Current Perspective Of Fungal Biocontrol Agent For The Management Of *Fusarium* Pathogen And Its Effects On Groundnut Plant

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By

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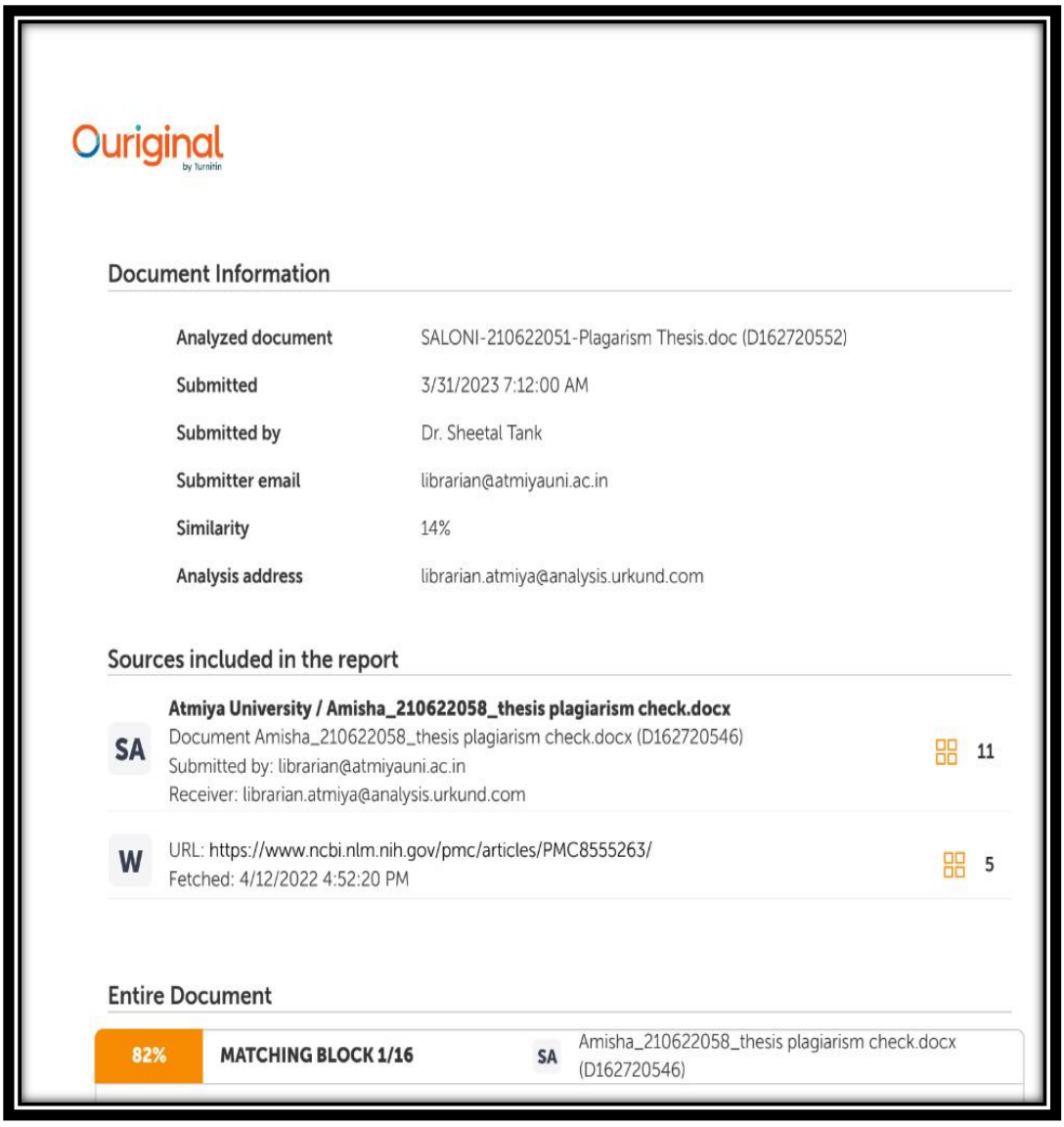

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This work (in part or in full) has not been submitted to any University for the award of an any Degree or a Diploma.

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APPENDIX

PDA	Potato dextrose agar media
PDB	Potato Dextrose Broth
DCPA	Dichloran Chloramphenicol Peptone Agar
LPCB	Lactophenol Cotton Blue Stain
RAR	Rajkot Agricultural Region
KRS	Kankot Region Soil
PVT.	Private
LTD.	Limited
C	Colony diameter of control pathogen
T	Colony diameter of the pathogen in inhibition on plate
rpm	Rotation Per Minutes
EC	Electrical Conductivity
OC	Organic Carbon
N	Nitrogen
K	Potassium
P	Phosphorous
Kg	Kilogram
h	Hectare
gm	Gram
mg	Milligrams
ml	Milliliter

W/W	Weight for Weight
pH	Potential of hydrogen
nm	Manometer
mins	Minutes
hr	Hours
V	Volume of extract
W	Weight of tissue
Fig.	Figure
i.e.,	That is
etc	And so, on
et al.,	Co-workers
sp.	Species
^{O}C	Degree Celcius

ABSTRACT: -

Groundnut is the third largest oil seed producer in Gujarat. Having the many beneficial effects of Groundnut, it is not much exported to other countries for its loss of production and poor quality due to contamination by various soil-borne pathogens. *Fusarium* is a soil-borne fungal pathogen generally causing fungal diseases in groundnut plants. Thus, it is necessary to control the pathogenicity of *Fusarium* species in the Groundnut Plants. A present investigation has been carried out to inhibit the pathogenicity of *Fusarium* species from the Groundnut plant through a biocontrol agent. Nowadays the use of fertilizers and chemicals has increased due to which several other problems have come into existence. Thus, to overcome this problem, in the current study, we are mainly focusing on biocontrol agents against soil-borne pathogens. The soil sample was collected from different regions of Rajkot, Gujarat where groundnut production is large in amount. For further study followed by serial dilutions up to 10^{-9} dilutions and direct inoculation on selective media for fungal isolation that are Dichloran Chloramphenicol Peptone Agar (DCPA) and Potato Dextrose Agar (PDA) that was supplemented with streptomycin to avoid bacterial contamination, followed by incubation for 5 to 6 days at 28^{O}C. Based on morphological and cultural characteristics, 20 isolates were obtained, out of which 2 fungal isolates were tentatively identified as *Fusarium species*. *Trichoderma species* were isolated and identified from the soil sample collected from Rajkot, Gujarat. Antagonistic screening activity was performed against *Trichoderma* species as it is known as the potent biocontrol agent against various pathogenic fungi. As a result, it was determined that *Trichoderma* was able to inhibit the growth of *Fusarium* species. Pathogenicity test was employed to identify the effects of pathogens on the number of leaves, root length, shoot length, and chlorophyll content for the Groundnut Plants. Statistical analysis through the standard deviation SD was carried out to check the potential of biocontrol agent over pathogen on Groundnut Plants. Results conclude that the potential of *Trichoderma* species as a biocontrol agent to manage several diseases caused by phytopathogenic soil-borne fungal pathogens and hence can serve as a good potent biocontrol source for the agricultural industry.

Keywords: *Fusarium* sp., *Trichoderma* sp., Biocontrol Agent, Groundnut, Antagonist, Statistical Analysis, Standard Deviation.

Chapter 1: INTRODUCTION

Groundnut is the third largest oil seed producer in Gujarat. Groundnuts are subjected to multiple soil-borne fungal pathogens. Despite having other beneficial effects groundnut is not much exported to other countries due to loss of product yield and poor quality of crop and being contaminated with soil-borne fungal pathogens like *Fusarium* (Lamprecht et. al. 2011). *Fusarium* is a genus of filamentous fungus widely distributed throughout the tropical regions of the world with major species ranging from endophytic to phytopathogenic fungal strains. Phytopathogenic species of *Fusarium* include some important plant pathogens associated with wilt and root rot diseases. *Fusarium oxysporum* and *Fusarium solani* are the two fungal phytopathogenic strains mainly isolated from soil that resides as conidia, mycelium, or chlamydospore. *Fusarium* sp. mainly attacks numerous plants that are important to human life and animal nutrition. It infects almost all the parts of plants and causes a reduction of economical yield and deprived product quality. Hence, it is necessary to control this problem caused by fungal infections. Farmers use chemical pesticides to reduce the pathogens and to protect the plant but this is harmful over a longer period of time causing other problems such as global warming and other health problems. Nowadays these plant diseases can be controlled by exploring microbial biocontrol agents having the ability to inhibit the growth of pathogens by showing various mycoparasite activities. Antagonism refers to the suppression of normal growth and its pathogenicity. Several fungal isolates mainly *Trichoderma sp.* which is found in normal rhizosphere soil regions have a significant role in bioremediation and are utilized as a biocontrol agent against myco-phytopathogens. The present investigation aims to explore the antagonistic activity of the *Trichoderma* strain against the *Fusarium sp.* plant fungal pathogen that can be considered a biocontrol agent for the reduction of plant diseases.

AIM:

The main aim of the current study is to study the effects of a Fungal Biocontrol agent against pathogenic *Fusarium* sp. in groundnut plants.

OBJECTIVES:

• Isolation and identification of fungal pathogenic species from different soil samples.

• To check the *in-vitro* antagonistic activity of fungal pathogens against potent species.

• Effect of growth of Biocontrol agent on groundnut.

• Effect of pathogenicity on groundnut plant.

