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Antifungal Activity of Trichoderma Viridae, Calotropis Gigantea Extract Against Fungal Pathogens of Jute Plant.

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Abstract

Jute is infected by more than 12 types of phytopathogenic fungi. Charcoal rot, anthracnose and Fusarium rot are major jute diseases of eastern Nepal. Hence, the objective of this study was to control three fungal pathogens viz; *Macrophominia phaseolinia, Fusarium solani and Colletotrichum species* using *Trichoderma viridae* and *Calotropis gigantea* extract. All fungal pathogens were isolated from jute field. Occurrence of each disease was checked. *Calotropis gigantea* extract as well as *Trichoderma* showed good antifungal activity. In this study, 7% methanolic extract solution of *Calotropis* showed 43.6% inhibition of *Colletotrichum*, 38.91% inhibition on *Fusarium solani* and 37.81% inhibition on *Macrophominia phaseolinia*. Similarly, *Trichoderma viridae* inhibited the *Fusarium solani* growth by 51.33%, *Macrophominia phaseolinia* growth by 39.50% and *Colletotrichum* growth by 36.12%. The antifungal activity of Calotropis extract against test and control was statistically significant ($p<0.001$). It is concluded that biological control agents like *Trichoderma viridae, Calotropis gigantea* can effectively reduce the fungal phytopathogens of jute and can be used as good alternatives to fungicides in farming.

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1. Introduction

Biological control of plant diseases includes the use of beneficial microbes and plant extracts to control phytopathogens which can be alternative to replace chemical pesticides [1]. Different mode of actions of biocontrol-active microorganisms in controlling fungal plant diseases include hyper parasitism, predation, antibiosis, cross protection, competition for site and nutrient and induced resistance [2]. Soil borne plant pathogenic fungi cause heavy crop losses all over the world. Numerous pests and pathogens play destructive activity in agriculture that leads to severe crop loss [3]. Jute is one of the most important fiber and cash crop of Nepal. Jute fiber is quite famous for its versatility, durability and used extensively for making fabrics [4].

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Jute plants suffer from more than 12 different diseases of which 10 are known to be seed borne [5].

Seed borne diseases like anthracnose, black band and stem rot caused by *Colletotrichum corchori*, *Botryodiplodia theobromae* and *Macrophomina phaseolina*, respectively have great negative role in reducing jute production [6]. The diseases are compelling crop to suffer are seedling blight, stem rot, leaf blight, root rot (*Macrophomina phaseolina*), anthracnose and leaf mosaic [7].

Biological control of soil-borne plant pathogens is a potential alternative to the use of chemical pesticides, which have already been proved to be harmful to the environment. Several strains of the fungus Trichoderma have been isolated and found to be effective biocontrol agents of various soil-borne plant pathogenic fungi under greenhouse and field conditions [8]. Trichoderma spp. are common

saprophytic fungi found in almost any soil and rhizosphere microflora and have been investigated as potential biocontrol agents because of their ability to reduce the incidence of diseases and common soil borne pathogens [9,10,11].

Strains of T. viride, Aspergillus niger (Strain AN-27) and some species of fluorescent Pseudomonas have been established as very effective biocontrol agents for stem and root rot in jute [7]. Study has shown that *Calotropis gigantea* solvent extracts effects on the growth of pathogenic fungi [12]. Therefore, the main aim of this was to determine antifungal activity of *Trichoderma viridae*, *Calotropis gigantea* extract against fungal pathogens of jute plant.

2.Materials and Methods

2.1 Sample Collection

Samples for the isolation of phytopathogenic fungi were collected from the jute field of Jute Research Program, Itahari, Nepal located at 110m from sea level. Suspected leaves and stems showing the symptoms of Charcoal Rot, Fusarium wilt and anthracnose were collected in sterile plastic bags. Around 5 gm Soil samples were collected for isolation of Trichoderma from six different plots of Jute field. Both type of samples were transported in ice box to microbiology laboratory of Central campus of Technology and processed within 2 hours.

2.2 Isolation and Identification

The collected plant samples were washed with sterile water followed by the surface sterilization with 1% sodium hypochlorite (HiMedia, India). Then, leaf and stem samples were cut into the pieces of size 1 cm. The sterilized pieces were cultured on potato dextrose agar (PDA) (HiMedia, India) at 25°C for 5 days.

1 gram of soil was taken from each sample and serially diluted in distilled water to 10^{-7} dilution. About 1 ml aliquot was taken from 10^{-5} and 10^{-6} dilution and spread on PDA and cultured at 25°C for 5 days. After that, the growing hyphal tips were excised and transferred to a new PDA medium to have pure cultures. Identification of the fungal cultures was done according to standard microbiological procedure [13].

