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Culture and application of *Trichoderma* species from Soil Sample of Ambikapur CG, India.

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ABSTRACT –

The antagonistic activity of the bio-control agent has long been recognized as an important factor in the management of soil borne plant diseases. Five of soil samples were collected from different parts of Chhatisgarh state in India to isolate *Trichoderma* species using serial dilution method. Soil samples containing *Trichoderma* species were analyzed for their pH , electrical conductivity, organic matter content , phosphorous and potassium values All seven isolates were further characterized by growing them at different temperatures (15^oC , 20^oC , 25^oC , and 30^oC) in vitro and in vivo antagonistic activity against *Rhizoctenia solani*. The casual agent of root rot of *Cicer arotinum* (gram) is discussed.

KEY WORDS

Soil fungus, *Trichoderma*, Fungus – purification, Bio – characterization of soil, Antagonism of fungi.

1. INTRODUCTION

The inappropriate and indiscriminate uses of fungicides and antibiotics have led to serious environmental threat to human life, specially in vegetables, spices, fruits and other cash crops because growers using large quantity of fungicides and antibiotics for the control of various diseases ignoring the persistency of these chemicals. The antagonistic activity of the bio control agents has long been recognized as an important factor in the management of soil borne diseases. *Trichoderma* species. is one of the important bio control agents and has been successfully used in the control of soil borne plant disease [1]

Bazgir and Okhovat (1996) reported the disease controlling potential by *Trichoderma harzianum* and *Trichoderma viride* in controlling *R. Solani* on beans (*Phaseolus vulgaris*). Both species of *Trichoderma* reduce the level of disease when added to soil one month before sowing.[1]

The potential of antagonistic organism is affected by several factors. Some of these are temperatures, pH, soil type and soil moisture. Jackson *et al* (1991) studied the effect of temperatures, pH and water potential on biomass production or hyphal extension of *Trichoderma* isolates and found that growth of all three isolates did not differ with temperatures and pH. Chhatisgarh have a wide crop diversity and several crops are being grown in the region however. Vegetable crops have got more area compared to the crops. Farmers are showing keen interest in using bio-control agents *i.e.* *Trichoderma* available in the area for the control of different diseases. Present research work was undertaken to isolate indigenous strains of *Trichoderma* and characterized them so that the mass scale production of effective and efficient strain of *Trichoderma* can be taken up to the farmers of Chhatisgarh region.[3]

There is a demand of this bio-control agent and several commercial formulations are available in the market. However, potential of commercially available *Trichoderma* in controlling diseases is found to be less due to one or two reasons. The viability of *Trichoderma* has been found to reduce due to long travel and poor transportation facilities. It has also been observed that local strains have got more potential to control the diseases as compared to strains of other area. It is also advisable to use local strains rather than strains of different area since, soil and climate affect the multiplication and disease controlling ability of the antagonist.[4]

2. MATERIAL AND METHOD-

1. SOIL SAMPLES COLLECTION AND ISOLATION –

The soil samples were taken from 0-5 cm soil depth from 05 places of a particular field and mixed together making one soil sample. All samples were brought the laboratory and analyzed using serial dilutions following Dhingra and Sinclair, 1992 [6]. One g soil sample was used for the isolation of *Trichoderma* isolates. After preparation of each soil sample streptomycin (30 ppm) and copper oxychloride (100 ppm) were added into 10 ml Soil solution.. 0.1 ml solution from it was added in Petri dishes having potato dextrose agar medium (Dhingra and Sinclair, 1992) and petri dishes were incubated at 25^oC₊₂. After three to five days of incubation, single colony was transferred to another petri dish and again incubated at 25^oC_{±2} for seven days. Pure culture of all isolates was stored in refrigerator and used for further study.

Figure 1 smashing of boiled potato and figure 2 represents to filtering of smashed figure 3 represents to mixing of dextrose and agar , figure 4 depicts to packing of PDA medium for sterilization figure 5 represents to sterilization process operating an autoclave. figure 6 represents to mixture of collected soil sample, figure 7 represents to incubation of soil sample figure 8 represents to mother culture and a fully grown *Trichoderma* sps is depicted in figure 9.