• Statistical approach

Chapter 2: REVIEW OF LITERATURE

2.1 Groundnut as the largest oil seed producer in India.

On the oilseeds map of the world, India occupies a prominent position concerning land area and production yield. India plays a part in about 10 percent of the world's oilseeds production. India stands 4th largest edible oil economy in the world (Fazlul *et.al.*2013). The survey report of the groundnut crop 2018 stated that the groundnut is a leguminous crop that is highly cultivated in tropical and subtropical regions. It is in demand for its high-oil edible seeds and economic value, and as such, it is the fourth dominant source of edible oil and the third dominant source of vegetable oil in the world. Groundnut is an important oilseed crop as well as an important agricultural export commodity. In India, though groundnut is cultivated in one or more seasons, about 80% of the complete land area and yield comes from the Kharif crop (Kharif -2018 survey of groundnut crop). Groundnuts are widely cultivated as important oil seed producers and economic crops; some factors affect groundnut production. Blind fertilization and continuous cropping have led to the accumulation of toxic substances and the epidemic of severe diseases, which have become the main constraints to groundnut production. Soil is not only the medium for the growth of the groundnut plant but also the nutrients that play an important role. The abundance of soil nutrients is directly related to the yield and quality of groundnuts (Wei *et.al.*2021). Continuous cropping and selective nutrient absorption make soil-borne pathogens cause diseases such as root rot, southern blight, and crown rot, as well as many pathogens of foliar diseases, that reside in the soil. *Fusarium* infestation of groundnuts generally causes root rot which results in browning and rotting of the roots, crumpling, and dry rot of the taproots (Wei *et.al.*2021).

For managing diseases and insect pests, the application of pesticides was widely practiced by farmers in all five states. 12 In Gujarat and Rajasthan, nearly all the farmers (98 to 99%) applied pesticides for their crop husbandry. The extent of farmers using pesticides although quite high in Maharashtra (88%), Karnataka (82%), and AP (77%) yet was not as high as in Gujarat and Rajasthan. Much depletion of pesticides and other chemicals later on harms the environment and damages the land as well as human health thus currently organic and microbial farming is a concern of the research.

2.2 Effect of Pathogens on Groundnut Plants

Groundnuts can be infected by microbial pathogens. Fungal pathogens include various diseases such as seed rot and seedling diseases, like root rot, stem rot, wilt diseases, blight, pod rot, foliar diseases, rusted leaf, and late leaf spots. Seed rot and seedling diseases may be caused by many soil-inhabited fungi colonizing the fungal spores in the soil. Various fungi causing the infection are *Aspergillus, Fusarium, Rhizopus, Penicillium sp*., *Verticillium, and Pythium.* Viral pathogens responsible for groundnut infection are *groundnut bud necrosis and tospovirus, Tobacco streak ilarvirus, Peanut mottle potyvirus, and Peanut clump furovirus.* some nematode pathogens cause root-knot disease, Kalahasti malady. Bacterial pathogens cause Collar disease, and root rot infection in groundnut (Faujdar *et.al* 1992) (Vinod *et.al.* 2016).

2.3 Effect of *Fusarium* pathogen in groundnut plant

Fusarium species constitute many strains that cause vascular wilt diseases to economically important crops throughout the world. Although sexual reproduction is unknown in the *Fusarium species*, horizontal gene transfer contributes to the pathogenic strains (Thomas et.al. 2017). *Fusarium oxysporum and Fusarium solani* are the most predominant *Fusarium* phytopathogen for groundnut plants. The fungus is mainly associated with groundnut, cumin, wheat, and sorghum causing infections and damaging the plant. The importance of the elimination of the growth of such phytopathogens and their associated effects on plants cannot be overemphasized considering the significant health hazards associated with their consumption in daily life.

Several infections such as root rot infection are caused by phytopathogenic fungi like *Fusarium sp.* due to which crop yield is reduced and the economic value also decreased. Though these pathogens are responsible to causes groundnut infection but *Fusarium* infection is dominating widely in groundnut plants. *Fusarium sp.* is able to cause wilt disease causing the lower end of the tap root to become brown to reddish brown. Secondary roots become brown and brittle. Leaves turn greyish green and plants dry. Due to the dominating infection of wilt, groundnuts are not much exported to foreign countries (Vinod *et.al.* 2016). Wilt disease infection is one of the main causes of yield reduction in groundnuts. Continuous cropping and frequent use with the increasing dosages of pesticides and chemical fertilizers made the pathogen's resistance to pesticides increase each passing year, thus the incidence of the disease increased exponentially. Thus, to overcome this scenario the use of potent antagonistic microorganisms, to control infection-causing microbes has become a current research trend.

2.4 Wilt infection control by the biocontrol agent

Soil-borne microorganisms are beneficial or harmful for complete plant growth and crop yield. All these microorganisms express the same habitat in the rhizosphere and interact with each other. *Fusarium* is one of the most dominating fungal pathogens in the soil causing yield loss due to suppression in plant growth and its development. Various chemical methods are being exploited for their management, which is costly and dangerous to the environment for imperishable development due to their toxicity. Control of *Fusarium* infection is difficult. Thus, biological control is one of the most effective manners to control it because of its very low effects of toxicity from the environmental point of view and it is cost-effective. Some bacteria and fungi act as biocontrol agents for different species as per their mode of action against the pathogen.

2.5 *Trichoderma* as biocontrol agent for *Fusarium*

The conclusions from the work of (Rojan. *et.al* 2010), assure the efficiency of *Trichoderma sp*. as a biocontrol agent against fungal soil pathogens and indicates the need for *Trichoderma-based* biocontrol agents to serve as an environment-friendly biocontrol agent. *Trichoderma* effectively controlled the pathogen and simultaneously increased the growth of plants. Antagonistic *Trichoderma* enhanced resistance against the phytopathogenic infection of the fungal species in groundnut.

As per Deepa et.al. (2021), physical and chemical approaches have been shown to reduce the effects caused by *Fusarium* but have proved to be ineffective during the production process. Hence, biological control methods using microorganisms, plant extracts, antioxidants, essential oils, phenolic compounds, and other advanced technologies have become an effective alternative for managing *Fusarium* and its effects. Similarly in the present work, *Trichoderma sp.* has been exploited as a biocontrol agent against *Fusarium sp.*

(Sun *et. al.* 2021) found that pre-inoculation with the root endophyte *Phomopsis liquidambaris* B3 could induce resistance to *Fusarium oxysporum* by activating the salicylic acid-dependent signalling pathway. Similarly in this current study, we have tried to overcome the problem by inducing a potent antagonist fungal strain against *Fusarium* in the Groundnut plant. Hence, the *Trichoderma* strain was employed as a biocontrol agent against Fusarium showing its mycoparasitic effects. *Trichoderma* behaving as a potential biocontrol agent shows its antagonistic activity as direct as well as indirect mechanism towards the pathogen. Here this current study

shows the direct mechanism of *Trichoderma* over the *Fusarium* pathogen through its mycoparasitic nature and inhibiting the growth of the pathogen.

Among the biocontrol agents, *Trichoderma* is a mainly symbiotic fungus that inhibits the growth of *Fusarium wilt* through its direct and indirect mechanism of mycoparasitism, completion, production of various lytic enzymes, and other antimicrobial activities along with enhancement in the growth of the host plant by the production of plant growth hormones. In the soil, *Trichoderma* releases various anti-mycoparasitic proteins, enzymes, and volatile and non-volatile compounds, that have the potency to solubilize nutrients and defend against pathogens. Due to these qualities, *Trichoderma* is a potential biocontrol agent against various pathogens under in-vitro laboratory conditions.

(Ishwar et.al. 2020). Important strains are emphasized for pot assay for, the further confirmation of the antagonistic activity of *Trichoderma* against *Fusarium* sp. The antagonistic activity showed the direct mechanism of mycoparasitism of *Trichoderma* as a biocontrol agent against *Fusarium* sp.

Chapter 3: - MATERIALS & METHODS

3.1 Material Media required:

3.1.1 Materials and Media Required for Isolation of Fungal Cultures

- Soil Sample
- PDA Potato Dextrose Agar
- DCPA Dichloran Chloramphenicol Peptone Agar
- PDB Potato Dextrose Broth

3.1.2 Materials Required for the Identification of Fungal Isolates

- LPCB Lactophenol Cotton Blue Dye
- Distilled water

3.1.3 Glass Wares and Other Instruments Required for The Current Study

- Beaker
- Petri plates
- Flask
- Glass Pipettes
- Test tubes
- Glass slides
- Coverslip
- Spatula
- Glass rod
- Spreader
- Autopipettes
- BOD Incubator
- Autoclave
- Waterbath
- Laminar Air Flow
- Bunsen Burner
- Wire loop
- Needle
- Microscope

3.2 Collection Of Soil Sample:

Soil samples were collected from rhizospheric regions from different areas of Rajkot, Gujarat; Atmiya Garden soil, Kankot, Rail Nagar, and Bedi regions of Rajkot. (Farzana et. al., 2021).

3.3 Processing Of Soil Sample:

1.0gm soil sample was thoroughly mixed in 10.0ml sterile distilled water for suspension and serial dilutions up to 10^{-1} to 10^{-5}. From this 0.1ml sample was spread onto Dichloran Chloramphenicol Peptone Agar DCPA medium plates and Potato Dextrose Agar PDA medium plates supplemented with 1% streptomycin to avoid bacterial contamination and incubated at 28^{O}C for 5 to 6 days.

3.4 Isolation Of Fungi From The Soil Samples:

PDA medium is the most frequently used selective media for the growth of fungal species by several workers who worked during their studies. The composition of the Potato Dextrose Agar PDA medium includes; 200.0gm potato infusion, 20.0gm dextrose, and 30.0gm Agar for 1000.0 ml of distilled water. The final pH was maintained at 5.6±0.2. Because of its simple nutritional composition and easy availability, it is more likely to support the growth of all types of fungi (Maheshwari et al.,1999) (Saha et al., 2010).

