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Antagonistic Assessment of *Trichoderma* spp. Against *Fusarium oxysporum* and *Aspergillus niger*

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Abstract

Many species of genus Trichoderma are used as an important source of biological agents.The potential efficiency of Trichoderma against the pathogenic fungi like Fusarium oxysporum and Aspergillus niger was evaluated on the fungal growth by cultural pattern in which radial growth extension rates were analysed. Isolation of Trischoderma spp. was made by the serial dilution technique of the soil sample. After 72 to 96 hour later, colonies of Trichoderma were seen on Petri dishes. The radial growth of experimental fungi viz. A. niger (66.24%) and F. oxysporum (52.4%) were inhibited by Trichoderma spp. due to secretion of antifungal compound and the nature of mycoparasitism, antibiosis and competition with tested fungi.

Key words : Trichoderma, Pathogenic fungi, Antifungal, *Asperillus niger, Fusarium oxysporum.*

Cereals, pulses, vegetable and other economic important crops are very essential for human beings as well as ecosystem. Fungi are a division of parasitic plant or plant like organisms. More than 10,000 species of fungi exist all over the world. It is a big challenge for the higher plants to survive healthy, because fungi have parasitic nature. Fungi present in every place where life is possible such as in soil, in water inside the tissue and on the animal skin. Fungi develop diseases depend on plant and species of fungi which cause a disease. For the control of fungus we generally use fungicide and several other chemicals but the fungicide affect the environment and play a major role in mutation in microorganisms and other flora and fauna. On the other hand we also go for disease resistant crop varieties. But the procedure of resistant variety development is time consuming process and take many months to years. Some ethical issues associated with GM crops. Another alternate source for the disease free crop is biological control. It is more effective and eco-friendly compare to

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the chemical pesticides. The word *Trichoderma* is derived from the Greek words trix and derma, which both imply skin with hair. All around the world, they are allowed to live in soil in both agricultural and natural settings. Trichoderma species are fungi that benefit farmers by improving crop yields and income while preserving the soil ecosystem. Products for sale that include unique strains of living Trichoderma species, primarily *Trichoderma harzianum*, have been produced for plant protection. They have been created in a way that makes it simple for farmers and producers to use them in the field. It is a biocontrol agent (BCA) that is risk-free and efficient for controlling a range of bacterial and fungal diseases, including downy mildew, stem rot, damping off, and wilt. However, *Trichoderma* works best in the soil and is a yield-booster that makes crops appear healthier, grow larger, and demonstrate superior root development. It is a biological degrader and competitor of fungal plant pathogens, which has evolved mechanisms for attacking other fungi in the root zone of a plant. Beyond that it also improves nutrient uptake of plants [4]. The genus *Trichoderma* comprises a large number of rhizocompetent filamentous fungal strains found in a large variety of ecosystems. Sometime *Trichoderma* act as a symbiont due to the nature of colony formation in root system of host plant. Some proteins like an expansin, Swollenin are helps to degrade or loosening the cell wall of host plant and some other proteins like Hydrophobin are helps to adhere on root surface[1].

Genus- *Fusarium :*

Genus *Fusarium* is a member of hyphomycetes occurs in soil and coupled with plants. *Fusarium* is a nacrotropic fungi it cause many disease in forest, agricultural and economically important crops. *Fusarium* may be survived in moderate climate[7,10,17]. *Fusarium* can survive on plant residue and act as a inoculum for next year[20]. A small number of species which are antagonistic to *Fusarium* among soil borne species. Several fungal species were examined for the ability to reduce the inoculum potential of *Fusarium* pathogens, mainly by reduction of biomass in plant residues colonized by *Fusarium*[3,9,11,12,13]. Some fungal species like *Gliocladium*, *Trichoderma* and species reduced the colonization of wheat and maize by pathogenic *Fusarium* species and suppressed the sporulation of the latter[12,22]. *Trichoderma* species have been examined for more than 50 years and are known as highly effective in biological control of a wide range of plant pathogens of soil origin. Hey are also known to produce over 120 secondary metabolites, in-cluding antifungal metabolites[5,22].

