Biological Control of Narrow Brown Leaf Spot and Leaf Smut Disease in Paddy Crops by Some Antagonistic Fungi

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Abstract

Rice is one of the most important crops worldwide. Fungal diseases in rice plants are raising concerns both in the field of research and production. Therefore, there is a definite requirement to find their control measures. In this study, an attempt was made to address the raised concerns by biologically controlling two of the least discussed diseases of the rice plants. An experiment to test potential of soil fungi as antagonists after treatment against the isolated fungal pathogens, Cercospora janseana and Entyloma oryzae was performed. In the present study, isolation of fungal plant pathogens were done from the infected plant collected from the rice field in Cauvery-Delta zone. The infected leaves were surface sterilized. Next, the efficiency of fungal isolates (ten) against Cercospora janseana and Entyloma oryzae were tested using dual culture method under in-vitro conditions. The culture filtrate test was performed to observe the maximum zone of inhibition at a particular concentration by the ten antagonistic fungi. Chemical fungicides like Carbendazim and Mancozeb were also tested using disc diffusion method. This test helped in comparison of the effects of biological control agents (antagonistic fungi) and chemical control agents (chemical fungicides). Results revealed that Trichoderma viride was found to be most effective as a biological control agent in all the tests when compared to other fungal species. All the test antagonists grew faster than the pathogens and produced inhibition zones which limited the growth of the fungal pathogens, Cercospora janseana and Entyloma oryzae. Gliocladium virens and Trichoderma harzianum also contributed in showing their antagonistic activity after Trichoderma viride. In conclusion, the three antagonistic fungi that were found to be highly efficient might be exploited commercially to biocontrol the narrow brown leaf spot and leaf smut disease. Further studies needs to be continued in this area of research.

Index terms— Cercospora janseana, Entyloma oryzae, Trichoderma viride, Biological control, Carbendazim, Mancozeb, rice.

1 I. Introduction

any types of crops get exposed to different species of pests, but only a few are taken into account (Pimentel et al., 1997). Plant pathogens, pest insects, and weed cause most reduction in world's food production, without application of pesticides.

Before harvest, losses due to pests are approximately 15% for pest insects, 13% for diseases, and 12% for weeds. Post-harvest, also a loss of food occurs due to other types of pests (Pimentel et al., 1997). Diseases in plants can be caused by a variety of fungi that result in significant losses on crops. Different types of fungi harm almost every tree, and each one attacks various kinds of plants. Approximately, more than 10,000 different species of fungi may cause diseases in plant varieties (Agrios, 2005).

Diseases caused by fungi can be reduced by the usage of inoculums and inhibition of its virulence mechanisms thereby, promoting genetic diversity in the crops (Strange et al., 2005). Mostly, fungicides cause acute toxicity,

whereas, few cause chronic toxicity as well (Goldman, 2008). Chemical pesticides usage leads to various environmental and health problems. International Labour Organization (ILO) has recorded that 14 % of occupational injuries occurs as a result of exposure to pesticides and other agrochemical constituents **??**ILO, 1996). World Health Organization (WHO) and United Nations Environment Programme surveyed that each year, up to three million workers in agriculture experience severe poisoning due to pesticides, of which about 18,000 die (Miller, 2004). Suitable improvement in technology results in productive use of natural resources, which is essential for agricultural development. Amongst all, one is the use of microbial antagonists.

According to previous reports, many microbial antagonists possess antagonistic activities against plant fungal pathogens, for example, Pseudomonas fluorescens, Agrobacterium radiobacter, Bacillus subtilis, B.cereus, B. amyloliquefaciens, Trichoderma viride, Burkholderia cepacia, Saccharomyces sp, Gliocadium sp.

A possible way of controlling plant diseases is the application of biological control which decreases the excessive use of agrochemicals and its health hazards effect. Many naturally occurring soil microbes aggressively attack plant pathogens and provide benefit to the plants by suppression of the disease and hence referred to as biocontrol agents. In addition to this, biological control agents also help to control insect, pests, and weeds.

Among several types of biological control agents available to be used in plants, screening of the appropriate biocontrol agent is necessary to be developed and commercialized further. Biocontrol agents possess multiple beneficial characters such as competence in the rhizosphere, antagonistic potential, and ability to produce antibiotics, lytic enzymes, and toxins.

2 II. Materials and Methods

3 a) Study Area

The study was mainly focussed and conducted in the Cauvery-Delta Zone to isolate the available plant pathogens from the infected paddy leaves grown in this region and find the antagonistic effect of different fungi on the isolated plant pathogens.

4 b) Sample collection of infected paddy plants

A survey was conducted in the above said areas during the crop season. Collection of diseased plants showing narrow brown leaf spot and leaf smut symptoms, from the Vadakovanur village in the Cauvery-Delta zone, Thiruvarur district, Tamil Nadu, India. A clean polythene bag stored the samples, and each sample was marked clearly to show details of the location and variety. In the laboratory, the samples got used for microscopic examination, isolation, purification and pathogenicity test.