2.3 Preparation of Plant Extract

C. gigantea leaves were collected from the different localities of Dharan Sub-Metropolitan City. Identification of plant was done with the help of Department of Botany, Central Campus of Technology. Dharan *C. gigantea* leaves were brought and shade dried for 2 weeks. Dried leaves were surface sterilized with 1% sodium hypochlorite. Leaves were washed with distilled water to remove excess sodium hypochlorite and dried in oven at 40°C to remove moisture. Dried leaves were powdered using the electric grinder. Powdered leaves of *C. gigantea* were soaked in methanol for seven days at room temperature and then filtered with autoclaved muslin cloth. The obtained filtrate was evaporated on rotary shaker and methanolic leaf extract of *C. gigantea* was obtained as gummy mass.

2.4 Determination of Phytochemical Constituents

A small portion of the dry extracts were subjected to the phytochemical test using methods to test for alkaloids, tannins, saponins, flavonoids and glycosides [14].

2.5 Antifungal Bioassay

Stock solution (20%) was prepared by the re-suspension of Methanolic gummy mass of *C. gigantea* leaves in distilled water. Five different concentrations of Potato Dextrose agar (PDA) (HiMedia, India) viz. 1.0, 2.5, 4.0, 5.5, and 7.0% were prepared by adding required amount of stock solution to PDA medium after autoclaving.

To avoid bacterial contamination 4 mg of chloramphenicol (HiMedia, India) was added in 1000ml of culture medium. Experiment was conducted in petri plates with three replicates for each concentration.

Five millimeter disks were cut from seven days old culture of *M. phaseolina, Fusarium solani and Colletotrichum sp.* with the help of surface sterilized cork borer and one disk was placed in the center of each petri plate. These plates were incubated at 25°C for five days in an incubator. Then after, fungal colony diameter in each plate was measured and percentage decrease in colony diameter of pathogens due to various extract concentrations over control was calculated using the following formula:

$$\% \text{ GOI} = \frac{\text{Growth in control} - \text{growth in test}}{\text{Growth in control}} \times 100$$

Where, GOI = Growth of inhibition

2.6 Dual culture assay using *Trichoderma*

Disc of 6 mm was taken from 5-day old PDA cultures of each *Trichoderma viridae* isolate and placed at the periphery of the PDA plates. 6 mm disk was taken from 5-day old culture of each fungal pathogen and placed on the opposite peripheral end. As a control Trichoderma was placed in similar manner on fresh PDA plates. Antagonistic activity was tested 5 days after the incubation by measuring the radius of pathogens colony in the direction of antagonistic Trichoderma colony. Distilled water was used as negative control. This study was carried out for three replications [15].

Growth inhibition is measured using formula:

$$\% \text{ GOI} = \frac{\text{Growth in control} - \text{growth in test}}{\text{Growth in control}} \times 100$$

Where, GOI = Growth Growth of inhibition

3. Data analysis

Data entry, checking and validation were done. All data were entered in MS Excel 2010 and finally analyzed by SPSS Software version 16.0. The p-value <0.05 was established statistically significant.

4. Results

4.1 Screening of plant Pathogenic fungi

The study was done in Central Campus of Technology. All the samples for study were obtained from jute field of Itahari. Out of 500 plants observed 40 plants showed the symptoms of diseases.

Samples were taken from stem and leaves of plant. Out of 11 samples, *Macrophominia* was isolated from 5 samples. Similarly, 20 plants showed the symptoms of Fusarium wilt. Out of the 20 samples taken *Fusarium solani* was isolated from 3 samples. Anthracnose was observed in 9 plants out of which *Colletotrichum species* were obtained from 4 plants.

4.2 Phytochemicals present in *Calotropis gigantea*

Calotropis gigantea plant is rich in various bioactive compounds. Tests were done to check the presence of different phytochemicals like alkaloids, saponins, cardiac glycosides and flavonoids.

Phytochemical tests showed the presence of glycosides and saponins whereas alkaloids, tannins and flavonoids were not detected (Table 1).

Table 1.Presence of different Phytochemicals

Compounds	Test result	Result
Alkaloids	Brown/reddish ppt. not formed	Absence of alkaloids
Tannins	Dark green solution not obtained	Absence of tannins
Flavonoids	Yellow ppt. was not obtained	Absence of flavonoids
Glycosides	Orange red ppt.	Presence of glycosides
Saponins	Creamy mix of small bubbles were appeared	Presence of Saponins

Fig.1a.Stem of infected jute plant with typical symptom of charcoal rot.