The serially diluted soil samples were used for the isolation of fungal culture on the Potato Dextrose Agar medium as well as DCPA Dichloran Chloramphenicol Peptone Agar medium plates. Soil dilutions were prepared by taking 1.0gm of soil sample in 10.0ml of sterile distilled water in test tubes for every sample. 10^{-3}, 10^{-4}, and 10^{-5} dilutions were employed to isolate fungal cultures from soil samples to avoid over-crowding of the fungal colonies in plates. 0.1ml serially diluted soil sample was spread on 1% streptomycin-containing potato dextrose agar medium and DCPA medium plates followed by incubation at 28^{O}C for 5 to 6 days. At higher dilutions, fungal colonies are easily isolated because they form a well-dispersed surface hyphal colony (Ratna et al., 2015).

3.5 Microscopic Identification Of Fungal Isolates:

For the microscopic identification of isolated fungal strains Lactophenol Cotton Blue staining method has been performed with the usage of a sterile inoculating needle and Bunsen burner. With the help of inoculating needle, a small loopful portion of fungal mycelium was placed onto the drop of Lactophenol Cotton Blue Stain on a glass slide covered with a cover slip. Then slightly

squashed the culture to avoid over-crowding of the mycelium. Then stained fungal isolates were observed under the light microscope (40X) for morphological identification (Aneja 2006). For 18S sequencing, the fungal isolates were provided for outsourced sequencing at Gene Explore Ahmedabad.

3.6 Soil Analysis:

Various physicochemical parameters are employed for soil analysis. The physical analysis includes the color of the soil and Dry weight and wet weight which are determined before and after autoclaving of soil samples. The chemical parameters include pH, Electrical Conductivity (EC), Organic Carbon (OC), Nitrogen(N), Phosphorus (P), and Potassium (K) for soil samples (G. Stotzky et. Al. 1963). Complete soil analysis was carried out by outsourcing at INVITRO LABORATORIES PVT.LTD. Rajkot, Gujarat.

3.7 *In-vitro* Antagonistic Activity Of *Trichoderma sp.* Against Soil-borne Fungal Isolates:

The antagonistic activity of *Trichoderma sp*. was evaluated against soil-born phytopathogens *Fusarium sp*. A dual culture method has been performed for the determination of the antagonistic activity of *Trichoderma sp.* against fungal pathogens *Fusarium sp.* on Potato Dextrose Agar medium. Approximately at an equal distance, the mycelium was employed from *Trichoderma sp.*, and test fungal cultures were placed on a PDA medium in the same petri dish, which was around 4 cm away from each other individually. All the culture-inoculated petri plates were incubated at 28°C for 5 to 7 days. After the completion of the incubation period plates were observed for antagonistic activity of *Trichoderma sp.* against soil-borne fungal pathogens. The percentage of growth inhibition of soil-borne fungal isolates was determined by the method of Watanabe (1984).

$$\%\ \text{of Growth Inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Colony diameter of control pathogen

T = Colony diameter of the pathogen in inhibition on plate

3.8 Fungal Suspension For Spore Analysis:

Freshly prepared PDA slants were used to prepare a fungal suspension. 5.0ml sterile distilled water was poured in a slant inoculated with *Fusarium* and *Trichoderma* isolates. Under aseptic conditions, fungal colonies were scraped using a sterile swab and the prepared suspension was poured into another tube for spore count per ml of suspension. (Pablo. et.al 2015)

3.9 Seed Treatment:

The well-isolated fungal cultures were inoculated on a PDA slant and incubated at 28°C for 4 days. After the completion of the incubation period, the seeds were soaked with the same fungal suspensions and one combination for the seed treatment experiment (A. Karthick *et. al.,* 2008).

i. Uninoculated Seed–Groundnut (Control)
ii. Seeds +*Fusarium sp.* suspension–Groundnut (Pathogenic)
iii. Seeds+ *Trichoderma sp.* suspension–Groundnut (Biocontrol)
iv. Seeds+ combination of *Fusarium sp.* and *Trichoderma sp.* suspension–Groundnut (combination)

Groundnut seeds were properly surface sterilized by 1.0% sodium hypochlorite solution and soaked for 30 mins in prepared *Fusarium* suspension, the soaked seeds were spread onto the moist chamber with a sterile cotton pad within the Petri plates to absorb the excess amount of suspension and allowed to air-dry overnight (Rudresh et al., 2005). *Trichoderma* suspension was applied as a coating over the seeds (Nawar, 2007). The treated groundnut seeds were planted in each pot filled with 200gm of sterile autoclaved soil. The pathogenicity was determined after proper growth of the shoot, root, and leaves of the groundnut plant.

3.10 Pathogenicity Test:

An experiment had been carried out by using agricultural soil at *in-vitro* culture conditions of the Microbiology Department, Atmiya University, Rajkot, Gujarat. Suspension of the more frequently isolated fungi, i.e., *Fusarium sp., and Trichoderma sp.* was prepared. Soil infection was attained by mixing the suspension of each fungal isolate with the soil and water was sprayed regularly for five to six days before seed plantation and the same amount of soil sample was kept as control treatment. Five seeds were sown in every pot containing 200gm of sterile autoclaved soil and watered whenever needed. Pots were used with each treatment to study the effect of tested fungi

and fungal biocontrol agents with survived plants were calculated at 20 days after planting, respectively (Shaban & Bramawy, 2011).

i. 200 gm Soil + Uninoculated Groundnut Seeds– (Control)
ii. 200 gm Soil + Groundnut Seeds + Inoculated with *Fusarium sp.* suspension– (Pathogenic)
iii. 200 gm Soil + Groundnut Seeds + Inoculated with *Trichoderma sp.* suspension– (Biocontrol)
iv. 200 gm Soil + Groundnut Seeds + Inoculated with a Combination of *Fusarium sp.* and *Trichoderma sp.* suspension– (Combination)

3.11 Effects Of The Pathogen As Well As The Biocontrol Agent On Groundnut Plant:

Effects of pathogenic fungal strain *Fusarium* sp. on the Groundnut plant was analyzed after 20 days of incubation and the difference in the various parameters such as the number of leaves, root length, and shoot length was observed for Groundnut plant infested with *Trichoderma sp.* as a biocontrol agent.

3.11.1 Effect Of Pathogen In Comparison With Biocontrol Agent Over The Number Of Groundnut Plant Leaves

The effect of the pathogen was checked for groundnut plant leaves. After the incubation of 20 days, the healthy plant was uprooted from the pot, and the number of leaves was measured in cm. The structural patterners of leaves were also measured to identify the cause of infection in a pathogenic plant verse a healthy plant.

3.11.2 Effect Of Pathogen In Comparison With Biocontrol Agent Over The Root Length Of Groundnut Plant

Similarly, the effect of the pathogen over the biocontrol agent was analyzed to check the difference in parameters for the root length of the groundnut plant. After proper incubation of 20 days, the plants were uprooted from the pot to measure the root length and check the cause of pathogenicity.

3.11.3 Effect of Pathogen In Comparison With Biocontrol Agent Over The Shoot Length Of Groundnut Plant

The effect of the pathogen was determined for the shoot length the of groundnut plant. The shoot length was analyzed with the pathogenic plant in comparison with the control Trichoderma-

infested plant. Shoot length was analyzed to check the efficiency of a plant to withstand the growth of pathogenicity.

3.12 Estimation Of Chlorophyll Content For Groundnut Plant Leaves:

Chlorophyll estimation was done by spectrophotometric analysis. Where 0.5 gm of groundnut plant leaves were taken out and cut down into small pieces. Crushed them with 10.0 ml of 80% acetone with the help of a mortar and pestle. Centrifugation the crushed solution at 10,000 rpm for 10 minutes and finally transfer the supernatant to a fresh tube. Take absorbance against 80% acetone as a blank. Take the absorbance of the sample at 645 nm and 663 nm wavelength. Chlorophyll was determined by the following formula:

$$\text{Chlorophyll a} = \frac{12.7 \times \text{O.D}(663) \ - 2.69 \times \text{O.D}(645)}{1000 \times \text{W}} \times \text{V}$$

$$\text{Chlorophyll b} = \frac{22.9 \times \text{O.D}(645) \ - 4.68 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

$$\text{Total Chlorophyll} = \frac{20.2 \times \text{O.D}(645) \ - \ 8.02 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

3.12.1 Estimation of Chlorophyll a Content for Groundnut Plant Leaves

Chlorophyll a content was determined by spectrophotometric analysis where10.0ml 80% acetone was used to crush the 0.5 gm of groundnut leaves. Chlorophyll a content was determined by the following formula;

$$\text{Chlorophyll a} = \frac{12.7 \times \text{O.D}(663) \ - 2.69 \times \text{O.D}(645)}{1000 \times \text{W}} \times \text{V}$$

3.12.2 Estimation of Chlorophyll b Content for Groundnut Plant Leaves

Chlorophyll b content was determined by spectrophotometric analysis where 10.0ml 80% acetone was used to crush the 0.5 gm of groundnut leaves. Chlorophyll b content was determined by the following formula;

$$\text{Chlorophyll b} = \frac{22.9 \times \text{O.D}(645) \ - 4.68 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

3.12.3 Estimation of Total Chlorophyll Content for Groundnut Plant Leaves

Total Chlorophyll content was determined by spectrophotometric analysis where 10.0ml 80% acetone was used to crush the 0.5 gm of groundnut leaves.Total Chlorophyll content was determined by the following formula;

$$\text{Total Chlorophyll} = \frac{20.2 \times \text{O.D}(645) \; - \; 8.02 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

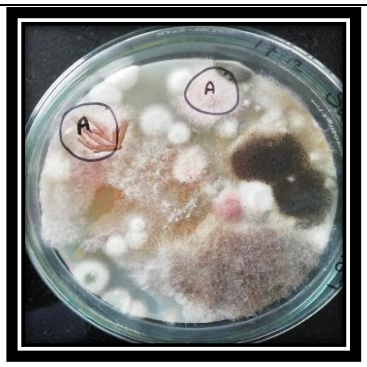
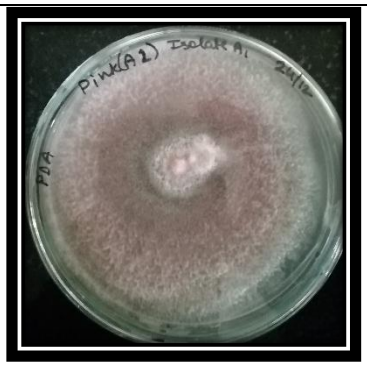
3.13 Statistical analysis of data:

All the experimental studies were conducted in triplicate and the data were analysed using the standard deviation (± SD) for the value.