5 c) Collection of soil sample

In paddy, collection of the rhizospheric soil sample, from Thirukkanurpatti, Thanjavur district, Cauvery-Delta zone to isolate Trichoderma, Aspergillus, Penicillium, Rhizopus, Fusarium, Gliocladium species. After sample collection, samples were brought to the laboratory and stored in the refrigerator for further biological analysis. (Waksman, 1922) Surface sterilization of the infected paddy leaves was done using 0.1% mercuric chloride solution, 1% sodium hypochlorite solution (1 min) and 70% ethanol wash (1 min). Potato Dextrose Agar medium was prepared and poured into the Petri plates. Surface sterilized sample was dried after placing between two sterile filter papers. Then inoculated or impressed (Impression method) in sterile Potato Dextrose Agar medium (PDA) and incubated at 27±2 0 C for 72 hours. To avoid bacterial contamination Streptomycin @ 100 ppm was added in the medium.

6 d) Isolation of Fungal Pathogens

7 e) Isolation of native antagonistic mycoflora from

rhizosphere of paddy i. Serial Dilution Technique (Aneja, 2002) The collected soil sample was serially diluted to isolate the fungal population. The soil sample was diluted in a conical flask containing 90 ml of sterile distilled water and mixed thoroughly to make 1:10 dilution (10 -1). Then 10 ml of diluted sample was transferred to the next flask and serially diluted into the series of conical flasks having 90 ml of sterile distilled water with sterile pipettes, up to 10 -6 . Consider 10 -4 to 10 -6 dilutions for the fungal isolation. The soil sample was taken from a container and subjected to serial dilution followed by pour plate method.

ii. Pour Plate Method (Johnson et al., 2001) Pour plate technique provides the maximum probability of recovering all bacteria present in a given sample. The process includes the addition of molten agar to the inoculums in a Petri plate, mixing it by rotating the plate and then allowing solidification of the medium with the inoculums. Since the lowest area of the agar contains a lesser quantity of oxygen, even anaerobic organisms can be effectually enumerated. Potato dextrose agar medium was used in pour plate method. In sterilized petri plates, 1 ml of sample was transferred from 10 -1 to 10 -6 dilution. Finally, the cooled medium was poured into the sample containing plates and incubated at 27±2 0 C for 72 hours.

The composition of Potato Dextrose Agar medium (pH -5.6): Potato (Peeled) -200g Dextrose -20g Agar -15g Distilled water -1000 ml iii. Preparation of PDA (Potato Dextrose Agar Medium) (Aneja, 2002) 200g of potatoes

were made into thin slices and boiled with 1000 ml distilled water for extraction; 15g of agar was mixed in 200 ml distilled water, and melted. In potato extract, this melted agar solution was mixed; to this mixture, 20g of dextrose was also added. On addition of distilled water, the final volume of medium was made up to 1000 ml. The pH (medium) was maintained as 5.6. The medium was sterilized in an autoclave at 121 0 C for 15 minutes. Addition of a pinch of Streptomycin just before pouring the medium into Petri plates is necessary to prevent the bacterial growth.

iv. Identification of Fungi a. Lactophenol Cotton Blue Technique ? On top of the glass slide, a drop of lactophenol cotton blue was placed. A small tuft of the fungus probably with spores and spore-bearing structures was transferred into the globule using an inoculation needle. ? The material was teased using the two inoculation needles. Fungal material was then flooded with stain. ? The fungal preparation was mounted with coverslip, taking care to avoid trapping air bubbles in the stain. It was then examined under the microscope.

8 b. Identification Manual

Identification of individual fungi are done on the basis of spore morphology, cultural characteristics and also using standard manuals like The Manual of Soil Fungi (Gilman, 1957), Microscopy and Photomicrography: a working manual (Smith, 1994), Ainsworth and Bisby's Dictionary of the Fungi (Hawksworth et al., 1995), Practical Mycology: manual for identification of fungi (Funder et al., 1968), Dictionary of the Fungi (Kirk et al., 1983), Biology of Conidial Fungi (Cole et al., 1981) and Laboratory Manual for Identification of Pathogenic fungi (Hazen et al., 1972).

v. Pathogenicity Test (Sakthivel et al., 1987) a.

9 Inoculum Preparation

The healthy paddy plants were planted in pots filled with a sterile potting mixture containing soil, sand and farmyard manure in the ratio of 1:1:1 and grown under greenhouse conditions. Cercospora janseana and Entyloma oryzae were cultured in Potato Dextrose broth in Roux bottles using mycelial plugs (3 mm) taken from the advancing margin of 7 days old culture of the isolate. The isolates were allowed to grow at 25 0 C±2 0 C for 14 days and the mycelial mats were used for pathogenicity tests. The mycelial mats were harvested, weighed and homogenized in a mixer blender and made into a suspension. Inoculation of suspension of 5 ml containing 1g ml/l over the soil surface around onemonth-old healthy paddy plants was performed. The ones without inoculums served as control. Evaluation of the plants was done by recording the development of black powdery spores on leaves and subsequent spotting and yellowing of the leaves. Observation of symptoms of the narrow brown leaf spot and leaf smut and recording of results was done at regular intervals.