Figure 1 Smashing Potato



Figure 2 Composition PDF

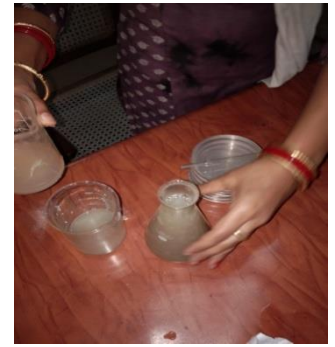


Figure 3 Composition PDF



Figure 4 Packing of PDF



Figure 5 Sterilization of PDF



Figure 6 Soil sample

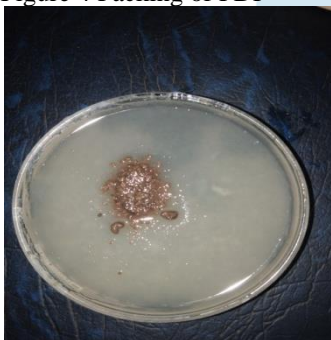


Figure 7 Incubation of Soil



Figure 8 Mother Culture

Figure 9 *Trichoderma*

2. ANTAGONISTIC ACTIVITY OF ISOLATES OF TRICHODERMA AGAINST RHIZOCTONIA SOLANI

The test pathogen *Rhizoctonia solani* was isolated from the root rot samples of gram from Jashpur area and maintained in potato dextrose agar medium. Mycelial discs of *R. solani* and – *Trichoderma* isolates were inoculated on P.D.A. medium at the opposite ends of petri dishes and incubated at $25^{\circ}\text{C} \pm 2$. Mycelial growth (in mm) of both pathogen and antagonist was recorded after 48 hr and 96 hr of incubation. Hyper parasitic activity of each isolate was recorded by measuring the over growth of *Trichoderma* isolates on *R. solani*.

In vivo, sick soil was prepared by mixing the inoculums of *R. solani* (multiplied on half boiled rice grains) in the soil 5g / pot and incubated for seven days. In these plastic pots (containing sick soil infested with *R. solani*) , different isolates of *Trichoderma sps.* are shown for testing of the disease controlling potential. Three treatments control included in the experiment. First ,control untreated seeds of gram were sown in pots having sick soil (control inoculated) .Second , control untreated seeds were shown in the pots having inoculums free soil. and third, control seeds treated with hexaconazole, an effective fungicide (Tiwari, 1995) were sown in pots having sick soil (control protected). Ten seeds were sown in each pot with four replications. Pre- emergence and post emergence mortality was recorded for the different isolates including control.

3. RESULT AND DISCUSSION --

1. EFFECT OF DIFFERENT TEMPERATURE ON THE MYCELIAL GROWTH OF TRICHODERMA SPS.s.-

Data presented in Table 1 indicated that, influence of different temperatures (15°C , 20°C , 25°C , and 30°C) on mycelial growth of *Trichoderma sps.* Was varied. Growth of all isolates was faster at same at 25°C and 30°C and took 96 hrs. to complete. However, growth rate of all isolates was faster at 30°C . Whereas, at low temperatures (15°C), isolate no.1 grew faster compared with other isolates. Isolate no. 2, 3, 4 and 5 found to be sensitive at low temperatures. Growing trend of different isolates

At different temperatures are therefore indicated and that isolate can be successfully used as bio-control agent effect on biomass production. Jackson *et. al* (1991) reported that, temperature and pH had significant occurred between 20°C , and at pH range between 4.6 and 6.8. Two isolates of *T. viride* grew at 50°C but no isolates grew at 40°C .

Table 1: Effect of different temperature on the mycelial growth (Length in mm) of different isolates of *Trichoderma* sps.