Genus- *Aspergillus :*

Aspergillus niger causes a disease in number of plants. It is a toxigenic fungi. When the *A. niger* grow on host plants it secretes a fungal products coarsely aflatoxins, which act as carcinogens[6]. Cereals can be very susceptible to toxigenic fungal growth in the field, during storage or during processing. Their presence in stored products can significantly decrease quality and economic value of the harvested grain. Commercial feedstuff in Argentina is an important component in modern animal husbandry. Their composition includes mixtures of home grown cereals mainly maize and other additives. It has been

demonstrated that in Argentina the most significant mycotoxin from poultry feeds was aflatoxin B1. The toxin levels found in the majority of the analysed samples were higher than the values of international regulations[2]. Control strategies are necessary to minimize the contamination in maize and poultry feeds. Microbial biocontrol agents have emerged as the most effective alternatives with several products on the market for controlling plant pathogens and pests of agricultural and horticultural crops pre- and postharvest. Yeasts may act as antagonistic micro-organisms thereby considerably decreasing the growth of filamentous spoilage fungi both in vitro and under conditions of storage[18,19,21]. Nevertheless some strains can increase aflatoxin production. According to Petersson[19], microbial agent with high potentiality of biocontrol should not enhance mycotoxin production during microbial interaction process. To select a promising biocontrol agent it is necessary to isolate micro-organisms from similar ecological niches and consider the relationship between microbial interaction and environmental factors. In the storage agro ecosystem, complex interactions occur between biotic and abiotic factors than impact on growth, toxin production and biological behaviours. Interactions between *Aspergillus* spp. and other fungal colonizers modify fungal growth of stored products in the absence of synthetic pesticides. Index of dominance (ID) showed the interspecific interactions between microbial species under a set of environmental conditions that interactions were shown profoundly influenced by water activity, temperature and nutrient substrate[8,15]. Changes in the environmental factors cause an impact that can be decisive to determine the coexistence level or dominance of species in a certain ecological niche[16].

Sample collection :

Soil sample are collected from various field; where vegetables crops are growing. Some samples were taken from Godavari farm house, Badi; here we take soil from rhizospheric zone of tomato and chilli. Some other samples were collected from Rajasthan Agriculture College Udaipur. Soil sample from botanical garden of Department of Botany also taken. Soil sample were placed into polybegs and stored at 4°C temperature until further processing.

Isolation of *Trichoderma* from the soil :

Isolation of *Trichoderma* spp. was made by the serial dilution technique of the soil sample. Composition of TSM media for one litre is shown in table-1.

Table-1. Chemicals required for composition of TSM media

Chemicals	**Amount**
Glucose	3.0 gm
Agar	15 gm
Chloramphenicol	0.25 gm
KCl	0.15 gm
NH_4O_3	1.0 gm
$MgSO_4$	0.20 gm
KH_2PO_4	0.90 gm
Rose bengal	0.15 gm
PNCB (Pentachloronit-robenzene)	0.3 gm
Distilled water	1 L

After 72 to 96 hour later, colonies of *Trichoderma* were seen on Petri dishes. These

colonies are purified by sub culturing on PDA (Potato Dextrose Agar) media. *Trichoderma* species was identified on the basis of their morphological characteristics. These identified and purified cultures are stored at 4ºC temperature for further process.

PDA media formation :

Firstly take a 200 gm peeled fresh potato and boil in double distilled water up to 400 ml aprox 30 minute and extract the potato extract using muslin cloth as a filter. Add agar and dextrose at appropriate amount and set the pH using 1N NaOH and 1N HCl. pH may be 5.6 and pour in 250 ml flask up to 150 ml. Add powder of antibiotic tablet in media for prevention of bacterial contamination. Provide steam sterilization using autoclave on 121ºC and 15 psi pressure for 15 minutes. For the formation of PDA chemicals are required and those are tabulated in table-2.

Table-2. Chemicals required for the composition of PDA

Chamicals	**Amount (1 Lit)**
Agar	18 gm
Dextrose	16 gm
DDW	1000 ml
1N NaOH	10 ml
1N HCl	10 ml
Chlortetracycline	40 mg

Sterilization and Inoculation :

For fungal culture we must require aseptic condition. Previously washed glass vials and PDA are full with microbes and virus. These microbes and virus affect the growth of fungal pathogen. So firstly, all the microbes should remove from all the glass vials and PDA media; these purpose we use autoclave (an instrument which provides steam sterilization) for removal of all contaminators at 121ºC temperature and 15 psi pressure for 15 minutes.