10 b. Reisolation of the pathogen

The plants which got infected by the fungi and showed symptoms of narrow brown leaf spot and leaf smut after 14 days (International Rice Research Institute, Philippines, 1988) were collected and used for the reisolation of the pathogens to prove the pathogenicity. The infected sample portions (infected paddy leaves) gathered in the laboratory were used for isolation. These were washed thoroughly with tap water to remove the impurities present on the leaves. Small pieces excised from the diseased portions along with some healthy parts were surface sterilized with 0.01% mercuric chloride or with 75% ethanol for 1-3 minutes, then washed for three times in sterile distilled water and transferred on to PDA Petri plates (90 mm diameter).

vi. Dual Culture Test This test was used to study the reduction in the growth of pathogens and inhibition zone formed due to the antagonistic activity of the biocontrol agents. The biocontrol agents Trichoderma viride, Trichoderma harzianum, Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Penicillium notatum, Penicillium chrysogenum, Rhizopus sp., Fusarium sp., and Gliocladium virens were selected to study the antagonistic activity against Cercospora janseana and Entyloma oryzae isolated from the infected paddy leaves. The Potato dextrose agar medium was prepared and poured into the Petri plates. After solidification, 6 mm diameters of the pure culture of each biocontrol agents were placed on the PDA medium in opposite direction against pathogenic fungi. The plates were incubated at 27±2 0 C for 15 days, and the results were noted at every 72 hours on 3, 6, 9, 12 and 15 th days respectively. In the control experiment, the test antagonists got replaced with sterile agar plugs. The growth of the pathogens was recorded in both the test and control experiments. Colony interaction was determined using dual culture method. The growth inhibition was calculated of the colony of the test pathogens and antagonistic fungi:Percentage inhibition of growth = ????? 1 ?? ×100

r=Measurement of growth of the pathogenic fungi from the center of the colony up to the core of the plate in the absence of antagonistic fungi. r 1 =Measurement of growth of the pathogenic fungi from the middle of the colony towards the antagonistic fungi.

The colony interaction assessment between test pathogens and soil fungi done following the model proposed by Porter (1924). Five types of interactions grade as proposed by Skidmore et al., 1976 The biocontrol agents were inoculated into the potato dextrose broth at 27 0 C with intermittent shaking at 150 rpm. The metabolites were collected from 12 days and filtered. The sterilized filtrates were amended in PDA to make 5%, 10%, and 15% concentration in Petri plates. The solidified agar plates were inoculated at the center with 6mm diameter

mycelia disc of the pathogen and incubated at 27 0 C for seven days. The Petri plates without filtrate served as control. The colony diameter was measured, and calculation of percentage inhibition of radial growth was done.

The percent inhibition of growth can be calculated as:% of inhibition of growth = Growth in control ? Growth in treatment Growth in control × 100

viii. Disc Preparation (Kirby Bauer et al., 1966) The Whatman No.1 filter paper, used for the disc preparation; the disc size was 6mm. The commercially available chemical fungicides namely, Carbendazim (50% wp) and Mancozeb (75% wp) were used. 0.3 gm of fungicides were diluted with 10 ml of sterile distilled water and added into the discs, and the discs were maintained in a hot air oven at 45 0 C till it reached required concentration.

ix

11 . Disc Diffusion Method

The PDA medium was prepared and sterilized at 121 0 C for 15 minutes and allowed to cool to approximately 50 0 C. Next, the medium was poured into the sterile Petri plates. After solidification, the isolated pathogens were swabbed on the agar plate with the help of sterile cotton buds. After disc preparation, the discs were placed on the PDA medium. Control plates containing only the isolated pathogens, without the introduction of chemical fungicides were also maintained. The Petri plates were stored in an incubator at 27±2 0 C for 7 days. After the incubation period, the results were recorded. The efficacy of fungicides was expressed as percent of radial growth over control, which was calculated by using the formula (Vincent et al., 1947):I= (C-T/C) x100

Where, I = Percent inhibition over control C = Radial growth in control T = Radial growth in treatments

12 x. Statistical Data Study

The entire test and all the data of the parameters were statistically analyzed using random sampling and expressed as Mean ± S.D. (Gupta et al., 1971). Duncan Multiple Range Test (DMRT) is a test used to evaluate the significant differences between treatments (P?0.05). ANOVA analysis is performed with the SPSS statistics software.

13 III. Results

14 a) Sample Collection

The present study was carried out to isolate the fungal species of pathogens from narrow brown leaf spot disease and leaf smut disease occurring in the infected paddy crop field located at Vadakovanur village in the Cauvery-Delta Zone, Thiruvarur district, Tamil Nadu, India. The physicochemical parameters of the soil sample collected from the same place were analyzed, and the morphological analysis was done to identify the fungal species present in the soil. The growth of Entyloma oryzae towards the center of the plates in the absence of any antagonistic fungus (control) was 28 mm, measurement taken within 72 hours.

A.terreus: Aspergillus terreus, P.notatum: Penicillium notatum, P.chrysogenum: Penicillium chrysogenum, R.stolonifer: Rhizopus stolonifer, F.oxysporum: Fusarium oxysporum.

15 IV. Discussion

Our study indicated that the antagonistic effect of T.viride was better than other species of isolated antagonistic fungi for the inhibition of the fungal pathogens, Cercospora janseana and Entyloma oryzae, causing narrow brown leaf spot and leaf smut diseases in paddy leaves. A high amount of moisture content, organic matter, and temperature, along with neutral pH was recorded while measuring the physicochemical parameters of the soil. In dual culture test, T.viride showed maximum % of inhibition (75%) against the tested pathogens, C.janseana and E.oryzae in comparison to other antagonistic fungi. Compared to other soil fungi in the Culture filtrate test, T.viride exhibited maximum control effect at 15% concentration rather than 5% and 10% concentration on the tested fungal pathogens, C.janseana and E.oryzae. From the commercial fungicides aspect, Carbendazim showed the maximum zone of inhibition compared to Mancozeb for the tested fungal pathogens, C.janseana and E.oryzae. Besides Trichoderma viride, Gliocladium virens and Trichoderma harzianum can also be used for controlling of the plant pathogens. Thus, the control of leaf borne paddy crop plant disease is possible through the use of antagonistic microorganisms as well as with the use of fungicides in the form of soil drenches.

