Studies on biodiversity of entomopathogenic fungi isolated from all the agro-climatic zones of Karnataka

S. Shubha, G.B. Santoshgowda, A. Anantha Rama

Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur, Karnataka, 584102, India, Email: shubhad2003@yahoo.co.in

ABSTRACT

Experiment was conducted to study the biodiversity of entomopathogenic fungi present in the ten agro climatic zones of Karnataka. A systemic survey was made to isolate entomopathogenic fungi and to study *murda* complex incidence in the chilli growing areas on different agro climatic zones of Karnataka. Maximum fungal biodiversity was observed in Southern transitional zone and least was observed in North East transitional zone and North Eastern dry zone. The 13 fungal isolates were isolated from the soil samples collected during the survey. Most common genera isolated from all zones includes *Penicillium*, *Aspergillus*, *Acremonium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Talaromyces*, *Trichoderma* and *Cladosporium macrocarpus*. In this isolates two of them were recognized as entomopathogens namely *Metarrhizium anisopliae, Verticillium lecanii* and rest others *Neozygites floridiana* and *Nomuraea rileyi* were antagonistic saprophytes. No other fungus belonging to commonly known entomopathogenic groups was noticed. Among the groups, the two fungi, *Fusarium* isolate and *Aspergillus* sp. were in equal proportion, four *Fusarium* isolates, four *Aspergillus* isolates and single isolates of each *Verticillium lecanii, Metarhizium anisopliae, Neozygites floridiana, Penicillium* and *Nomuraea rileyi* was found. Among 13 isolates *Verticillium lecanii, Metarhizium anisopliae* and *Fusarium* isolate was tested on the sucking pests like thrips and mites. In the *in vitro* condition *Fusarium* isolate caused 86.6 per cent mortality, *N. floridana* 80 %, *M. anisopliae* 13.3 % mortality and *V. lecanii* 3.33% mortality on *S. dorsalis*. The results obtained revealed that the biodiversity of entomopathogenic fungi is more in the soils having high organic matter and rich in fertility status.

Keywords: Murda complex, chilli, *Scirtothrips dorsalis*, *Polyphagotarsonemus latus*

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the important crop grown for green and ripe dry fruits throughout the tropics and warm temperate regions of the world. India is the world's largest producer and exporter of chilli. The consumption rate of chilli is also highest in India. It also exports dry chilli and chilli products to the countries like Sri Lanka, Bangladesh, South Korea, USA and Japan. In Karnataka, chilli is one of the major remunerative cash crop and is grown throughout the year. With respect to chilli insect pest, a total of 21 species of pests have been reported to inflict damage to chilli in India [1]. The actual number is much more encountered 51 and 39 species of pests attacking transplanted and nursery crops respectively in Karnataka [2], among these thrips, aphids, whiteflies and mites have been identified as major pests. Chilli crop mainly suffers from "murda" syndrome which has been attributed by the attack of a tiny tarsonemid mite, *Polyphagotarsonemus latus* Banks [3] and thrips, *Scirtothrips dorsalis* Hood [4,5] or both [6]. To overcome from all these insect pest there is a need for developing ecofriendly insect pest management strategies, because of excess use of chemical insecticides which is causing deteriorate

Research Article, Acta Biologica Indica 2014, 3(1):574-579

pISSN 2319-1244, eISSN 2279-0160

effect on the environment. With this objective to isolate efficient entomopathogen the study was carried out on biodiversity of entomopathogenic fungi isolated from all the ten agro climatic zones of Karnataka.

MATERIALS AND METHODS

Roving survey to isolate entomopathogens

To study the biodiversity of entomopathogens, a systemic survey was carried out in chilli growing areas of all the agro climatic zones of Karnataka. The zones such as North eastern transition zone, North eastern dry zone, Northern dry zone, Central dry zone, Eastern dry zone, Southern dry zone, Southern transitional zone, Northern transitional zone, Hilly zone and Coastal zone. From all these zones major chilli growing areas was selected and soil and foliage samples were collected to isolate soil borne entomopathogenic fungi and phytogenic entomopathogenic fungi respectively. Soil samples were collected from the base of chilli plants up to a depth of 10 cm with soil augar (7.5 cm diameter) [7,8] and placed separately in plastic bags and labelled. These samples were brought to the laboratory, pooled and mixed thoroughly and used to isolate Entomopathogens. Leaves with large thrips and mites population were randomly collected and placed in polythene bags.