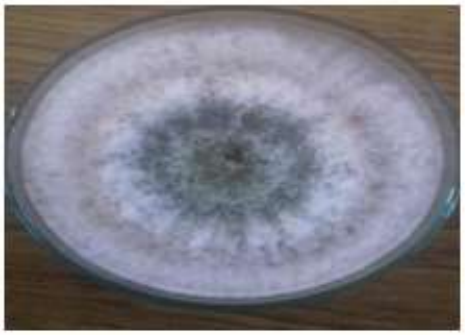

Fig.1b. 7 days old culture of *Trichoderma viridae*

Fig.1c. Growth inhibition by methanolic extract of *Calotropis gigantea* leaves.

4.4 Dual Culture Technique

The antagonistic *Trichoderma viridae* was evaluated for its antagonistic effect against *M. phaseolinia, Colletotrichum species and Fusarium solani* under in vitro conditions by dual culture technique as explained in materials and methods. Length of fungal colony on test was recorded, percent inhibition was calculated and results obtained are presented in Table 2.

4.5 Evaluation of *Calotropis gigantea* extract against pathogens

The methanolic extract of *Calotropis gigantea* was evaluated for its antifungal activity under in vitro conditions by poisoned food technique. Study was done using the concentrations 1%, 2.5%, 4%, 5.5% and 7% of methanolic extract. The results are presented in the bar diagram (Figure 2).

Table 2. Bio efficacy of *Trichoderma viridae* against *Macrophominia phaseolinia, Colletotrichum Species and Fusarium solani*

Isolate (M)	RG (mm)	C (mm)	% I	Isolate (C)	RG (mm)	C (mm)	% I	Isolate (F)	RG (mm)	C (mm)	% I
M1	31.22	51.61	39.50%	C1	31.13	48.43	35.945%	F1	23.98	49.1	51%
M2	32.88	48.33	31.96%	C2	33.20	49.37	32.72%	F2	22.07	45.14	51.33%
M3	35.93	53.08	32.33%	C3	35.26	50.40	30.28%	F3	27.53	47.93	43.40%
M4	31.23	50.78	38.43%	C4	31.94	50	36.12%				
M5	29.98	49.25	39.1%								

Note: M- *Macrophominia phaseolinia*, C- *Colletotrichum* Species, F- *Fusarium solani*.
RG- Radial growth, % I- Percentage Inhibition, C- Control

