Biological Management of Sootymold Disease on *Butea monosperma* (Palash) at Jabalpur

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Abstract

Fungal Disease of *Butea monosperma* a important medicinal plant clearly indicates that the plant is attacked by many fungi including sootymold disease severe incidence of sootymold was recorded at all the sampling site given in the paper. A total 15 fungi were recorded, among these *Trichoderma viride* FCNBM#15 imposed maximum inhibition to the growth of all fungi of the sootymold complex. Culture filtrate toxicity of *Trichoderma viride* FCNBM#15 was found significant on *in-vitro* growth of sootymold fungi. So *Trichoderma viride* FCNBM#15 was the most effective antagonist against the test sootymold fungi.

KEYWORDS : *Butea monosperma*, *Trichoderma viride* ,fungi, Culture Filtrate Toxicity

INTRODUCTION

Butea monosperma, popularly called as Palash is one of the important forest plant of high economic important forest plant of high economic values. It is considered a home of several Gods. More or less all the part of plant is used as medicines, insecticides and for dyes values in tribal areas. These are used for the production of cheap leaf plates and cups for rural feast. Leaves are also used in wrapping of tobacco to makes biddies. The plant is severely suffered by very serious disease including Sootymold disease which covers entire leaves and growing tips also. In spite of paramount significance of the plant, no serious efforts has yet been made by pathologists to control this disease. The plant is attacked by several fungi disease including Sootymold . The name itself descriptive as Sootymold is a black, powdery coating on commonly given to a condition that is not truly a disease, but a black coating on leaves branches and fruit made up of fungal growth. A black velvety coating made up of fungal strand is formed on the surface of leaves twigs and fruit. Sootymold comprises a mixture of many fungi growing on sugary honey dew, secreted by aphids, scales of whitefly and other insects which suck sap from their host plant. They are saprophytic fungus associated only on surface of leaves. it is normally considered to be a cosmetic or aesthetic problem. In spite of paramount significance of the plant, no serious efforts has yet been made by pathologists to control this disease. However, Sootymold disease reduces the economic values of the plants and also hampered photosynthetic areas of the leaves. The disease occur mainly in January, the plant disease impact negatively through economic loss. (Rivera *et al.*, 2002)

MATERIAL & METHOD

(1) Survey & Collection of Plant samples

Thorough systematic and periodical survey will be at in and around of Jabalpur. Diseased samples of the target plant were collected different location of Jabalpur *viz.*

Dumna, Sitapahadi, Pachpedi, Barela, Vehicle factory, and brought to the laboratory for further processing.

(2) Preservation of Samples

For proper drying, diseased plants samples collected during survey were kept between two sheets of blotting paper and placed on a flat surface and kept pressed with light weight material paper was changed regularly after every 24hrs till complete dryness.

(3) Recovery of the Pathogens and other Non-pathogens

The samples brought from the field were further analysed for the isolation of fungi. For quantitative estimation and comparing the fungal flora of different diseased samples "Pour plate" method were followed (Walksman, 1922; Brierly *et al.*, 1927). Serial dilutions of the samples was prepared. 10gms of each sample was over dried and grinded in vortere mixer. These sample was placed in 125ml Erlenmeyer flask containing 100mlsterile distilled water. The flask containing the suspension was shaken well with the help of rotary shaker (Yorko's) for 30mins. at medium speed and normal temperature. This was kept undisturbed for one hour and then 10ml. of clear suspension was transferred to another flask containing 90ml of sterile distilled water. It was repeated to obtain further dilutions (Stevens, 1981 ; Tuite, 1969; Agrawal and Hasija, 1986).Each dilution, 1ml. of suspension was transferred aseptically into pre-sterilized petriplates and approximately 10 - 15ml. of potato - dextrose - agar medium (Agrawal and Hasija, 1986; Martin, 1950)

(4) Identification of Fungi

The identification was done after studying the morphological and cultural characteristics with the help of manuals, monographs and paper of various workers (Mano harachary *et al.*, 2004).