16 V. Conclusion

In our research findings, we concluded that fungal antagonists like Trichoderma and Gliocladium are potential biocontrol agents that can be explored to provide productive and safe means to manage paddy crop diseases. The present study showed that three species of fungi, i.e. Trichoderma viride, Gliocladium virens and Trichoderma harzianum suppressed the growth of Cercospora janseana and Entyloma oryzae, the cause of narrow brown leaf spot and leaf smut disease in paddy crops. Rice (Oryza sativa L.) being a main cereal crop with high demand worldwide should be prevented from disease-causing plant pathogens as it results in increased yield losses of paddy crops.

In definite areas, farmers still rely on the use of synthetic fungicides to control plant diseases. However, the misuse of these chemicals may cause serious environmental and health problems. Therefore, these chemical

fungicides must be replaced with biocontrol agents for the prevention of plant diseases. Thus, it can be concluded that fungal biocontrol agents being harmless to the animals and human beings (no side effects), cheaper than chemicals and highly potent will hold significant value in the field of agriculture. [1]

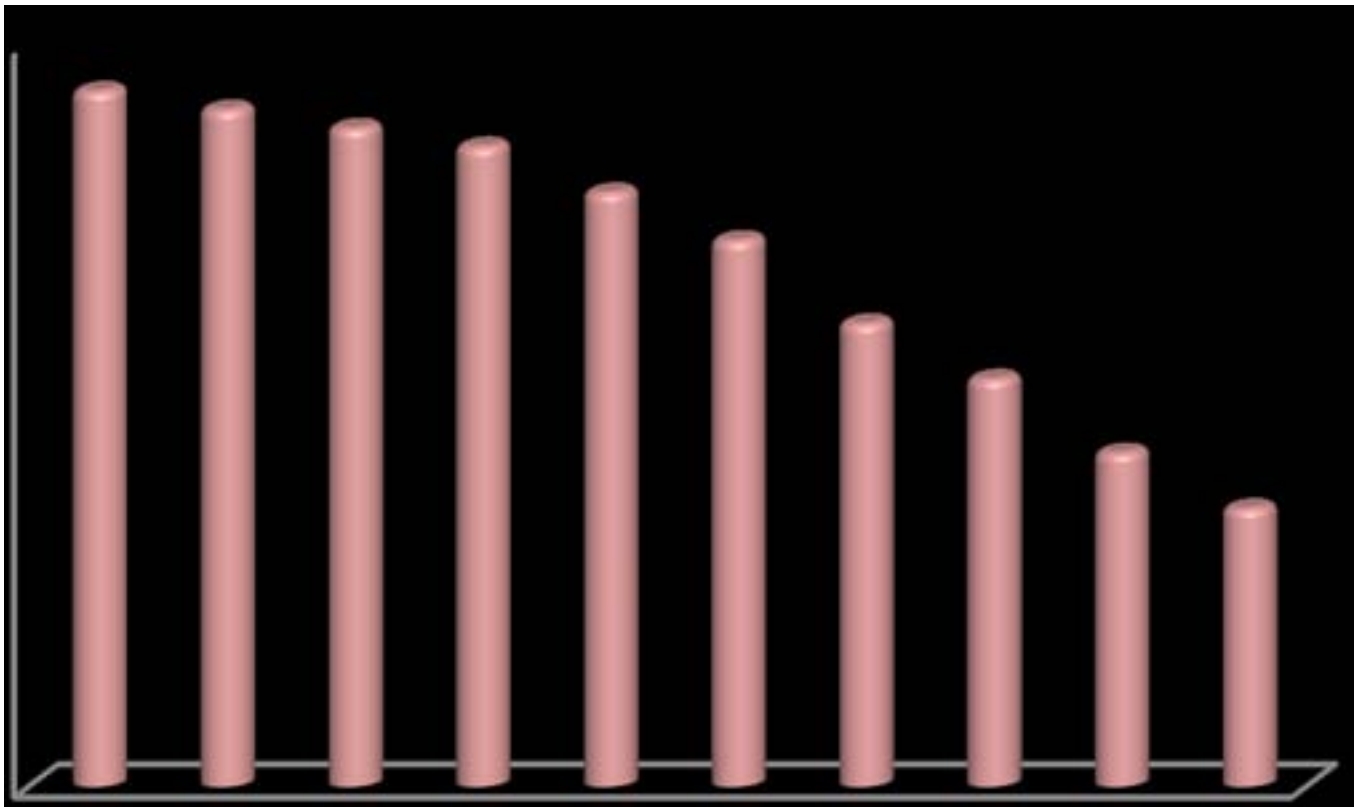

Figure 1: Mean

Figure 2: Fig. 1 :