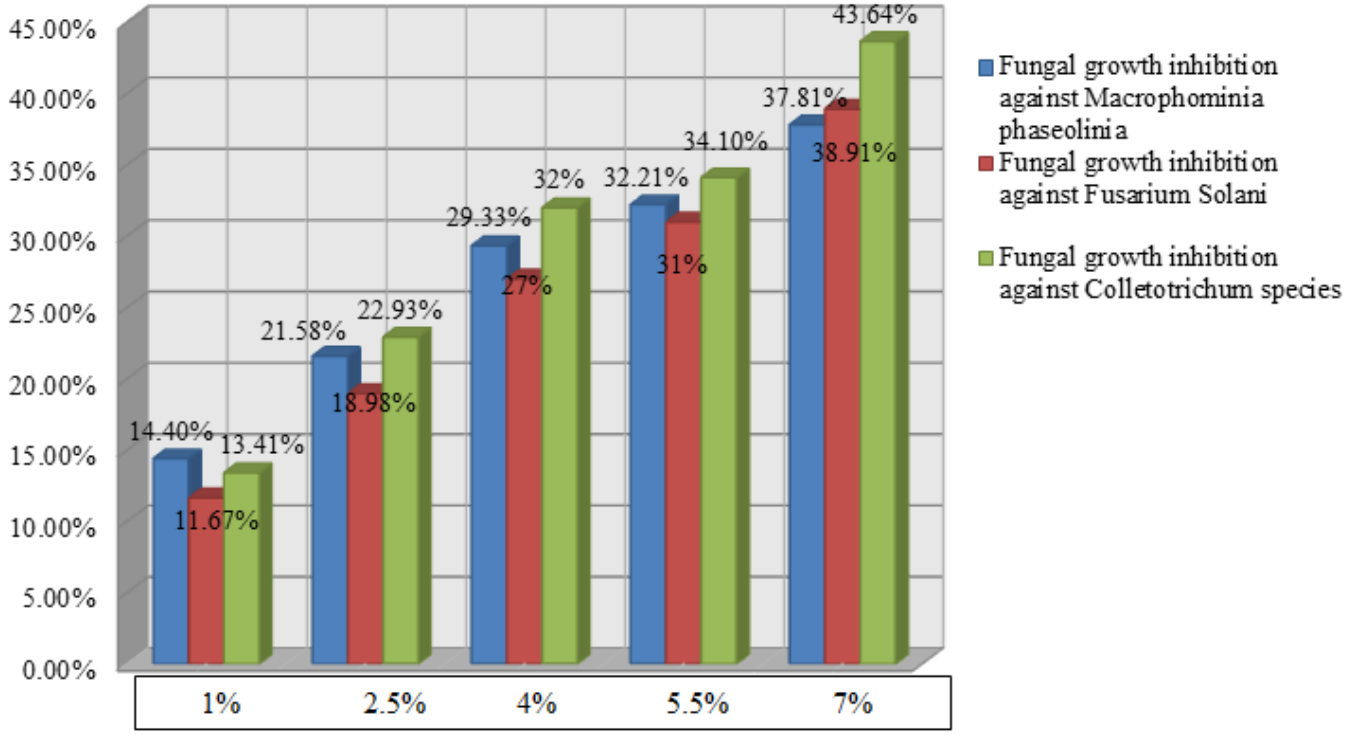

Fig.2. Evaluation of Calotropis extract against phytopathogens

5. Discussion

Jute (*Corchorus olitorius and C. capsularis*) is an important fiber crop next to cotton. Fiber produced from jute is durable and biodegradable. A number of phytopathogens attack on jute causing a minor harm to death of plant[16]. Charcoal rot is also referred to as dry weather wilt or summer wilt, because the plant symptoms appear under heat and drought stresses [17]. During the present study periods, three phytopathogenic fungi causing charcoal rot, anthracnose and Fusarium wilt were isolated from the Jute plants. *Macrophominia phaseolinia* was found to be associated with charcoal rot disease. Similarly, *Colletotrichum* species were found to be responsible for anthracnose and *Fusarium species* were found to be associated with wilt.

Trichoderma spp. are common saprophytic fungi found in almost any soil and rhizosphere microflora and have been investigated as potential biocontrol agents because of their ability to reduce the incidence of diseases and common soil borne pathogens [9, 10,11]. To study the antagonistic action of Trichoderma with fungal pathogens, *Trichoderma viridae* was isolated and studied. As these fungi are soil and seed borne antagonistic action of *Trichoderma viridae* was tested against them. Strains of T. viride, have been established as very effective biocontrol agents for stem and root rot in jute [7,18]. Seed and soil borne are difficult to control with commercial fungicides. So, the use of antagonistic soil fungi can be helpful in controlling plant disease. Formulation and use of Trichoderma with seeds before sowing helps to decrease the loss caused by fungi. The Highest growth inhibition was seen in *Colletotrichum species* by 43.64%, Fusarium solani by 38.91%, *Macrophominia phaseolinia* by 37.81% using the extract of 7% *Calotropis* extract concentration. Also the control on

growth at 5.5% concentration was satisfactory. As the *Calotropis gigantea* is easily everywhere it can be purified and used for controlling fungal pathogens of plants [19].

Most of the fungal infection in plant occurs due to water stress. Studies have showed that management of different pathogenic fungi of jute can be done by frequent irrigation of fields. Some plant pathogenic soil fungi have a complex relationship with the host, and infection may be hampered at low soil moisture, while high soil moisture may reduce symptom expression and improve yields [20]. Synergistic use of control agents can help in better control of these phytopathogens. Destruction and removal of infected plant or early treatment can reduce the spread of jute disease. Crop rotation also may reduce the occurrence of the disease. However, the sclerotia of fungi can remain dormant in soil for long time. So, crop rotation doesn't effectively protect the crop. Use of synthetic chemicals not only hampers the beneficial organisms but also causes negative impacts on human health. Now, more emphasis should be given in the control of fungi using biological control agents and plant products.

6. Conclusion

From this study it is concluded that *Trichoderma viridae, Calotropis gigantea* are an effective biological control agent which is ecofriendly and beneficial from the farming perspective in comparison to chemically synthesized fungicides to control pests of jute plants. Moreover, it is harmless and nonpathogenic to humans and animals. It is concluded that biological control agents and plant products can effectively reduce the fungal population and can be used as alternatives to chemical fungicides in farming. The need for commercial formulation of these biopesticide and its rational use are essence to elevate agriculture sectors and ecosystem.

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