Soil baiting technique to isolate entomopathogens

Soil baiting technique [8] was followed to isolate fungi from collected soils and also foliage sampling was done to search for any diseased cadavers of thrips under microscope (10X). Infected thrips and mites appearing lethargic, moribund, colour changed larvae having unusual dark spots on the epidermis were separated and used for isolation of fungi. All the isolated fungi were subjected to initial identification based on the morphological characteristics following manual and an internet based interactive key specific for each organism.

Bioassay on fungal pathogenecity

Bioassays were conducted to select the potential fungi pathogenic to thrips and mites following the description of Butt and Goettel [9]. Fungal cultures were grown in SDYE and incubated at 25±2°C 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 necessary serial dilutions under phase contrast microscope [10].

Bioassay on thrips (*Scirtothrips dorsalis*)

The method used by Parker [8] was followed. Conidial suspension of the fungi (600 ml each) was applied to two sterile filter papers (Whatman No. 1-7 cm diameter) and 10 second instar thrips were placed on one of the papers. The 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 attached closer due to the moisture. Control assay was conducted using sterile distilled water instead of conidial suspension. These petri dishes were kept at 25±1°C and 79±6% RH. Three replicates were maintained. Mortality was assessed after five days. Dead larvae of thrips were first surface sterilized using 5% NaOCl for three minutes and were rinsed in sterile distilled water thrice. The specimens (dead larvae) 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.

Serial dilution was done to prepare different spore concentrations of the isolates, viz., 2.1×10^4, 2.1×10^5, 2.1×10^6, 2.1×10^7, 2.1×10^8 and 2.1×10^9 per ml. Mortality of the thrips was recorded daily. Treatments mortality was adjusted with the control mortality using Abbott's formula: P = (T-C/ 100-C) × 100 (T- Corrected per cent mortality in treatment; C- Observed per cent mortality in treatment). The LC_{50} and LC_{90} values of the isolates were calculated using Log probit analysis. The analytical software SPSS (Version 10.0), and the graphical software ORIGIN were used.

Bioassay on mites (*Polyphagotarsonemus latus*)

The "Detached-leaf bioassay method" used [11] to test the virulence of *Verticillium lecanii* and *M. anisopliae* on aphids (*Myzus persicae, Aphis gossypii* and *Aphis citricola*) was followed to conduct bioassay on mites. Fourth tender leaf of chilli infested heavily with mite was detached along with the petiole and placed in a plastic vial. The active stages of mites present on both sides of the leaf were counted using stereo binocular microscope (10X). Spore suspensions were sprayed on the leaves using an atomizer and were air dried for few seconds in a laminar flow hood. The bottom side of the plastic vial was then glued on a circular card strip and the whole setup was kept inside a plastic bottle. The card strip helped to keep the vial upright preventing the leaf coming in contact with the sides of the bottle. The setup was incubated at 26±2°C and mortality was recorded daily. Three replicates were maintained.

RESULTS AND DISCUSSION

Among all the chilli fields examined during survey, upward leaf curl caused by thrips was observed, whereas downward curl caused by mites was noticed in selective zones such as Central dry zone, Southern transitional zone and Eastern dry zone. Total 13 different fungal species were isolated in the study. The fungal isolates such as *Penicillium, Aspergillus, Acremonium, Fusarium, Mucor, Paecilomyces, Talaromyces, Trichoderma*, *Cladosporium macrocarpus* and entomopathogens such as *Metarrhizium anisopliae, Verticillium lecanii* and rest others *Neozygites floridiana* and *Nomuraea rileyi* were antagonistic saprophytes. The fungi recovered were found unevenly distributed among these zones (Table 1). More number of entomopathogens were recovered from the soils of Central dry zone, Eastern dry zone, Southern dry zone, Southern transitional and Northern transitional zones, Hilly zones. Because, soils of this zones contains high organic matter and rich in fertility status whereas in Northern dry zone and North east transitional and Coastal zones resulted poor recovery of entomopathogens from soils and from foliage samples *Fusarium* isolate was recovered from *Scirtothrips dorsalis, Polyphagotarsonemus latus* and *Aphis gosypii*. *Verticillium lecanii* and *Metarrhizium anisopliae* were recovered from *Scirtothrips dorsalis*.