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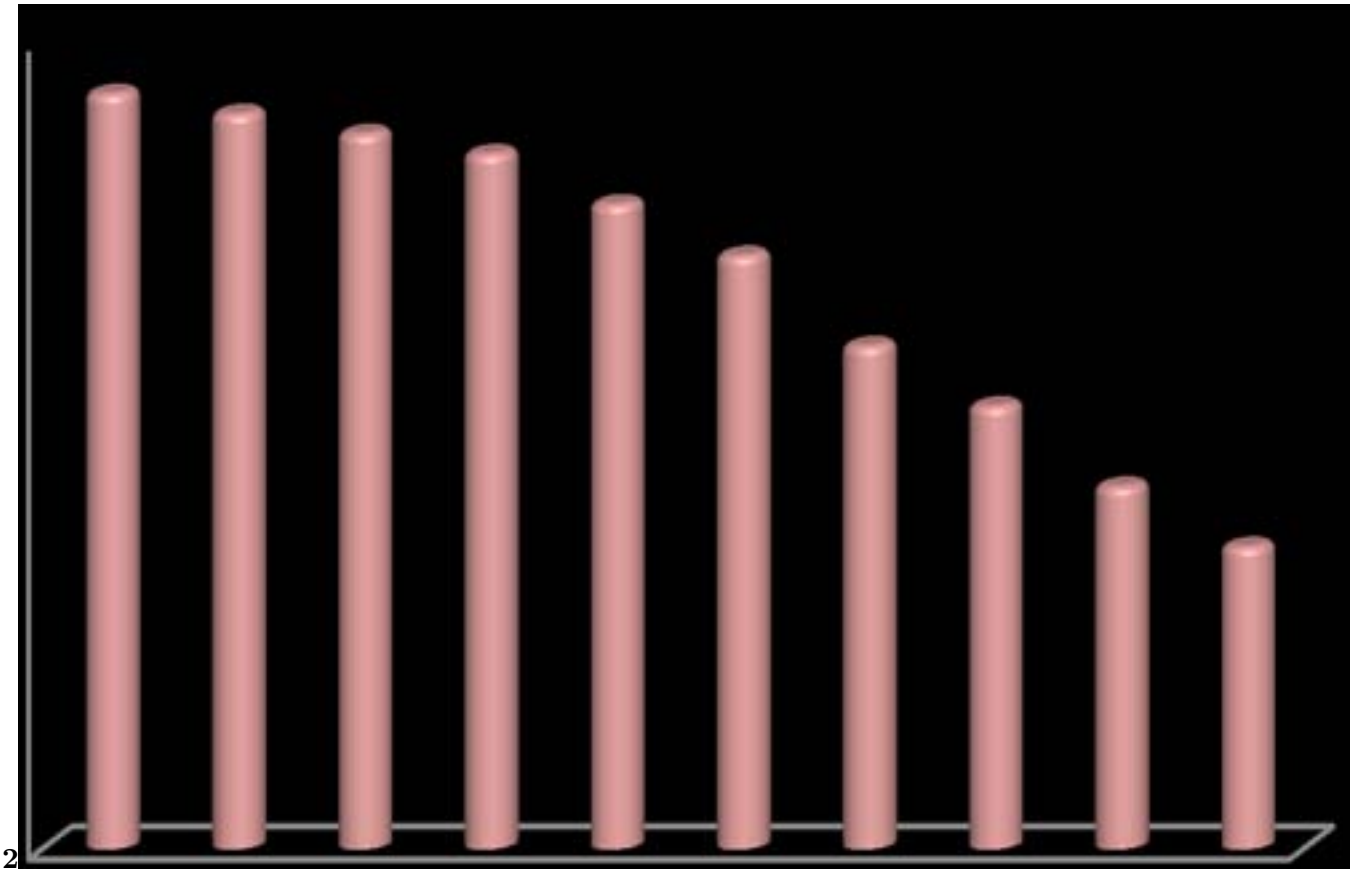


Figure 3: Fig. 2 :

Figure 4: Fig. 3 :