Isolates / Length	15°C		20°C		25°C		30°C	
	72hrs	96hrs	72hrs	96hrs	72hrs	96hrs	72hrs	96hrs
T 1	30.mm	45mm	64mm.	90mm	61mm	74mm	46mm	72mm
T 2	25mm	41mm	50mm	81mm	36mm	51mm	47mm	75mm
T 3	24mm	40.mm	50.mm	77.mm	38mm	54.mm	46.mm	79mm
T 4	32mm	49mm	48.mm	85mm	45mm	60.mm	42mm	83mm
T 5	23mm	39.mm	46mm	74mm	41mm	56 mm	46mm	85mm

2. *IN VIVO* ANTAGONISTIC ACTIVITY OF ISOLATES OF TRICHODERM SPS. AGAINST RHIZOCTONIA SOLANI AT 25°C

Data presented in Table 2 on antagonistic activity indicated that, all isolates could restrict the growth of *R.solani* to its half growth stage.. Hamed *et al* (1996) studied the efficiency of antagonistic microorganisms as biological agents causing wilt and root-rot. *Trichoderma harzianum*, which showed strong *in vitro* antagonism to *Corticium rolfsii*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. sesami* and *F. oxysporium*, were evaluated. *T.harzianum* was the most antagonistic microorganism to wilt and root-rot disease pathogens. The application of *T.harzianum* to sesame reduced infection by the pathogenic fungi.[4]

Table 2 : Mycelial growth (mm) of *Rhizoctonia solani* and isolates of *Trichoderma* sps. after different incubation periods.

Isolate	5days		6days		7days		8days	
	<i>R.solani</i>	<i>Trichoderma</i>	<i>R.solani</i>	<i>Trichoderma</i>	<i>R.solani</i>	<i>Trichoderma</i>	<i>R.solani</i>	<i>Trichoderma</i>
T1	59mm	40mm	63mm	49mm	73 mm	67 mm	83 mm	86 mm
T2	56mm	44mm	66mm	52mm	68mm	62mm	77mm	73mm
T3	58mm	48mm	68 mm	57 mm	70 mm	66 mm	80 mm	75 mm
T4	60 mm	45 mm	70 mm	57 mm	80 mm	66 mm	88 mm	75 mm
T5	58 mm	46 mm	68 mm	61 mm	78 mm	73 mm	89 mm	80 mm

Bazgir and Okhovat (1996) reported the disease controlling potential of *Trichoderma harzianum* and *T. viride* in controlling *Rhizoctenia solani* on beans (*Phaseolus vulgaris*). Both species of *Trichoderma* reduced the level of disease when added to soil one month before showing. *In vivo*, it was observed that all isolates could delete control the mortality of gram seedlings caused by *R. solani*. However *Trichoderma* isolates no 1 and 2 were found to be more effective in controlling dry root rot of chickpea caused by *Rhizoctonia solani*. *Trichoderma* isolates no 3 was showing weak antagonistic activity against the pathogen as comparatively higher mortality per cent was recorded (Table 3) *in vitro* it was observed that isolate no.5 possessed high antagonistic activity while it did not control the disease effectively compared to other isolates, his might be due to weak multiplication in the soil.

Table 3 : Mortality percentage of *Cicer arotinum* (gram) seed treated with different isolate of *Trichoderma* spp. against. *R. solani*.

<i>Trichoderma</i> Isolates	Mortality per cent		
	After 10 days	After 20 days	After 30 days
T1	10	15.	32.
T2	35.	62	67.
T3	30	75.	80
T4	20	25	50
T5	17.	35	40

3. STRUCTURE OF TRICHODERMA SPECIES:

Figure 9 represents the structure of the thallus of the *Trichoderma*. It is composed of well – developed mycelium of septate-branched hyphae. The hyphae may be simple and short at earlier stage, or long and much branched at maturity i.e. conidial stage. The hyphal cells are usually multinucleate. They possess perforated septa. Through these perforations cytoplasmic streaming and nuclear migration take place from cell to cell. The nature of septa is very much similar to that of the Ascomycetes which suggests affinities between the deuteromycetes and the Asciomycetes.