The bioassay conducted to screen the fungal isolates against *S. dorsalis* (Table 2) revealed that the *Fusarium* isolate caused 86.6 per cent mortality, *N. floridana* 80 per cent, *M. anisopliae* 13.3 per cent mortality and *V. lecanii* 3.33 per cent mortality. The per cent mortality of the second larval instar of *S. dorsalis* recorded against different spore concentrations of *Fusarium* sp. using "paper-thrips-paper sandwich" method are summarized (Table 3). The *Fusarium* sp. isolates at 2.1×10^8 and 2.1×10^9 spores/ ml caused 63.35 and 76.65 per cent mortality of larvae, respectively. The lethal spore concentration of the isolate to cause 50 per cent (LC_{50}) and 90 per cent mortality (LC_{90}) were calculated as 2.7×10^7 and 2.96×10^{12} spores per ml.

Out of the three fungal isolates tested on *P. latus*, the *Fusarium* sp. caused 94.4 per cent mortality, while *V. lecanii, M. anisopliae* showed no mortality. Other mycopathogens were also tested. However none of them were found to infect *P. latus* (Table 4). The per cent mortality recorded against different spore concentrations of the *Fusarium* sp. isolate is presented (Table 5).

Different spore concentrations of the *Fusarium* sp. isolate differed significantly pertaining to the mortality of mites. The concentrations at 2.1×10^{9} and 2.1×10^{8} spores/ml of the *Fusarium* sp. isolate were found to cause highest mortality of *P. latus*. The concentration at 2.1×10^{7} spores /ml recorded next best (48.21%). LC_{50} and LC_{90} values of the *Fusarium* sp. isolate were calculated as 8.68×10^{6} and 1.75×10^{10} spores/ml respectively.

Table 1. Fungal isolates from different agro-climatic zones of Karnataka.

Fungal isolates	Agro-climatic zones of Karnataka									
	1	2	3	4	5	6	7	8	9	10
Acremonium sp.	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	+	-	-	+	+	+	+	+	+	-
Cladosporium macrocarpus	-	-	-	-	+	+	+	-	-	-
Fusarium sp.	+	+	-	+	+	-	+	+	+	+
Metarrhizium anisopliae	-	-	-	-	-	+	+	-	+	-
Mucor sp.	+	+	-	+	+	+	+	+	+	+
Neozygites floridiana	+	+	+	+	+	+	+	+	+	-
Nomuraea rileyi	+	+	+	+	-	+	+	+	+	-
Paecilomyces	-	-	+	-	+	+	+	+	+	+
Pencillium notatum	+	-	+	-	+	+	+	+	+	+
Talaromyces sp.	-	-	-	-	+	+	-	-	+	+
Trichoderma hamatum	+	-	-	-	+	+	+	+	+	+
Verticillium lecanii	+	+	-	+	+	+	+	+	-	+

+ = Present, - = Absent; 1- North eastern transition zone, 2 - North eastern dry zone, 3 - Northern dry zone, 4 - Central dry zone, 5 - Eastern dry zone, 6 - Southern dry zone, 7 - South transition zone, 8 - North transition zone, 9 - Hilly zone, 10 - Coastal zone.

Table 2. Pathogenicity of fungal pathogens on *S. dorsalis*.

Fungus	Pre count of active stages of thrips	Number of thrips infected	% of thrips infected 5 DAT*
Acremonium sp.	30	Nil	-
Aspergillus nidulens	30	10	33.3
Cladosporium macrocarpus	30	Nil	-
Fusarium sp.	30	26	86.6
Metarrhizium anisopliae	30	4	13.3
Mucor sp.	30	Nil	-
Neozygites floridiana	30	24	80
Nomuraea rileyi	30	Nil	-
Paecilomyces	30	Nil	-
Pencillium notatum	30	Nil	-
Talaromyces sp.	30	Nil	-
Trichoderma hamatum	30	Nil	-
Verticillium lecanii	30	1	3.33

*DAT- Days After Treatment

Table 3. Per cent mortality of *S. dorsalis* due to *Fusarium* sp. isolate using paper-thrips-paper sandwich method.