Inoculation is the next step which is done under an instrument called laminar air flow chamber. LAFC provide aseptic condition to transfer an inoculum to appropriate media. In this step different concentration of serial dilution test-tubes were taken and sample water spread throughout the media. Inoculation method is as following-

Firstly, we clean the laminar air flow chamber and operator's hand surface using cotton and absolute alcohol. After than give at least 20 to 30 min. UV treatment to all instruments which is used in laminar except biological material because UV causes mutation. It is necessary to turn off the UV light and on the blower and florescent light after than Pouring the PDA media in Petri plate and allow solidify and then spread water sample on the PDA media and sealed with paraffin and this Petri-plates Put into incubator for 3-4 days. Measure the growth using zone scale.

Sub- culture :

Colonies obtained through inoculation, a number of species were obtained in a Petri plate. All the species of fungus require to pure its culture. All the procedure is same to inoculation method but, the inoculam were taken from previously inoculated Petri plate using cork- borer or needle. Petri plate sealed with paraffin and place into incubator from 72 to 96 hours.

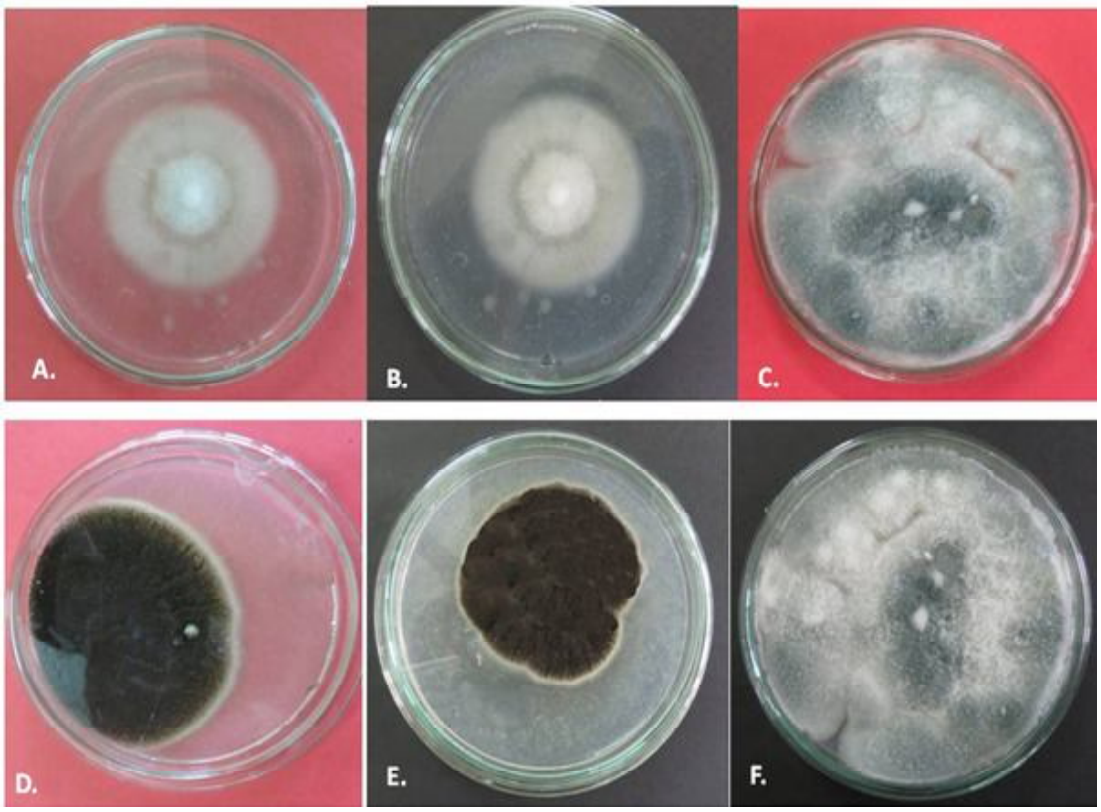

Figure 1. Picture A & B – Pure culture of *Fusarium oxysporum*; Picture C & D – Pure culture of *Aspergillus niger;* Picture E & F – Pure culture of *Trichoderma* spp.