(5) Preparation and Maintenance of Culture:

Fungi isolated were aseptically transferred to agar slants and by repeated sub-culturing pure cultures of different isolates were obtained. The stock culture of the micro-organisms were maintained on the PDA slants and stored at a low temperature in the refrigerator. The other slants were kept in the incubator (BOD) at 28 ± 1°C and were routinely transferred into the fresh slants for experimental purposes.

(6) Distribution of Fungi :

Study the distribution of fungi isolated earlier from the various samples the percentage frequency and the abundance of various species were detected. Frequency as introducted Raunkier (1934) indicates the number of sampling units in which a given species occurred and thus expresses the distribution or dispersion of various species in a community. From this, percentage frequency is calculated as follows.

$$\text{Percentage frequency} = \frac{\text{Number of sampling units in which the species occurred frequency}}{\text{Total number of units studied}} X\ 100$$

$$\text{Percentage abundance} = \frac{\text{Total Number of colonies of a species in all abservations abundance}}{\text{Total number of colonies}} X\ 100$$

(7) Culture Filtrate Toxicity:

The culture of each isolated strain was inoculated separately in presterilized plate containing medium. After six days, two agar blocks (5mm diameter) of fungi, cut with the help of sterilized cork borer from actively growing cultures were separately transferred into 150ml Erlenmeyer flask containing 50ml autoclaved CDY broth. Each experiment was done in triplicate. Flasks were incubated in KUMAR's BOD incubator for 15 days at 28 ± 2^0C. Each flask was shaken every alternate day to maintain homogenous growth in liquid medium. After incubation, the cultures were filtered through Seitz filter paper to remove all the bacterial and all other contamination.

"Poisoned food technique" suggested by Sharvell ,(1956) and Neely (1971) was followed to test the toxicity of the culture filtrates. Two concentration *i.e.* 2ml and 4ml in 20ml of autoclaved, cooled CDYA medium was poured separately into presterilized petriplates. CDYA plate devoid of culture filtrate served as control. Each plate after solidification were inoculated with the test pathogen (5mm disc from seven days old culture) and kept for incubation at 28 ± 2^0C in BOD incubator. The percentage growth inhibition was calculated by Vincent formula as discussed earlier. (Mishra, 2013)

Culture Filtrate Toxicity of each antagonist (2ml and 4ml were also added in Erlenmeyer flask containing autoclaved, cooled 50ml CDYA broth separately. These flasks were seeded with 5mm agar blocks of test pathogen separated from seven days old cultures grown on PDA medium, CDYA broth devoid of culture filtrate served as control. After incubation period of seven days, cultures were filtered through Whatman filter paper no 42 and oven dried at 60°C. Inhibition in dry weight was calculated.Composition of Cziapek - Dox - Yeast - Extract agar medium

$NaNO_3$	-	2.0gm.
K_2HPO_4	-	1.0gm.
$MgSO_4 . 7H_2O$	-	0.5gm.
HCl	-	0.5gm.
Sucrose	-	30gm.
Ferrous sulphate	-	0.01gm.
Yeast extract	-	1.0gm.
Agar – Agar	-	20gm.
Distilled Water	-	1000ml.

RESULTS & DISCUSSION

(a) Distribution of fungi

During survey of the field at in and around of Jabalpur, it was noticed that sootymold and other disease were more prevelant during January to March in *Butea monosperma* trees. A servere incidence of sootymold was recorded at all the sampling sites. A total of 15 fungi were recorded from six

different sampling sites Table 1 Maximum number of fungi were recorded from the sample collected from Pariyat, which was followed by Dumna. Minimum number of fungi were recorded at the Barela sampling site. It may be due to physiochemical conditions at Pariyat, site was found to be highly conducible for fungal growth and colonization. *Aspergillus niger* FCNBM#07 showed maximum frequency in all the selected sampling sites. It was followed by *A. flavus* FCNBM#06, *Trichoderma viride* FCNBM#15, *Absidia corymbifera* FCNBM#01.