Due to lack of information about the normal sexual stage thes fungus can not assigned to the three main classes of fungi which have already been described. Since these fungi apparently lack sexual stage, they are called fungi imperfecti or deuteromycetes.

According to the rules of nomenclature, a name based on the perfect stage always takes precedence over one based on an imperfect stage, regardless of which name is the older.

The conidial forms produce on conidiophores arising directly from the somatic hyphae which are bright – coloured. The somatic hyphae are loose separate; innate and closely aggregated to form sporogenous structures.

In the loose hyphal forms, conidiophores grow at separate treads. Conidia are borne externally on the conidiophores which are in cluster

The complexity of soil environment and the diversity of its population make sampling and assessment of the microbial population very difficult. There are three main methods : cultural studies, direct examination, and activity measurement. No information on the species of organisms is obtained by these methods. Cultural examination method is time consuming and it is difficult to locate and count organisms in opaque soils. Activity measurement study is even more difficult and inconclusive .

The behavior and distributional pattern of micro – organisms depend on the nutrients available, temperature, moisture, gas content and depth of soil. Generally there is decrease in numbers with depth.

The microorganisms that inhabit the soil exhibit different types of associations interactions which depend upon the biotic components of soil. An association is **natural** when two different species of microorganisms occupy the same environment without affecting each other and without producing metabolic end products that are inhibitory. When an association involves symbiotic relationship between two species it is called **symbiotrophism**. A relationship between organisms in which one partner receives benefit, the other is not affected is designated as **commensalism** encountered in association between fungi and bacteria, where fungi breakdown cellulose to glucose. Many bacteria are unable to utilize cellulose, but they utilize the fungal breakdown products of cellulose. The association is said to be antagonism when one species affects the environment for another species by producing antibiotics or other inhibitory substances. There may be competition among species. A relation between microorganisms may also be parasitic when one organism lives in or on another organism encountered in case of Gram – negative bacteria *Bdellovibrio bacteriovorus* which is widespread in soil and sewage. Again viruses which attack bacteria, fungi, and algae are intracellular parasites.

Isolation experiments suggest that in general, species of *Mucor*, *Penicillium*, *Trichoderma* and *Aspergillus* predominate and those of *Rhizopus*, *Zygomycetes*, *Fusarium*, *Cephalosporium*, *Cladosporium* and *Vorticillium* are also often present in the soil. Addition of organic matter to a soil stimulates the growth of fungal flora in soil in the same way as it does the zymogenous bacterial population. The aerobes and their mycelium is interwoven among, the soil particles and it binds these together, thus improving the texture of soil. Again the major part of the fungal flora occurs in the upper soil horizons where there is most organic matter.

Algal species living in soil include flagellate, coccoid, or filamentous ones. Some of the common algae are: *Oscillatoria*, *Nostoc*, *Anabaena*, *Cylindrocapsa*, *Chlorococcum*, *Chlorella* and certain diatoms (e.g. *Hantzschia* and *Navicula*). The growth of soil algae affects the surface soil by depletion of some nutrients. The algal biomass on surface soil is great. The soil fauna contains numerous protozoa and representative of metazoa. The smallest species, protozoa and nematodes are widely distributed in the soil. The former generally occur in the water film surrounding soil particles and the latter usually in the soil of the upper horizons among plant roots. The more micro – organisms there are in the soil, the more productive it is. These soil inhabiting microorganisms play important roles in the soil. Some of them are :

- (a) decomposition of organic matter, transformations in the soil and maintenance of soil fertility balance;
- (b) improvement of soil fertility, reclamation of barren soil and check soil erosion; and
- (c) biological control of disease and afforestation.

4 CONCLUSION:

We conclude from the present study that isolate no1 can be promoted for mass production as bio-control agent as it grew well not only at all temperatures, but also has higher antagonistic activity and disease controlling potential *in vitro* and *in vivo* against *Rhizoctonia solani* causing root rot in chickpea against *R. solani* . Isolation of isolates of *Trichoderma* from the other parts of Chhattisgarh state will lead to identification of more potential isolates and which can be useful in controlling the diseases of different crops in the region.-

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