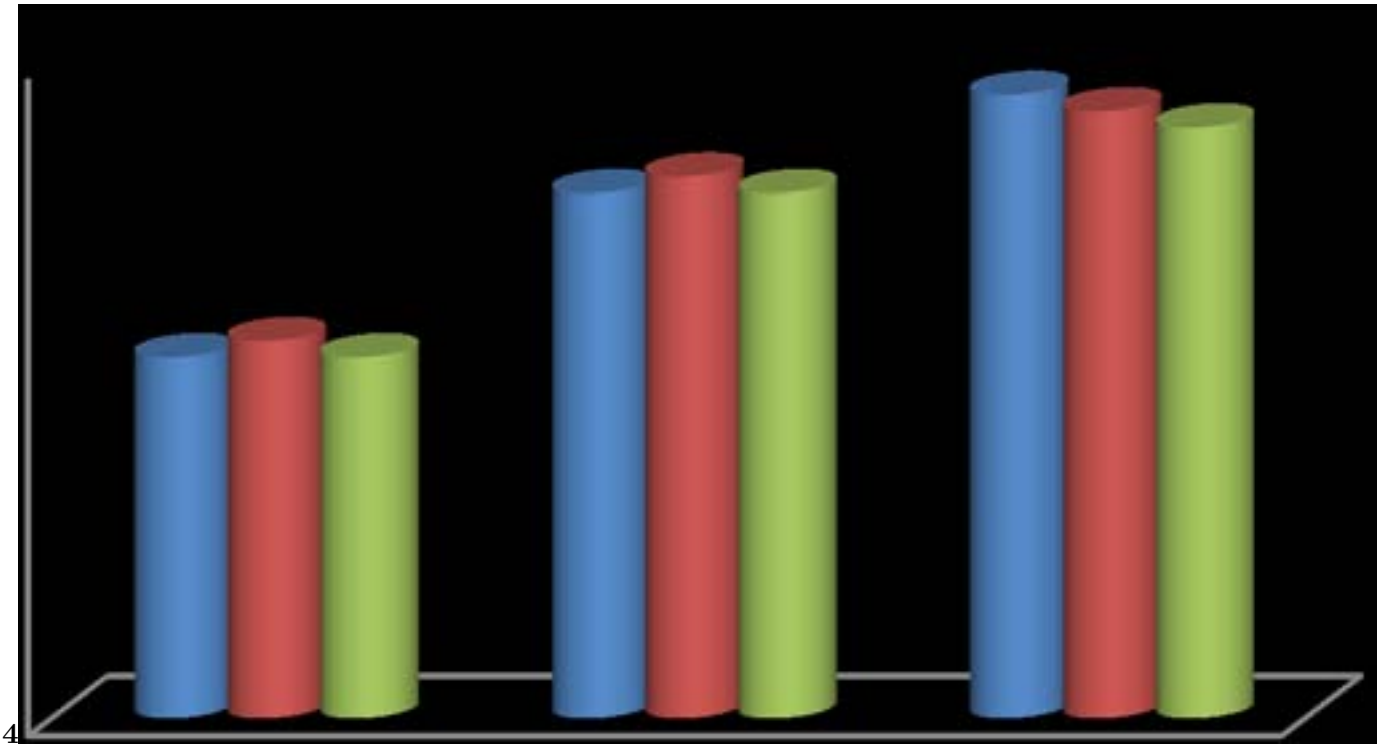


Figure 5: Fig. 4 :

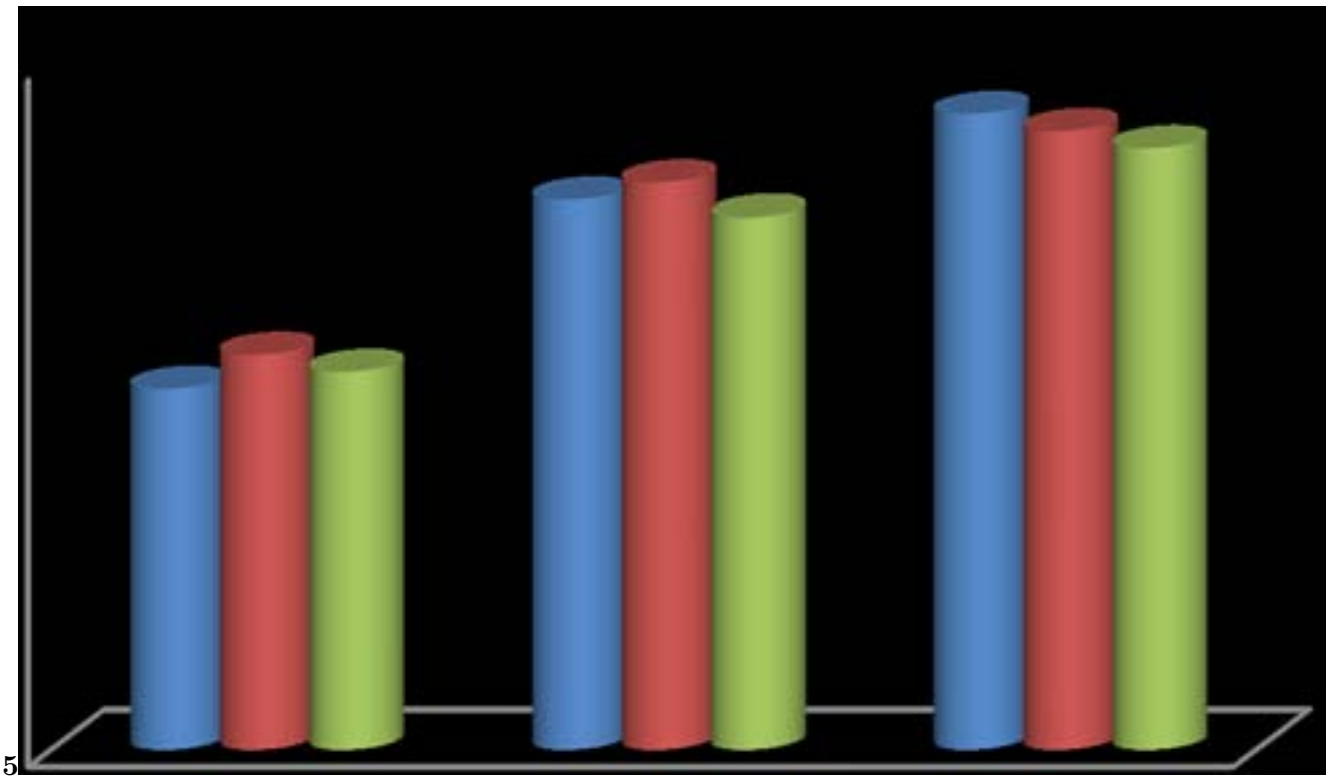
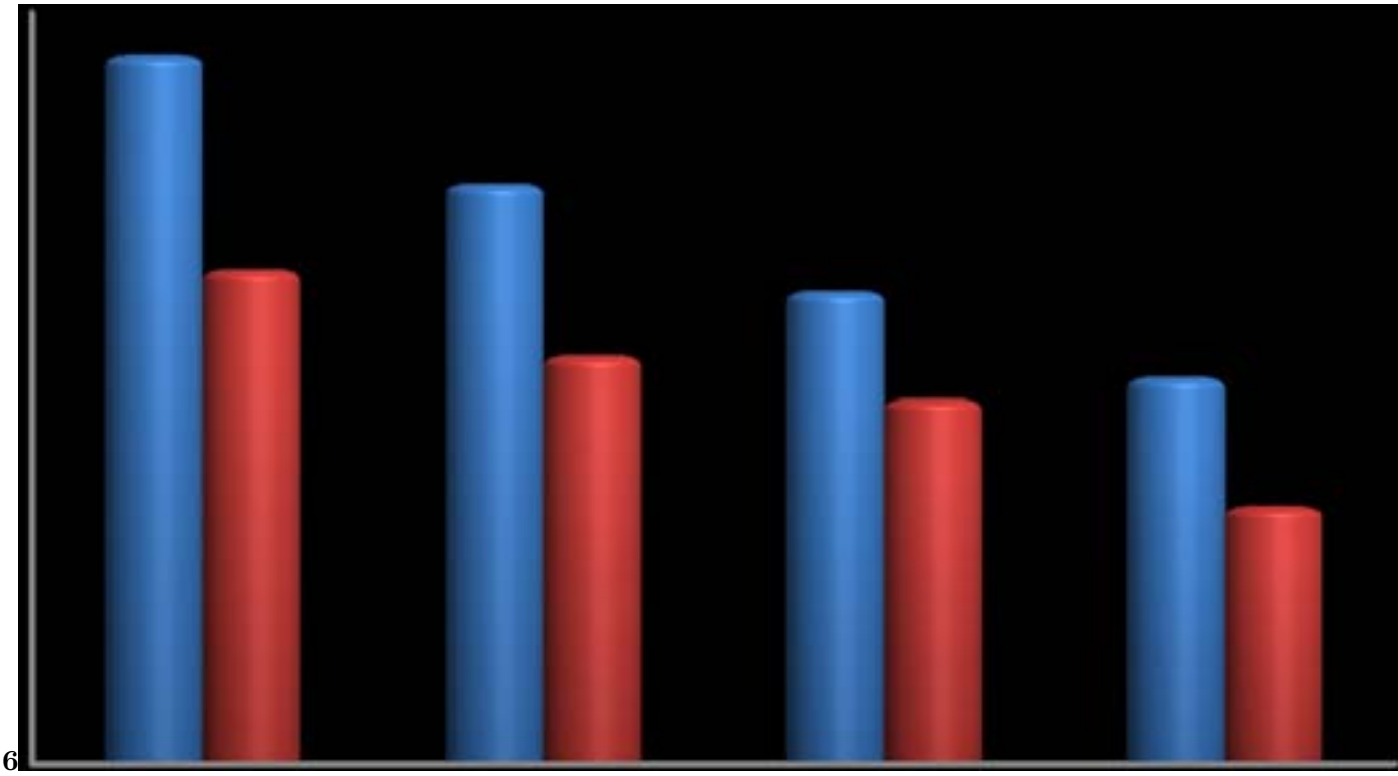