Chapter 4: - RESULT & DISCUSSION

4.1 Isolation Of Fungi From Soil Samples:

By performing the serial dilution of soil samples collected from 4 different regions Rail Nagar, Kankot, Bedi, and Atmiya University Garden, Rajkot Gujarat. After proper serial dilution of the soil sample, the diluted soil samples were spread on to PDA medium containing Streptomycin to avoid other bacterial contamination and incubated for 5 to 6 days at 28℃. After proper incubation, 20 isolates were obtained out of which 3 fungal species were isolated as pure fungal isolates.

	
Isolation of Fungi from the Soil sample	Isolation of Fungi from the Soil sample
	
Pure Fungal Isolate (A)	Pure Fungal Isolate (B)

Pure Fungal Isolate

Fig 1: Isolation Of Pure Fungi From Soil Sample

4.2 Morphological Identification Of Pure Fungal Isolates:

By performing serial dilution of soil sample 20 isolates were obtained out of which 2 fungal isolates were tentatively identified as *Fusarium sp.* on basis of morphological identification through lactophenol cotton-blue staining technique. The microscopic identification was carried out and observed as white-colored colonies having raised pigment-producing mycelium without wrinkled structure on PDA plates and it appeared as septate microconidia with short branched fascicles through a 40x microscope. Which was tentatively identified as *Fusarium* sp. Whereas another isolated fungal colony was observed as pink color forming raised mycelium without wrinkled structures on it. When observed under a 40x microscope it appeared as short branched septate sickle-shaped forming conidiophore and which was tentatively identified as *Fusarium* sp. *Trichoderma* isolates were provided by the Department of Microbiology, Atmiya University, Rajkot. The provided *Trichoderma* strain was identified by lactophenol cotton-blue staining which developed a dark green colony with pustules fringed by mycelium around as frequently branching with ampulliform phialide conidia-shaped subglobose or obovoid-shaped structure. Tentatively identified as *Trichoderma harzanium*. 18S sequencing was carried out at Gene Explore, Ahmedabad for further confirmation of pure fungal isolates and to determine the properties of fungal isolates. Accession number obtained from NCBI for 18S gene sequencing GenBank: OQ654012.1.

Table 1. Morphological And Microscopical Characteristics Of Fungal Isolates On Potato Dextrose Agar media

Sample	**Colony Color**	**Front view, Arial Mycelia/ Vegetative/ Arise**	**Area (cm)**		**Pigmentation**	**Wrinkle Furrow**	**Microscopic Observation**	**Identified Fungal Isolates**
			Height	**Width**				
RAR1	Dark green or pustules fringed by sterile mycelium	Vegetative	9	9	Dull Yellow	NO	Conidiophore Frequently branching, Ampulliform Phialide, conidia shape- Sub globose to obovoid	*Trichoderma harzanium*
KRS 1	White	Arise	7.7	6	NO Pigmentation	NO	Conidiophore short, unbranched	*Fusarium* sp.

							septate macrospores sickle-shaped	
KRS 2	Pink to Purple	Arise	7	6	Purple to Pink reverse mycelium pigmentation	NO	Short branched septate sickle-shaped forming conidiophore	*Fusarium* sp.

	
Identification of Pure Fungal Isolate	Microscopic observation of *Trichoderma harzanium* through LPCB Staining

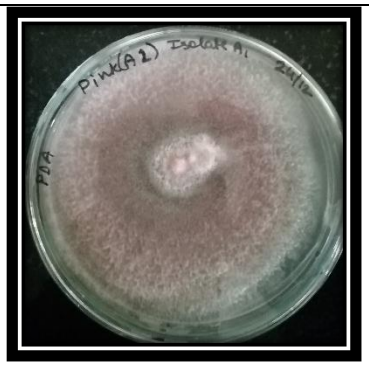
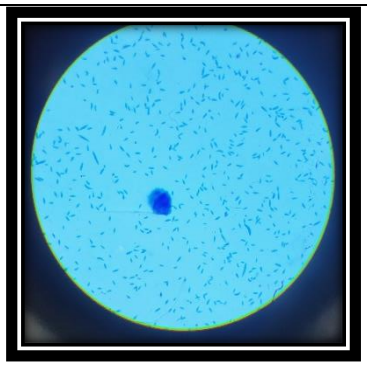
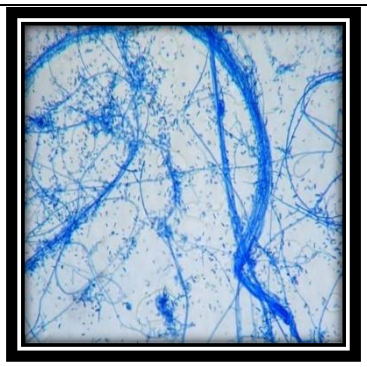
	
Identification of Pure Fungal Isolate (A)	Microscopic observation of *Fusarium sp.* (A) through LPCB Staining
	
Identification of Pure Fungal Isolate (B)	Microscopic observation of *Fusarium sp.* (B) through LPCB Staining

Fig 2: Identification Of Pure Fungi Isolated From Soil Sample

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Phylogenetic Tree

Figure. Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 1.30590943 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method [2] and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 501 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [3].

References:

1. Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

2. Nei M. and Kumar S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

3. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Molecular Biology and Evolution 33:1870-1874.

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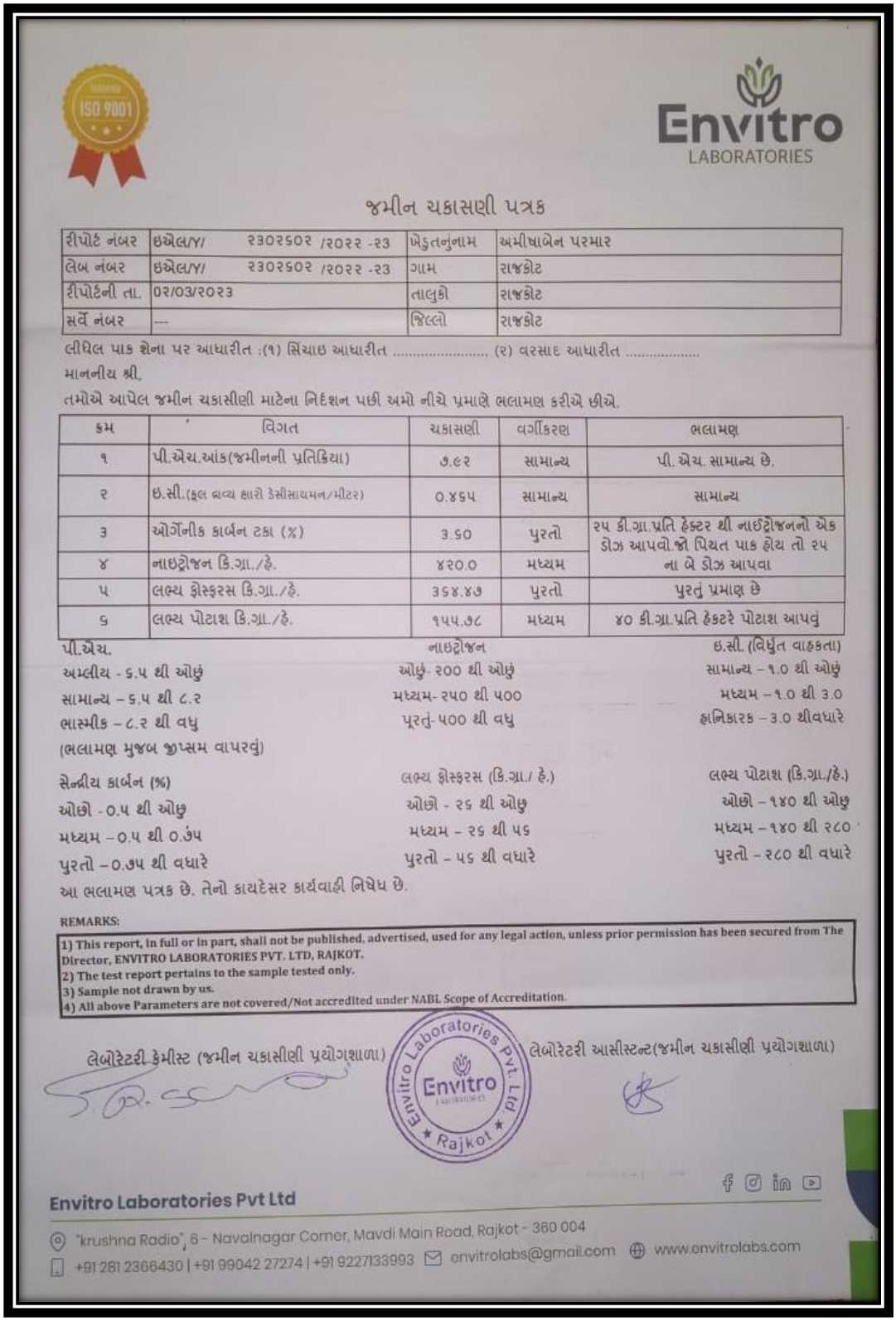
Fig 3: Phylogenetic Tree Analysis For Sequenced Fungal Culture

4.3 Soil Analysis:

The soil analysis was carried out to check the soil quality. Parameters included physical and chemical parameters such as dry and wet weight, pH, color, electrical conductivity (EC), organic carbon (OC), nitrogen content (N), Phosphorus (P), and Potassium (K) of the soil sample. The physical parameter measured as the color of the soil which was black to brown suggests the fertility of the soil. pH was determined by using a pH meter and calculated as 7.5 which indicates healthy soil. Dry and wet weight was determined before and after autoclaving the soil samples respectively. Complete soil analysis was carried out by INVITRO LABORATORIES PVT.LTD. Rajkot, Gujarat.