Many well known Sootymold fungi viz. *Capnodendron trichomericola* FCNBM#04, *Acremonium persinium* FCNBM#02, *Alternaria alternate* FCNBM#03, *Cephalosporium sp.* FCNBM#09, and *Phoma sp.* FCNBM#08 were also frequently-encountered in the samples. Amongst these *Alternaria alternata* FCNBM#03, *Curvularia lunata* FCNBM#1l, *Fusarium oxysporum* FCNBM#12 and *Penicillium nigricans* FCNBM#14 were well known plant pathogens. Similar observation regarding variation in composition sootymold disease have also been reported by (Cooke, 1976). Occurrence of very high fungal populations in samples might be because of availability of nutrients and required elements.

(b) Culture Filtrate Toxicity

Inhibition of *in-vitro* growth of Sootymold was also determined by Poisoned Food Technique. It is evident from the data recorded in Fig. 1 clearly indicate that a varying degree of inhibition in cell-free culture filtrate obtained from fermented of *Trichoderma viride* FCNBM#15 had significant impact on *in-vitro* growth of Sootymold fungi. It was varied greatly with different fungi.

Cell free culture filtrate of *T. viride* FCNBM#15 imposed maximum inhibition on the growth of *Curvularia lunata* FCNBM#11 which was followed by *Capnodendron trichomericala* FCNBM#04 at higher canceatration. Inhibition was also significant incase of *Fusarium oxyaporum* FCNBM#12, *Mucor racemoium* FCNBM#13, *Phoma sp.* FCNBM#08, *Aspergillus fumigates* FCNBM#05. More or less similar observation were also recorded at lower concentration.

Similar obseration have also recorded by many earlier workers. Dennis and Webster (1971a) reported that the extent of growth inhibition depends on the concentration of the culture filtrate used. Production of the anti-fungal metabolite might be the main inhibitory factor in the culture filtrates (Mishra, 2013). Similar observations also recorded with different fungi studied by them.

Conclusion

On the basis of above observations it can be concluded that the strain *i.e. Trichoderma viride* FCNBM#15 have been very high bio-potential against the sootymold disease of Palash (*Butea monosperma*). We can be developed for the management of this disease through.