Figure 6: Fig. 5 :



6

Figure 7: Fig. 6 :

1

SL. No.	Soil Characteristics	Amount
1.	Colour	Pale Brown
2.	Texture	Clay Particles
3.	Temperature	47 0 C
4.	pH	7.31
5.	Moisture	40.03%
6.	Organic Carbon	0.30%
7.	Organic Matter	0.420%
8.	Organic Nitrogen	0.080%

Figure 8: Table 1 :

2

SL. No.	Name of the organisms
1.	Trichoderma viride
2.	Gliocladium virens
3.	Trichoderma harzianum
4.	Aspergillus niger
5.	Aspergillus flavus
6.	Aspergillus terreus
7.	Penicillium notatum
8.	Penicillium chrysogenum
9.	Rhizopus stolonifer
10.	Fusarium oxysporum

Figure 9: Table 2 :

3

SL. No.	Name of the organisms
1.	Cercospora janseana
2.	Entyloma oryzae

Figure 10: Table 3 :

4

Sl. No.	Growth response of the antagonist and the test fungus	T.viride	Antagonistic fungi tested (mm)		G.v
1.	Colony growth of the pathogen towards antagonist (mm)	8	9	10	12
2.	Colony growth of the pathogenic fungi growing away from the antagonistic fungi (mm)	20	10	15	13
3.	% growth inhibition of the pathogenic fungi near the zone of interaction (mm)	75.0	73.8	72.3	57.1
	Colony growth of the antagonist in Control, i.e.				
4.	Growth towards the center of the plate in the absence of the pathogen (mm)	30	28	24	35
5.	Colony growth of the antagonist towards the pathogen (mm)	20	18	16	15
6.	Colony growth of the antagonist away from the pathogen (mm)	40	42	34	23
7.	% growth inhibition in the zone of interaction	24.6	27.8	28.8	46.4

[Note: T.viride: Trichoderma viride, G.virens: Gliocladium virens, T.harzianum: Trichoderma harzianum, A.niger: Aspergillus niger, A.flavus: Aspergillus flavus.]