Table 2: Soil Analysis

Parameters analysed	**Data Generated**
Color	Black to Brown
pH	7.5
Weight	525gm
Electrical Conductivity (EC)	0.465
Organic Carbon (OC)	3.60%
Nitrogen (N)	420.0kg/h
Phosphorous (P)	364.47kg/h
Potassium (K)	155.78kg/h



Envitro LABORATORIES

જમીન ચકાસણી પત્રક

રીપોર્ટ નંબર	ઇએલ/Y/ ૨૩૦૨૬૦૨ /૨૦૨૨ -૨૩	ખેડુતનુંનામ	અમીષાબેન પરમાર
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રીપોર્ટની તા.	૦૨/૦૩/૨૦૨૩	તાલુકો	રાજકોટ
સર્વે નંબર	---	જિલ્લો	રાજકોટ

લીધેલ પાક શેના પર આધારીત :(૧) સિચાઇ આધારીત ..... (૨) વરસાદ આધારીત .....

માનનીય શ્રી,

તમોએ આપેલ જમીન ચકાસીણી માટેના નિર્દેશન પછી અમો નીચે પ્રમાણે ભલામણ કરીએ છીએ.

ક્રમ	વિગત	ચકાસણી	વર્ગીકરણ	ભલામણ
૧	પી.એચ.આંક(જમીનની પ્રતિક્રિયા)	૭.૯૨	સામાન્ય	પી. એચ. સામાન્ય છે.
૨	ઇ.સી.(ફુલ ઘ્રાવ્ય ક્ષારો ડેસીસાયમન/મીટર)	૦.૪૬૫	સામાન્ય	સામાન્ય
૩	ઓર્ગેનીક કાર્બન ટકા (%)	૩.૬૦	પુરતો	૨૫ કી.ગ્રા.પ્રતિ હેક્ટર થી નાઇટ્રોજનનો એક ડોઝ આપવો.જો પિયત પાક હોય તો ૨૫ ના બે ડોઝ આપવા
૪	નાઇટ્રોજન કિ.ગ્રા./હે.	૪૨૦.૦	મધ્યમ	
૫	લભ્ય ફોસ્ફરસ કિ.ગ્રા./હે.	૩૬૪.૪૭	પુરતો	પુરતું પ્રમાણ છે
૬	લભ્ય પોટાશ કિ.ગ્રા./હે.	૧૫૫.૭૮	મધ્યમ	૪૦ કી.ગ્રા.પ્રતિ હેકટરે પોટાશ આપવું

પી.એચ.	નાઇટ્રોજન	ઇ.સી. (વિદ્યુત વાહકતા)
અમ્લીય - ૬.૫ થી ઓછું	ઓછું- ૨૦૦ થી ઓછું	સામાન્ય – ૧.૦ થી ઓછું
સામાન્ય – ૬.૫ થી ૮.૨	મધ્યમ- ૨૫૦ થી ૫૦૦	મધ્યમ – ૧.૦ થી ૩.૦
ભાસ્મીક – ૮.૨ થી વધુ	પૂરતું- ૫૦૦ થી વધુ	હાનિકારક – ૩.૦ થીવધારે

(ભલામણ મુજબ જીપ્સમ વાપરવું)

સેન્દ્રીય કાર્બન (%)	લભ્ય ફોસ્ફરસ (કિ.ગ્રા./ હે.)	લભ્ય પોટાશ (કિ.ગ્રા./હે.)
ઓછો - ૦.૫ થી ઓછુ	ઓછો - ૨૬ થી ઓછુ	ઓછો – ૧૪૦ થી ઓછુ
મધ્યમ – ૦.૫ થી ૦.૭૫	મધ્યમ – ૨૬ થી ૫૬	મધ્યમ – ૧૪૦ થી ૨૮૦
પુરતો – ૦.૭૫ થી વધારે	પુરતો – ૫૬ થી વધારે	પુરતો – ૨૮૦ થી વધારે

આ ભલામણ પત્રક છે. તેનો કાયદેસર કાર્યવાહી નિષેધ છે.

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Envitro Laboratories Pvt Ltd ★ Rajkot ★

લેબોરેટરી આસીસ્ટન્ટ(જમીન ચકાસીણી પ્રયોગશાળા)

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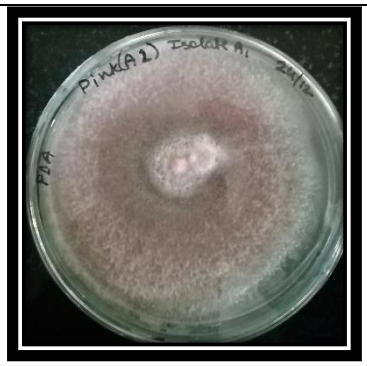
Fig 4: Soil Analysis Report Conducted By Envitro Laboratories, Rajkot

4.4 Antagonistic Activity Of *Trichoderma sp.* Against Most Pathogenic Fungal Strains i.e., *Fusarium sp.* Isolates:

An *In-vitro* antagonistic activity of *Trichoderma sp.* was carried out against specific isolated soil-borne fungal culture i.e., *Fusarium sp.* by dual culture plate method on PDA media, incubated at 28˚C for 3 to 4 days. The results revealed *Trichoderma sp.* showed mycoparasitism for both the *Fusarium sp.* 94.11% growth inhibition for both selected soil-borne fungal isolates was calculated from the given formula. With both the soil-borne fungal isolates *Trichoderma sp.* shows antagonism as mycoparasitism that completely inhibits the growth of both fungal phytopathogen. Thus, we can conclude that the *Trichoderma* sp. acts as a potential source of biocontrol agent for soil-borne pathogenic fungal isolates. Similarly concluded in (Shen et.al.2021) *Trichoderma* inhibiting many fungal pathogens.

		
Dual culture method of *Fusarium sp.* (A) and *Trichoderma sp.*	Control *Fusarium sp.*(A) without *Trichoderma sp.*	Control *Trichoderma sp.* without *Fusarium sp.*
		

Dual culture method of *Fusarium sp.*(B) and *Trichoderma sp*.	Control *Fusarium sp.* (B) without *Trichoderma sp.*	Control *Trichoderma sp.* without *Fusarium sp.*
		
Dual culture method of *Fusarium sp. and Trichoderma sp*. with control		
Fig 5: Antagonistic Activity By Dual Culture Method		

4.5 Fungal suspension for spore analysis:

At optimum growth conditions, fungal culture was sufficiently grown on a PDA medium tube. The freshly prepared slants were used to prepare fungal spore suspension. Where about 0.5 ml of distilled water was poured over fungal mycelium to scrap out the mycelium using a sterile swab. The prepared suspension was poured into another tube to count the spores per ml of suspension.

4.6 Seed Treatment:

Properly surface sterilized groundnut seeds were soaked for 30 mins in prepared *Fusarium* suspension, left in the moist chamber with a sterile cotton pad within the Petri plates to absorb the excess amount of suspension, and allowed to air-dry overnight. *Trichoderma* suspension was applied as a coating over the seeds. The treated groundnut seeds were planted in each pot. The pathogenicity was determined after proper growth of the shoot, root, and leaves of the groundnut plant. Contamination in groundnut plants can occur during any stage of farming i.e., pre-harvesting, harvesting, post-harvesting, storage, and transportation. thus, in the present study, the biocontrol agent *Trichoderma* suspension was coated over groundnut seeds and left for a few minutes. The coated seeds were shown hence, the disease can be prevented in its preliminary stage which finally leads to crop production.