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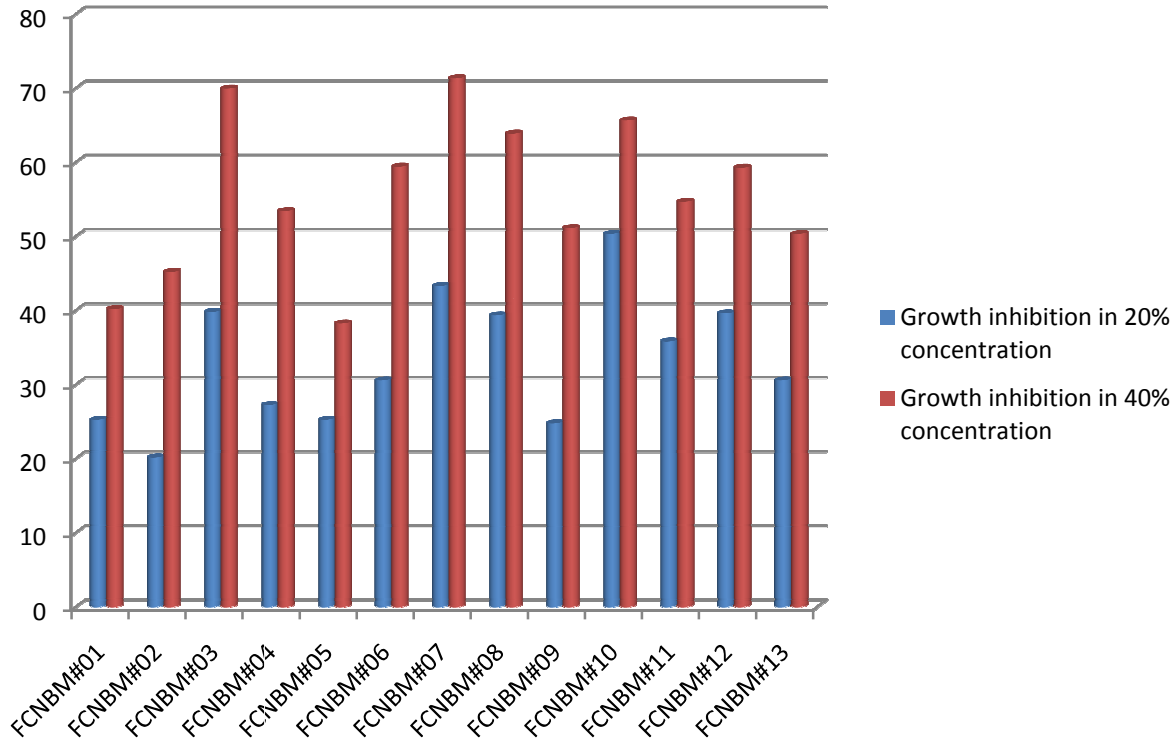
Table (1). Distribution of fungi associated with sootymold of *Butea monosperma*								
S. No	**Name of the fungi**	**No. of fungus**	**Frequency (%)**					
			A	**B**	**C**	**D**	**E**	**F**	**G**
1	*Absidia corymbifera*	FCNBM#01	22.22	30.56	16.67	11.11	05.56	00.00	03.24
2	*Acremonium persinium*	FCNBM#02	11.11	16.67	00.00	00.00	05.56	00.00	00.00
3	*Alternaria alternate*	FCNBM#03	25.00	05.56	11.11	00.00	05.56	00.00	07.78
4	*Capnodendron trichomericola*	FCNBM#04	16.67	05.56	2.78	00.00	11.11	5.56	00.00
5	*Aspergillus fumigates*	FCNBM#05	77.78	50.00	30.56	27.78	44.44	33.33	58.11
6	*Aspergillus flavus*	FCNBM#06	68.11	50.00	55.56	00.00	00.00	00.00	22.22
7	*Aspergillus niger*	FCNBM#07	98.00	93.00	85.11	86.20	81.00	80.00	74.52
8	*Phoma sp.*	FCNBM#08	33.33	22.22	11.11	27.78	19.56	00.00	03.24
9	*Cephalosporium sp.*	FCNBM#09	11.11	22.22	00.00	11.11	00.00	05.56	00.00
10	*Chaetomium globosum*	FCNBM#10	68.11	30.56	00.00	00.00	27.78	05.56	00.00
11	*Curvularia lunata*	FCNBM#11	80.56	16.67	11.11	27.78	33.33	16.67	13.89
12	*Fusarium oxysporum*	FCNBM#12	72.22	63.89	36.11	44.44	33.33	02.78	30.56
13	*Mucor racemoium*	FCNBM#13	33.33	19.44	05.56	00.00	00.00	00.00	03.24
14	*Penicillium nigricans*	FCNBM#14	38.89	11.11	00.00	16.67	00.00	00.00	00.00
15	*Trichoderma viride*	FCNBM#15	22.22	11.11	05.56	02.78	05.56	11.11	05.56

Sampling site

A. Pariyat
B. Dumna
C. Kalpi
D. Gwarighat
E. Bhedaghat
F. Sitapahadi
G. Pachpedi

FCNBM. Fungal Culture Number *Butea monosperma*

Fig (1). Effect of culture filtrate of *T. viride* FGCCBM#15 on radial growth of sootymold fungi



Absidia corymbifera FCNBM#01
Acremonium persinium FCNBM#02
Alternaria alternate FCNBM#03
Capnodendron trichomericola FCNBM#04
Aspergillus fumigates FCNBM#05
Aspergillus flavus FCNBM#06
Aspergillus niger FCNBM#07
Phoma sp. FCNBM#08
Cephalosporium sp. FCNBM#09
Chaetomium globosum FCNBM#10
Curvularia lunata FCNBM#11
Fusarium oxysporum FCNBM#12
Mucor racemoium FCNBM#13