S. dorsalis, P. latus* and *A. gossypii*. *N. rileyi* was isolated from *S. litura.* On larvae of *S. dorsalis,* the LC_{50} values of *F. semitectum* and *Fusarium* spp. isolate GM15 were 2.71 x 10^7 spores/ml and 7.66 x 10^7 spores/ml, respectively. On active stages of *P. latus,* the LC_{50} values of *F. semitectum* and *Fusarium* spp. isolate GM15 were 8.68 x 10^6 and 5.26 x 10^7 spores/ml, respectively. *F. semitectum* was more virulent than the isolate GM 15 on *S. dorsalis* and *P. latus.* The mite was highly susceptible to *F. semitectum* than thrips. These results can be used to control the thrips and mites in chilli and the mycopathogens, *Fusarium* spp. can be well incorporated as a viable biocontrol agent in an integrated chilli pest management programme.

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