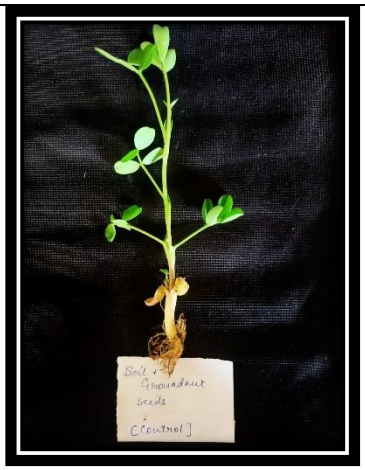
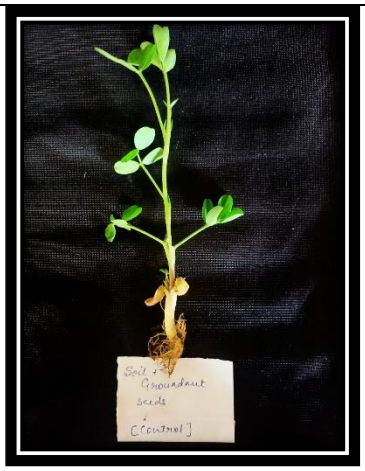
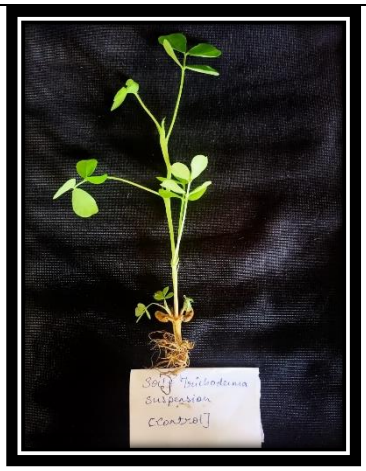
	
Groundnut seeds soaked in *Fusarium* suspension (Pathogenic)	Groundnut seeds soaked in *Fusarium* suspension (Pathogenic)
	
Groundnut seeds soaked in *Trichoderma* suspension	Groundnut seeds soaked with a combination of *Fusarium* and *Trichoderma suspension*

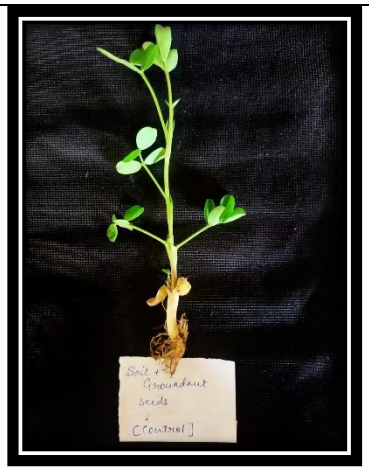
Fig 6: Seed Treatment Experiment

4.7 Pathogenicity testing:

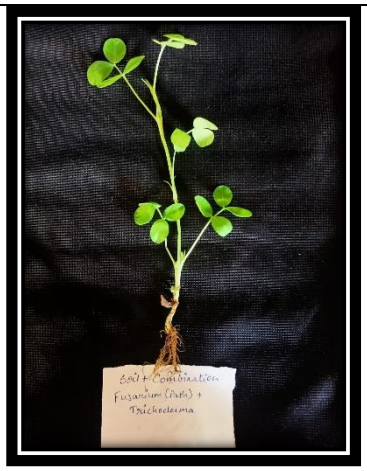
Soil infestation was attained by mixing the fungal suspension with the soil and water sprayed regularly for five to six days before seed plantation and the same amount of soil sample was kept as control treatment without any infestation of the pathogen. Five seeds were sown in each pot containing 200.0gm of pre-autoclaved soil and watered whenever needed. Pots were used with each treatment to study the effect of tested fungi and fungal biocontrol agents with survived plants were calculated at 15 to 20 days after planting, respectively. The resulting plant showed disease and weak root structure due to infection caused by the *Fusarium* pathogen. Some of the seeds were

unable to germinate and grow completely due to the pathogenic attack, there was no significant growth of root and shoot observed. The pathogenic plants showed a smaller number of leaves in comparison with the control, pathogenic plant leaves were too small and not able to open properly where the roots were very weak and thin indicating the contamination of the pathogen. Whereas the plant inoculated with *Trichoderma* suspension showed a greater number of leaves, properly opened and having branched roots. Indicating the potential of a biocontrol agent. Our results are similar to other investigators (Bardin et al., 2004; Infantin, et al., 2006; Mazen, et al., 2008; Baraka et al., 2009, Shaban & Bramawy, 2011).

	
Control plant without inoculation of fungal pathogen	Pathogenic plant with fungal infection
	
Control plant without inoculation of fungal pathogen	Control plant with *Trichoderma* infection



Control plant without inoculation of fungal pathogen



Groundnut plant inoculated with a combination of both pathogenic and biocontrol suspension

Fig 7: Pathogenicity Test

4.8. Effects Of The Pathogen As Well As The Biocontrol Agent On Groundnut Plant:

Effects of pathogenic fungal strain *Fusarium* sp. on Groundnut plant was analysed after 20 days of incubation and the difference in the various parameters such as number of leaves, root length, and shoot length was observed with the Groundnut plant infected with *Trichoderma sp.* as biocontrol agent.

Table 3. Effect of Pathogen As Well As A Biocontrol Agent On Groundnut Plant

Parameters	Number of leaves	Root length(cm)	Shoot length(cm)
Control	20.0	4.0	13.0
Pathogenic	16.0	2.0	4.0
Trichoderma	32.0	5.5	20.0
Combination	36.0	3.5	17.0

4.8.1 Effect Of Pathogen In Comparison With Biocontrol Agent Over Number Of Groundnut Plant Leaves

The effect of the pathogen was checked for groundnut plant leaves where we found the number of leaves was 16.0 in number. In comparison to pathogenic, the *Trichoderma-infested* groundnut plant leaves were higher than pathogenic plant leaves which were 32.0 in number. Whereas, in the combinational approach of pathogenic and biocontrol agent the number of leaves were 36.0 in number. When the structural patterns of leaves were observed, the pathogenic plant leaves were unable to open up completely and were smaller in size. When the leaves patterns and morphology for *Trichoderma* infested plant were completely opened and showed as a healthier plant in comparison to pathogenic groundnut plant. Thus, we can predict that the pathogenicity was reduced in presence of *Trichoderma* as a biocontrol agent.

Table 4. Effect Of Pathogen As Well As A Biocontrol Agent On Number Of Leaves Of Groundnut Plant Leaves

Parameters	Number of Leaves		Mean		SD
Control	20	22	19	19	1.52753
Pathogenic	16	13	15	13	1.52753
Trichoderma	32	30	35	30	2.51661
Combination	36	34	30	30	3.05505

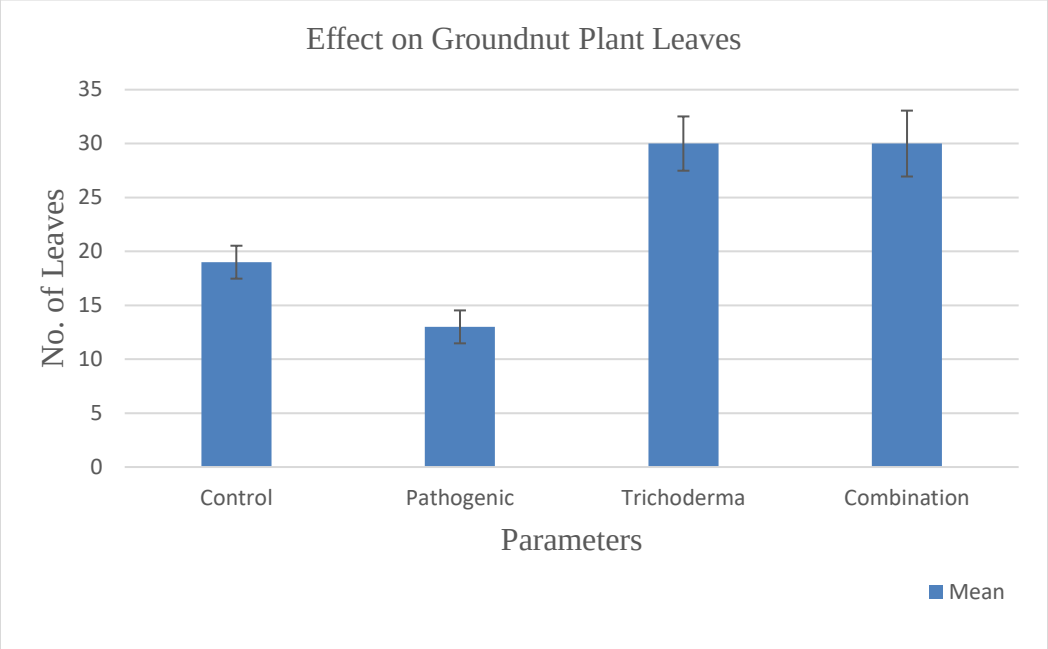


Fig 8. Effect Of Pathogen As Well As A Biocontrol Agent On Number Of Leaves Of Groundnut Plant Leaves

4.8.2 Effect Of Pathogen In Comparison With Biocontrol Agent Over Root Length Of Groundnut Plant

Similarly, the effect of the pathogen over the biocontrol agent showed the difference in parameters for the root length of the groundnut plant. When the root length was measured, the pathogenic groundnut plant showed lesser root length as compared to *Trichoderma* infested plant. The root length of control plant without any infection of pathogen was analysed as 4.0cm, pathogenic plant root length was measured as 2.0cm, the Trichoderma infested groundnut plant as 5.5cm and for the combinational approach measured as 3.5cm. these analysis shows the effect of pathogen on root length by a gradual decrement.