Figure 11: Table 4 :

5

Sl. No.	Growth response of the antagonist and the test fungus	Antagonistic fungi tested (mm) A.terreus			P.no
1.	Colony growth of the pathogen towards antagonist (mm)	15	11	10	18
2.	Colony growth of the pathogenic fungi growing away from the antagonistic fungi (mm)	24	15	14	27
3.	% growth inhibition of the pathogenic fungi near the zone of interaction (mm)	42.9	64.2	69.2	32.8
4.	Colony growth of the antagonist in Control, i.e. Growth towards the center of the plate in the absence of the pathogen (mm)	28	30	25	32
5.	Colony growth of the antagonist towards the pathogen (mm)	12	12	16	11
6.	Colony growth of the antagonist away from the pathogen (mm)	19	25	34	20
7.	% growth inhibition in the zone of interaction	64.3	42.9	37.8	63.7

[Note: The growth of Cercospora janseana towards the center of the plates in the absence of any antagonistic fungus (control) was 26 mm, measurement was taken within 72 hours. A.terreus: Aspergillus terreus, P.notatum: Penicillium notatum, P.chrysogenum: Penicillium chrysogenum, R.stolonifer: Rhizopus stolonifer, F.oxysporum: Fusarium oxysporum.]

Figure 12: Table 5 :

6

Sl. No.	Growth response of the antagonist and the test fungus	Antagonistic fungi tested (mm) T.viride			G.v
1.	Colony growth of the pathogen towards antagonist (mm)	8	9	10	12
2.	Colony growth of the pathogenic fungi growing away from the antagonistic fungi (mm)	20	10	15	13
3.	% growth inhibition of the pathogenic fungi near the zone of interaction (mm)	75.2	73.7	72.4	57.8
4.	Colony growth of the antagonist in Control, i.e. Growth towards the center of the plate in the absence of the pathogen (mm)	30	28	24	35
5.	Colony growth of the antagonist towards the pathogen (mm)	20	18	16	15
6.	Colony growth of the antagonist away from the pathogen (mm)	40	42	34	23
7.	% growth inhibition in the zone of interaction	24.6	27.8	28.8	46.4

[Note: T.viride: Trichoderma viride, G.virens: Gliocladium virens, T.harzianum: Trichoderma harzianum, A.niger: Aspergillus niger, A.flavus: Aspergillus flavus.]

Figure 13: Table 6 :

7

Sl. No.	Growth response of the antagonist and the test fungus	Antagonistic fungi tested (mm)			A.terreus P
1.	Colony growth of the pathogen towards antagonist (mm)	15	11	10	18
2.	Colony growth of the pathogenic fungi growing away from the antagonistic fungi (mm)	24	15	14	27
3.	% growth inhibition of the pathogenic fungi near the zone of interaction (mm)	43.0	64.6	69.9	32.4
4.	Colony growth of the antagonist in Control, i.e. Growth towards the center of the plate in the absence of the pathogen (mm)	28	30	25	32

Figure 14: Table 7 :

8

Sl. No.	Name of the Antagonist	on PDA medium Radial average growth of C.janseana (mm) at different c		
		5	10	15
1.	Trichoderma viride	10.4	7.6	4.2
2.	Gliocladium virens	12.8	10.4	3.0
	Trichoderma harzianum	14.2	12.4	10.9
4.	Penicillium chrysogenum	12.8	11.6	10.3
5.	Penicillium notatum	16.3	14.2	12.3
6.	Aspergillus niger	19.3	16.5	10.3
7.	Aspergillus terreus	18.4	15.4	14.2
8.	Aspergillus flavus	23.6	20.4	18.6
9.	Rhizopus stolonifer	10.0	12.0	10.2
10.	Fusarium oxysporum	30.2	28.3	22.4

Figure 15: Table 8 :

9

Sl. No.	Name of the Antagonist	Radial average growth of E.oryzae (mm) at different conce		
		5	10	15
1.	Trichoderma viride	10.4	7.6	3.8
2.	Gliocladium virens	12.8	10.4	6.4
3.	Trichoderma harzianum	14.2	12.4	8.2
4.	Penicillium chrysogenum	12.8	11.6	10.1
5.	Penicillium notatum	16.3	14.2	7.6
6.	Aspergillus niger	19.3	16.5	9.8
7.	Aspergillus terreus	18.4	15.4	14.3
8.	Aspergillus flavus	23.6	20.4	15.3
9.	Rhizopus stolonifer	10.0	12.0	6.8
10.	Fusarium oxysporum	30.2	28.3	20.0

Figure 16: Table 9 :

10

Sl. No.	Hours	Zone of Inhibition of Carbendazim (mm)	Zone of Inhibition of Mancozeb (mm)
1.	72	34 ± 0.1	24 ± 0.3
2.	120	27 ± 0.2	20 ± 0.2
3.	168	20 ± 0.8	18 ± 0.5
4.	216	18 ± 0.9	15 ± 0.7
		Values are expressed as Mean ± Standard Deviation	

Figure 17: Table 10 :

11

Sl. No.	Hours	Zone of Inhibition of Carbendazim (mm)	Zone of Inhibition of Mancozeb (mm)
1.	72	32 ± 0.1	22 ± 0.1
2.	120	26 ± 0.2	16 ± 0.2
3.	168	19 ± 0.8	12 ± 0.8
4.	216	17 ± 0.9	10 ± 0.9

[Note: are expressed as Mean ± Standard Deviation]

Figure 18: Table 11 :

Peak

#	Ret. Time	Area	Height	Area%	Height%
1	3.116	1815055	270073	31.754	47.834
2	3.262	3897232	294400	68.181	52.143
3	4.757	3700	130	0.065	0.023
Total		5715987	564603	100.000	100.000

Figure 19: Table Peak

This research work can be extended or studied further shortly by understanding the antagonistic mechanism in depth, improvement of strains and development of additional products of fungi biocontrol agents for the control of plant pathogens. Measures can also be taken for improving the potential of these agents by continual improvement in isolation, formulation and application methods, particularly in the field of crops.

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