Antagonistic activity :

The antagonistic activity was conducted to using Dual culture technique. The colonies of *Trichoderma* spp. and pathogen species were obtained. After 7 days, with the help of cork borer 6 millimetres mycelial plugs were taken from edge of each colonies (which species to be tested) transferred to PDA plates in Laminar air flow chamber. The antagonistic activity applied in more than one Petri plate of each species. The controls consisted of pure culture of *Aspergillus niger* and *Fusarium oxysporum*. The percent inhibition of mycelia growth over control was calculated by following equation-

$$\text{Percent inhibition (\%)} = \frac{\text{Growth in control - growth in treatment} \times 100}{\text{Growth in control}}$$

Results of the culture *Trichoderma* species clearly show the antagonistic activity against the *Fusarium oxysporum* and *Aspergillus niger* as shown in figure 2 & figure 3.

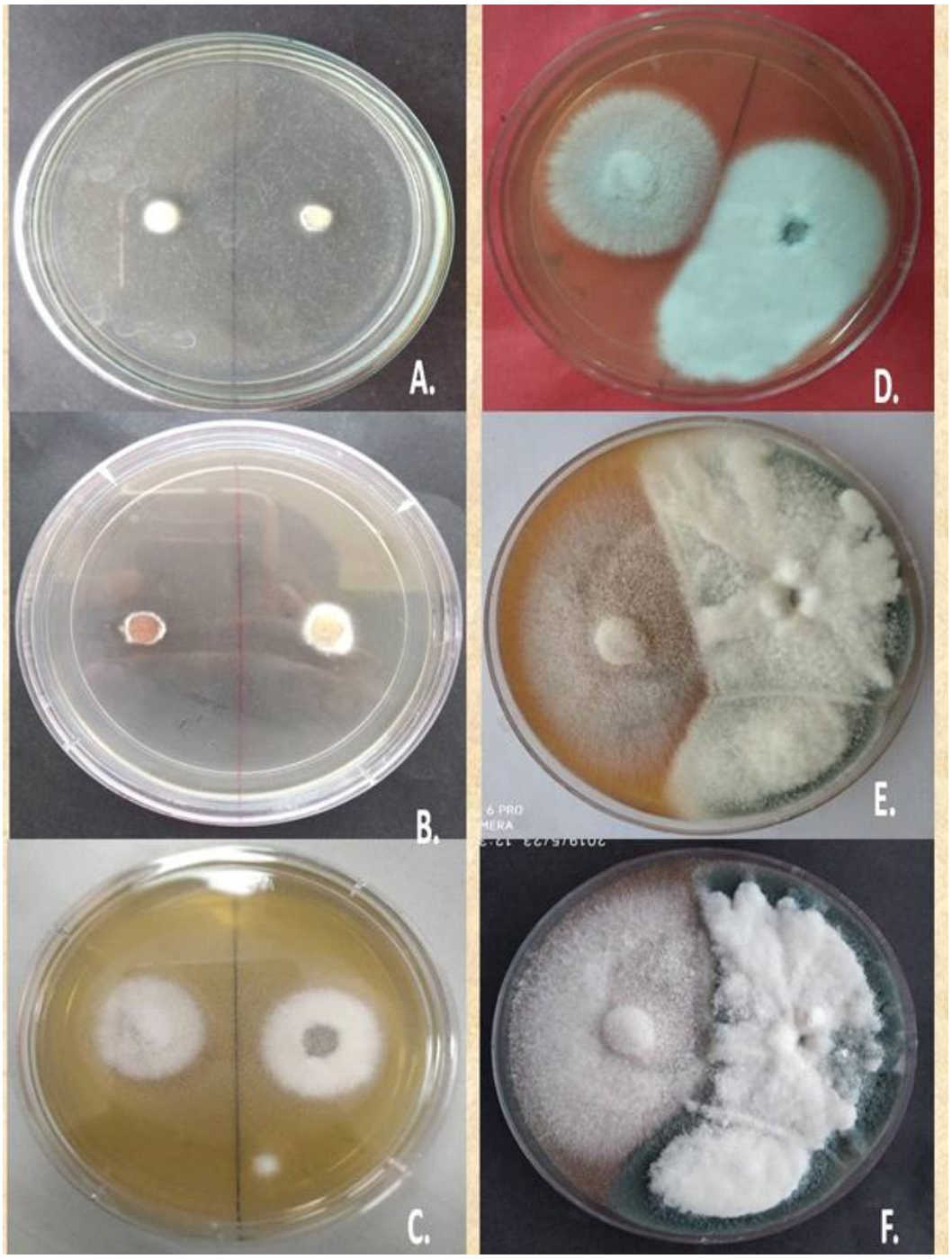

Figure 2. Antagonistic activity of *Trichoderma* spp. against *Fusarium oxysporum*

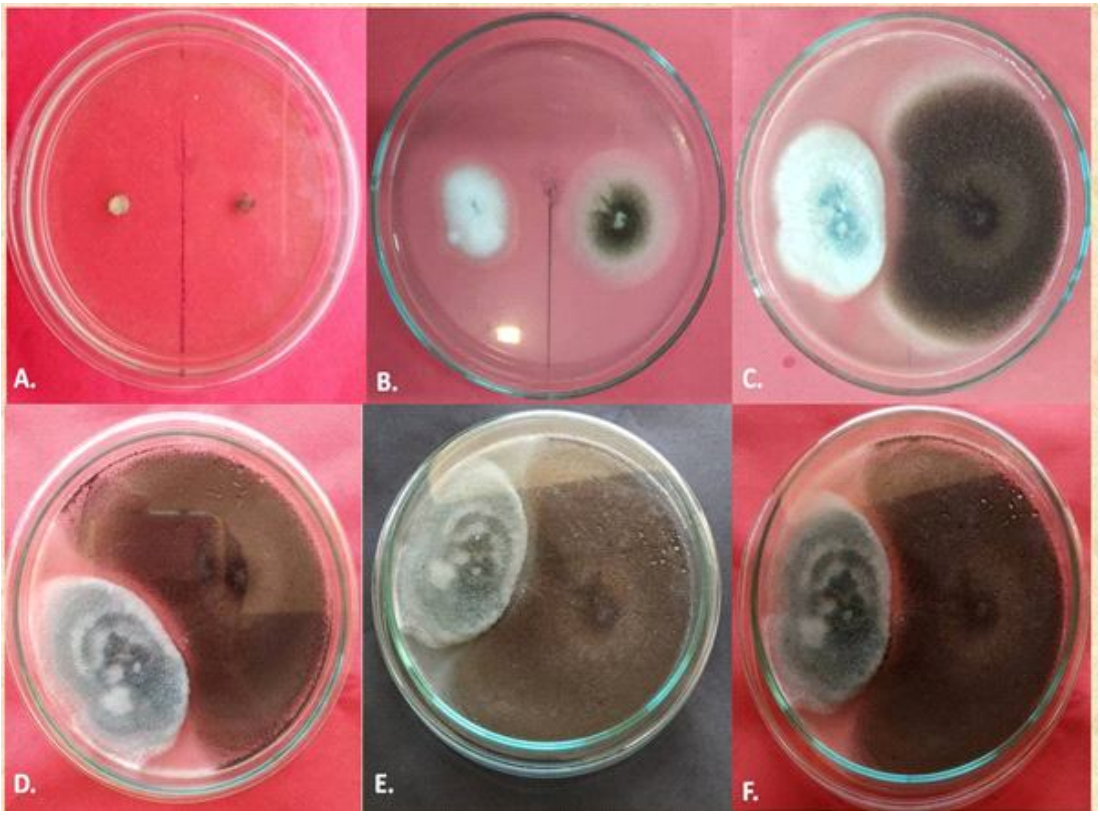

Figure 3. Antagonistic activity of *Trichoderma* spp. against *Aspergillus niger*

Observation Table :

Growth of *Fusarium oxysporum* and *Aspergillus niger* in control and in treated condition can be understand by the below mentioned table 3 and 4 as well as graph.