Table 5. Effect Of The Pathogen In Comparison With Biocontrol Agent Over Root Length Of Groundnut Plant

Parameters	Root Length(cm)		Mean		SD
Control	4.0	6.0	8.0	4.0	2.0

Pathogenic	2.0	4.0	6.0	2.0	2.0
Trichoderma	5.5	5.8	6.0	5.5	0.2517
Combination	3.5	3.8	4.0	3.5	0.2517

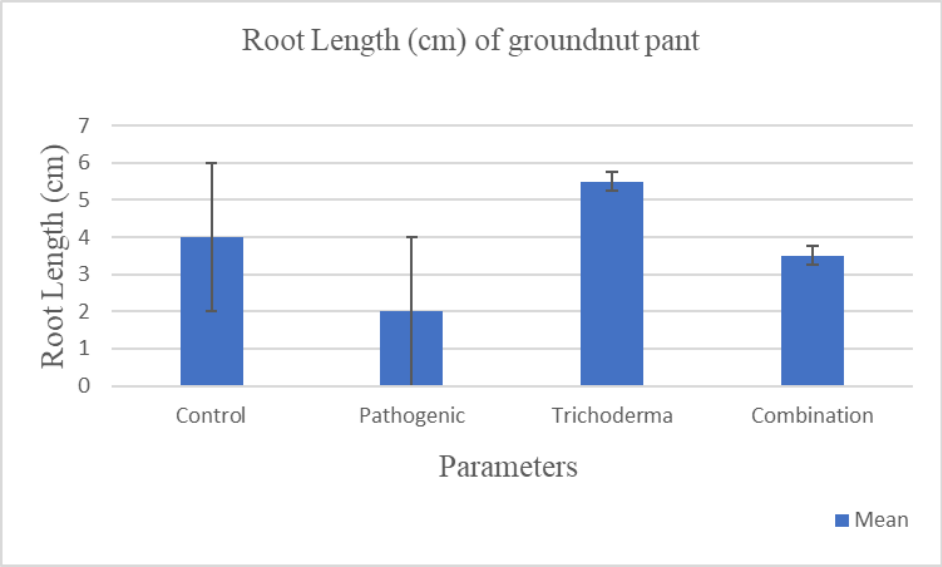


Fig 9. Effect Of Pathogen On Root Length For Groundnut Plant (cm)

4.8.3 Effect of Pathogen in Comparison with Biocontrol Agent Over Shoot Length of Groundnut Plant:

The effect of the pathogen was determined for the shoot length of the groundnut plant. The shoot length was analyzed with the pathogenic plant in comparison to control and *Trichoderma-infested* plant. The control plant determined the shoot length values as 13.0cm when compared with the pathogenic plant determined as 4.0cm which depicts the cause of the pathogen in groundnut plant. The groundnut plant was not able to grow properly the shoot and roots were very thinner and weak due to infection of pathogen. While the combinational approach showed 17.0cm of shoot length and the shoot were properly developed and strong enough to carry out a proper growth of groundnut plant. Thus, the effect of pathogen in groundnut plant shows its affect in growth of plant.

Table 6. Effect Of Pathogen As Well As A Biocontrol Agent On Shoot Length Of Groundnut Plant

Parameters	Shoot Length(cm)			Mean	SD
Control	13.0	15.0	16.0	13.0	1.527525
Pathogenic	4.0	6.0	8.0	4.0	2.0
Trichoderma	20.0	24.0	23.0	20.0	2.081666
Combination	17.0	20.0	19.0	17.0	1.527525

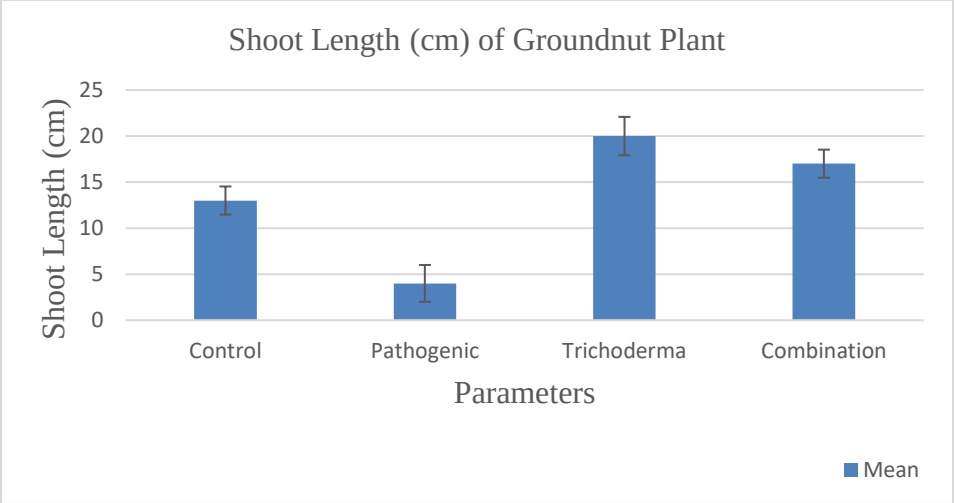


Fig 10: Effect Of Pathogen On Shoot Length For Groundnut Plant (cm)

5.1 Estimation of Chlorophyll Content in Groundnut Plant:

Chlorophyll is an important factor in plant development. The green color of a plant is due to its chlorophyll content. Chlorophyll content was estimated by spectrophotometric analysis. Where 0.5 gm of groundnut plant leaves were taken out and cut into small pieces. Crushed with 10.0 ml of 80% acetone using a mortar and pestle. Centrifugation of the crushed solution at 10,000 rpm for 10 minutes and the supernatant was transferred to a fresh tube. Take absorbance against 80% acetone as a blank. Take the absorbance of the sample at 645 nm and 663 nm wavelength. Chlorophyll was determined by the following formulas;

$$\text{Chlorophyll a} = \frac{12.7 \times \text{O.D}(663) \ - 2.69 \times \text{O.D}(645)}{1000 \times \text{W}} \times \text{V}$$

$$\text{Chlorophyll b} = \frac{22.9 \times \text{O.D}(645) \ - 4.68 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

$$\text{Total Chlorophyll} = \frac{20.2 \times \text{O.D}(645) \ - \ 8.02 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

Table 7. Estimation Of Chlorophyll Content For Groundnut Plant Leaves

Parameters	**OD at 645nm**	**OD at 663nm**
Control	0.262	0.161
Pathogenic	0.153	0.149
Trichoderma	0.247	0.243
Combination	0.130	0.134

Table 8. Statistical Analysis Of Chlorophyll Content In Groundnut Plant Leaves (mg/g tissues)

Parameters	**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophyll**
Control	0.025	0.1028	0.08002
Pathogenic	0.0296	0.05613	0.0379
Trichoderma	0.0484	0.0903	0.0608
Combination	0.02704	0.047	0.03104

5.1.1 Estimation of Chlorophyll a Content for Groundnut Plant Leaves

Chlorophyll content was determined by spectrophotometric analysis where 80% acetone was used to crush the 0.5 gm of groundnut leaves. Chlorophyll content dtermines 3 parameters chlorophyll a, chlorophyll b, and toatal chlorophyll content for groundnut plant leaves. Chlorophyll a content was determined by the following formula;

$$\text{Chlorophyll a} = \frac{12.7 \times \text{O.D}(663) \ - 2.69 \times \text{O.D}(645)}{1000 \times \text{W}} \times \text{V}$$

Table 9. Estimation Of Chlorophyll a Content For Groundnut Plant Leaves

Parameters	**Chlorophyll a**			**Mean**	**SD**
Control	0.025	0.022	0.026	0.022	0.002082
Pathogenic	0.0296	0.0292	0.0293	0.0292	0.000208
Trichoderma	0.0484	0.0482	0.0486	0.0482	0.0002
Combination	0.02704	0.02708	0.02706	0.02704	2.0

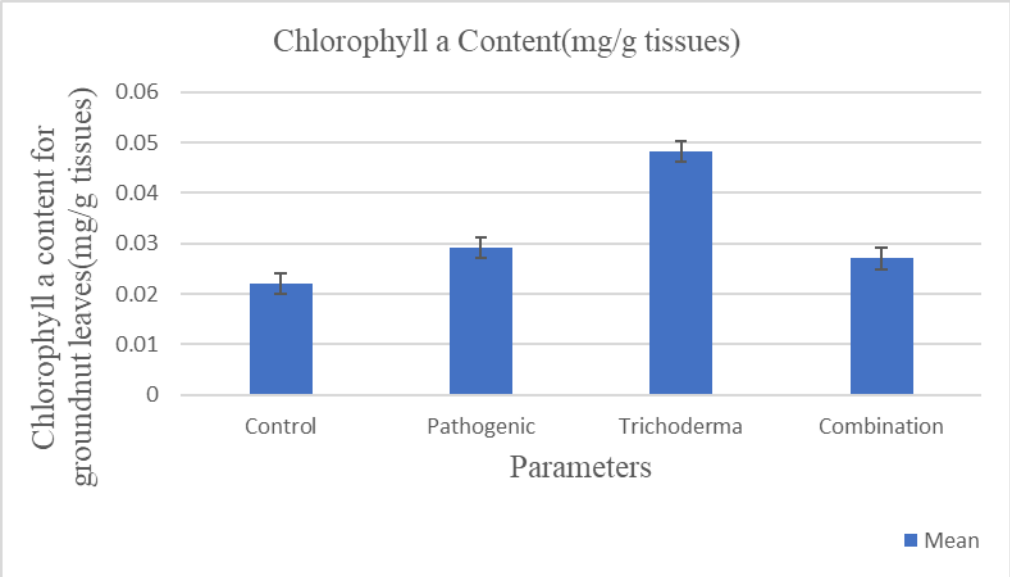


Fig 11: Estimation Of Chlorophyll a Content For Groundnut Plant Leaves

5.1.2. Estimation of Chlorophyll b Content for Groundnut Plant Leaves

Chlorophyll content was determined by spectrophotometric analysis where 80% acetone was used to crush the 0.5 gm of groundnut leaves. Chlorophyll content dtermines 3 parameters chlorophyll a, chlorophyll b, and toatal chlorophyll content for groundnut plant leaves. Chlorophyll b content was determined by the following formula;

$$\text{Chlorophyll b} = \frac{22.9 \times \text{O.D}(645) \ - 4.68 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

Table 10. Estimation Of Chlorophyll b Content For Groundnut Plant Leaves (mg/g tissues)

Parameters	**Chlorophyll b**			**Mean**	**SD**
Control	0.1028	0.1025	0.1023	0.1023	0.000252
Pathogenic	0.05613	0.05616	0.05619	0.05613	0.00002
Trichoderma	0.0903	0.0906	0.0909	0.0903	0.0003
Combination	0.047	0.042	0.049	0.042	0.003606