Spores per ml	Number of active stages of thrips	Number of active stages infected	Per cent mortality
2.1×10^4	20	2.67	13.25 (21.15)
2.1×10^5	20	5.00	25.00 (29.93)
2.1×10^6	20	6.33	31.65 (34.23)
2.1×10^7	20	8.33	41.65 (40.23)
2.1×10^8	20	12.67	63.35 (54.78)
2.1×10^9	20	15.33	76.65 (61.15)
F – Test			*
SEM±			(1.748)
CD @ P = 0.01			(5.389)

Table 4. Pathogenicity of fungal pathogens on *P. latus*.

Fungus	Pre count of active stages of mite	Number of mite infected	% of mite infected 5 DAT*
Acremonium sp.	83	Nil	-
Aspergillus niger	103	10	9.70
Cladosporium macrocarpus	88	Nil	-
Fusarium sp.	72	68	94.4
Metarrhizium anisopliae	93	Nil	-
Mucor sp.	84	Nil	-
Neozygites floridiana	69	Nil	-
Nomuraea rileyi	80	Nil	-
Paecilomyces	82	Nil	-
Pencillium notatum	64	Nil	-
Talaromyces sp.	71	Nil	-
Trichoderma hamatum	88	Nil	-
Verticillium lecanii	75	Nil	-

Table 5. Per cent mortality of *P. latus* due to *Fusarium* sp. isolate using "Detached leaf method".

Spores per ml	Number of active stages of mite	Number of active stages infected	Per cent mortality
2.1×10^4	116	14.30	12.32 (20.53)
2.1×10^5	122	27.67	22.68 (28.76)
2.1×10^6	111	39.67	35.74 (36.47)
2.1×10^7	112	54.00	48.21 (44.0)
2.1×10^8	133	92.67	69.68 (56.60)
2.1×10^9	117	90.00	79.92 (61.31)
F – Test			*
SEM ±			(1.255)
CD @ P = 0.01			(3.866)

The "Paper- thrips-paper sandwich" method of *S. dorsalis* and "Detached leaf" method of *P. latus* confirmed that *Fusarium* isolate was more virulent than other species against both the test organisms. Mycosis developed on the larvae of thrips and active stages of mites on three days after spraying. However, the time required to the fungus to cause 50 per cent mortality was lesser in mites than thrips. With support to our study Draganova [12] also reported that the natural occurrence of entomopathogenic *Fusarium* isolate as a effective biocontrol agent against eriophyid mite, *Aceria guerreronis* damaging coconut in Kerala, India [13]. Parker [8] opinioned that the *Fusarium* isolate, though classified as weak entomopathogen, should be bio assayed for pathogenicity against thrips. These findings clearly indicate that mites were more susceptible to the *Fusarium* sp. isolates than the thrips.

With these results it was concluded that, *Fusarium* isolate which was isolated showed positive results by suppressing the growth of the *Scirtothrips dorsalis* and *Polyphagotarsonemus latus* by mass multiplying this isolate we can use this entomopathogen to control the thrips and mites population in chilli crop and it can serve as effective biocontrol tool for ecofriendly management of insect pests against chilli.

REFERENCES

[1] Butani DK. Pesticides 1976, 10:38-41.
[2] Reddy DNR, Puttaswamy B. Mysore J. Agric. Sci. 1984, 17:246-251.
[3] Kulkarni GS. Agric. J. Ind. 1922, 22:51-54.
[4] Ayyar TVR. Agric. Livestock Ind. 1932, 2:391-403.
[5] Peiris JWL. Tropical Agric. 1953, 109:1-2.
[6] Puttarudraiah M. Mysore J. Agric. Sci. 1959, 34:93-95.
[7] Zimmerman G. Zonal Angew Entomology 1986, 102:213-215.
[8] Parker BL, Skinner M, Brownbridge, et al. Acta Hortic. 1996, 431:521-534.
[9] Butt TM, Goettel MS. Envrionmental Entomology 2000, 141-195.
[10] Hall RA. J. Invert. Pathol. 1976, 27:41-48.
[11] Yokomi RK, Gottwald TR. J. Invert. Pathol. 1988, 51:250-258.
[12] Draganova SA. Bulgerian J. Agric. Sci. 1999, 5:577-583.
[13] Gopal M, Saleena A, Mccoy CW. Curr. Sci. 2002, 82:22-24.