A. *Fusarium oxysporum :*

Table-3. Details of growth of *Fusarium oxysporum* in control and in treatment

Days	Control(in mm)	Treatment(in mm)	Percent inhibition (in %)
1	6	6	0
2	7	7	0
3	14	14	0
4	35	33.00	5.7
5	52	40.04	23
6	59	45.05	23.7

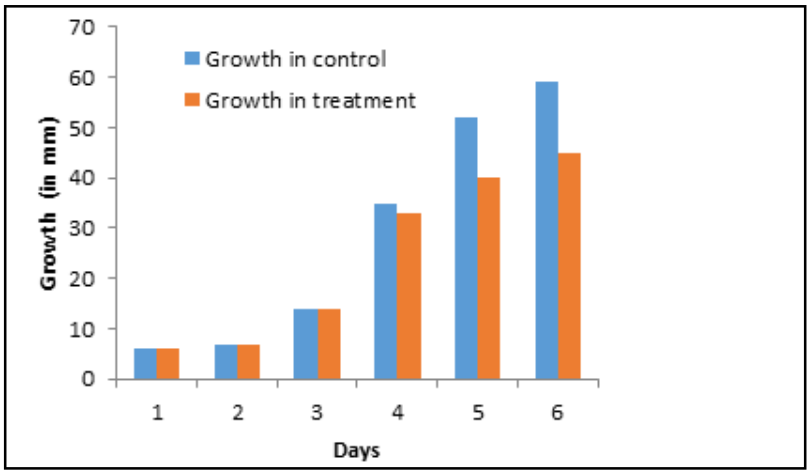

Figure 4. Showing graphical details of growth of *Fusarium oxysporum* in control and in treatment.

B. *Aspergillus niger-*

Table-4. Detailed of growth of *Aspergillus niger* in control and in treatment

Day	Control (in mm)	Treatment (in mm)	Percent inhibition (in %)
1	6	6	0
2	36	36	0
3	72.15	65.03	9.86
4	83.85	74.05	11.68
5	90	71.19	20.09
6	90	68.58	23.8

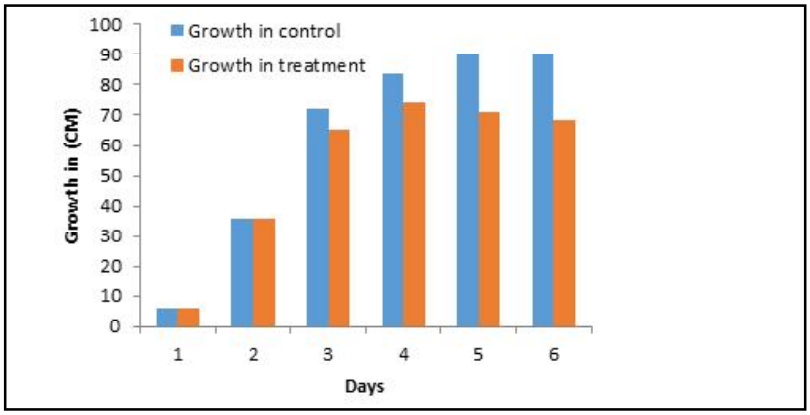

Figure 5. Showing graphical details of growth of *Aspergillus niger* in control and in treatment.

Antagonistic assessment of *Trichoderma spp.* against *Fusarium oxysporum* and *Aspergillus niger* is achieved under lab conditions. The radial growth of experimental fungi viz. *A. niger* (66.24%) and *F. oxysporum* (52.4%) were inhibited by *Trichoderma spp.* due to secretion of antifungal compound and the nature of mycoparasitism, antibiosis and competition with tested fungi.

In our ecosystem plants act as an energy convertor and spread this energy throughout ecosystem thus we need healthy plant and its life. We use chemical pesticides because its work instantly and prevent the plant to become diseased. Chemical pesticides are dangerous for our ecosystem as well as human-beings. That's why we chose the bio control agents to prevent the plant pathogens. This experiment explains that *Trichoderma spp.* act as a biocontrol agent and restricts the growth of *Aspergillus niger* and *Fusarium oxysporum* pathogen on Potato Dextrose Agar medium in the dual culture method.

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