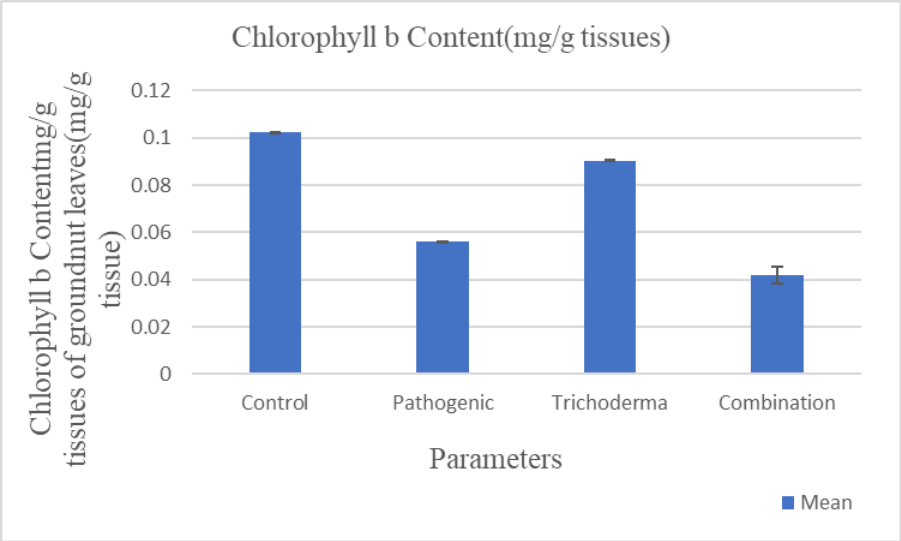


Fig 12: Estimation Of Chlorophyll b Content For Groundnut Plant Leaves

5.1.3. Estimation Of Total Chlorophyll Content For Groundnut Plant Leaves

Chlorophyll content was determined by spectrophotometric analysis where 80% acetone was used to crush the 0.5 gm of groundnut leaves. Chlorophyll content dtermines 3 parameters chlorophyll a, chlorophyll b, and toatal chlorophyll content for groundnut plant leaves. Chlorophyll b content was determined by the following formula;

$$\text{Total Chlorophyll} = \frac{20.2 \times \text{O.D}(645) \; - \; 8.02 \times \text{O.D}(663)}{1000 \times \text{W}} \times \text{V}$$

Table 11. Estimation Of Total Chlorophyll Content Of Groundnut Plant Leaves (mg/g tissues)

Parameters	Total Chlorophyll			Mean	SD
Control	0.08002	0.08004	0.08006	0.08002	0.00025
Pathogenic	0.0379	0.0376	0.0374	0.0374	0.000252
Trichoderma	0.0608	0.0603	0.0605	0.0603	0.000252

Combination	0.03104	0.03108	0.03102	0.03102	0.000251

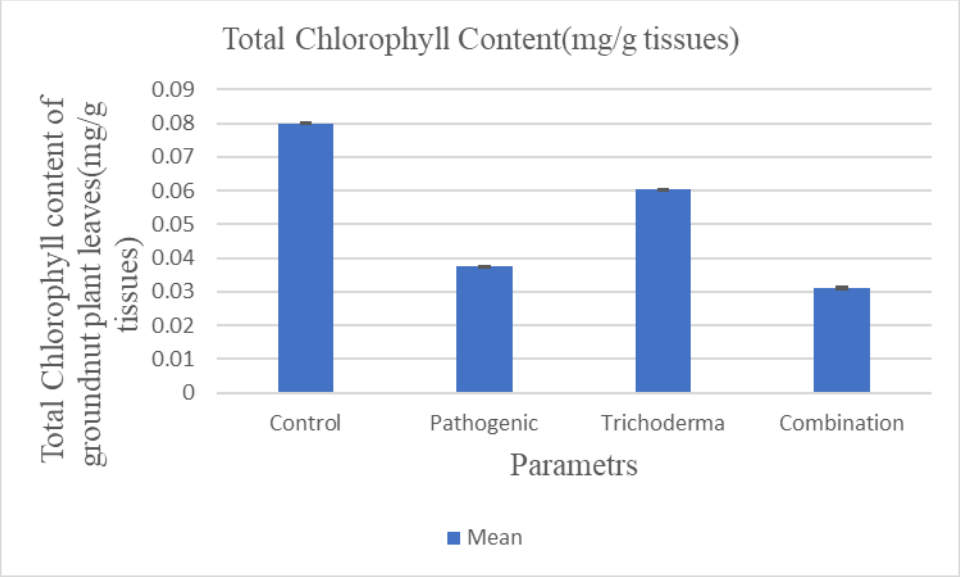


Fig 13: Estimation Of Total Chlorophyll Content For Groundnut Plant Leaves

Chapter 5: - CONCLUSION

In present study, effect of biocontrol agent against soil-borne fungal pathogens on groundnut plant has been studied. The *Trichoderma* as potent bio-control agent as it shows its mycoparasitic effects against *Fusarium* sp. causing infection in Groundnut plant. Trichoderma is used as a biocontrol agent due to its many beneficial uses like; disease control agent, plant growth promoter and play important role in bio-remediation. It interacts with the root and prevents infection caused by various fungal isolates. It helps the plant to protect from soil borne pathogens. *Trichoderma* potentially shows mycoparasitism against plant pathogens such as *A. flavus*, *Rhizopus* spp. and *Fusarium* spp. Contamination in groundnut plant can occur during any stage of farming i, e., pre-harvesting, harvesting, post-harvesting, storage, and transportation. So, in the current study, the *Trichoderma* as a biocontrol agent is being coated on Groundnut seeds to check its effects over pathogenic fungal isolate. Then after the seeds were grown so that the infection can be prevent or cure in preliminary stage which ultimately leads to healthy crop production. Thus, Trichoderma serves as a potential source of biocontrol agent and can be applied for agricultural field.it can be used as a biopesticide in agricultural field without any harmful effects.

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ACHIEVEMENTS

Sr. No.	Achievements	Organization	Date Of Achievement
1.	Microbial Identification using 18S rRNA Gene Sequencing	Gene Explore, Ahmedabad, Gujarat	06th February 2023
2.	NCBI Submission (*Fusarium* sp. isolate Fungi small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence)	GenBank: OQ654012.1	20th March 2023
3.	Paper Publication (ISOLATION AND IDENTIFICATION OF PGPR TRAITS FROM SOIL SAMPLES OF THE SAURASHTRA REGION)	Indian Journal of Scientific Research	31st January 2023
3.	Poster Presentation on the topic (EXPLORATION OF POTENT ANTAGONIST TRICHODERMA STRAIN AGAINST SOIL-BORN PHYTOPATHOGEN FUSARIUM SPECIES AND ITS EFFECTS ON GROUNDNUT SEEDS)	Atmiya University, Rajkot, Gujarat	11th February 2023
4.	Attended National Conference on Emerging Paradigm In Agricultural Microbiology	Atmiya University, Rajkot, Gujarat	11th February 2023

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Fusarium sp. isolate Fungi small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: OQ654012.1
FASTA Graphics

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Change region shown

Customize view

Analyze this sequence
Run BLAST
Pick Primers
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Related information
Taxonomy

Recent activity
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- Fusarium sp. isolate AT7A internal transcribed spacer 1, partial sequen Nucleotide

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ISOLATION AND IDENTIFICATION OF PGPR TRAITS FROM SOIL SAMPLES OF THE SAURASHTRA REGION

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) is an eco-friendly and potent microorganism that can serve as both a nitrogen fixer and a biocontrol agent. Hence it can be used as a substitute for chemical fertilizers and pesticides. So, in the present investigation, total 9 rhizospheric soil samples (2 from Veraval, 1 sample from Khijadiya, 1 from Rajkot, and 3 samples from Morbi) from a different region of Saurashtra have been collected. Serial dilutions method was employed followed the by spread plate method for the isolation of rhizospheric bacterial strains. Total 41 bacterial strains were isolated from soil samples among which 4 bacterial strains potentially act as rhizobacteria. They were screened by various growth promotion tests such as the HCN test, Ammonia test, siderophore production, IAA production, and chitinase assay. KS2, KC8, KC9, and KC11 show the highest results for all these tests. So, these traits can be further used as potential biofertilizers to promote the growth of plants. According to the results of test these traits may belong to *Azatobacter* sp., *Bacillus* sp., and *Pseudomonas* sp.

EXPLORATION OF POTENT ANTAGONIST TRICHODERMA STRAIN AGAINST SOIL-BORN PHYTOPATHOGEN FUSARIUM SPECIES AND ITS EFFECTS ON GROUNDNUT SEEDS

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Abstract:

Groundnut is the third largest oil seed producer in Gujarat. *Fusarium* is a soil-borne fungal pathogen generally causing fungal diseases in groundnut plants. Thus, it is necessary to control the pathogenicity of *Fusarium* species in groundnut. A present investigation has been carried out to inhibit the pathogenicity of Fusarium species from groundnut. The soil sample was collected from different regions of Gujarat where groundnut production is large in amount. For further study followed by serial dilutions up to 10^-9 dilutions and direct inoculation on selective media for fungal isolation that are Dichloran Chloramphenicol Peptone Agar (DCPA) and Potato Dextrose Agar (PDA) that was supplemented with streptomycin to avoid bacterial contamination, followed by incubation for 7 days at 27C. Based on morphological and cultural characteristics, 20 isolates were obtained, out of which 2 fungal isolates were tentatively identified as *Fusarium solani* and *Fusarium oxysporum*. *Trichoderma harzanium* was isolated and identified from the soil sample collected from Junagadh, Gujarat. Antagonistic screening activity was performed against *Trichoderma* species as it is known as the potent biocontrol agent against various pathogenic fungi. As a result, it was determined that *Trichoderma* was able to inhibit the growth of *Fusarium* species. And can be used as a biocontrol agent in agricultural fields.

Keywords: Biocontrol agent, *Trichoderma sp.*, *Fusarium sp.*, Groundnut seeds.

Introduction :

Groundnut is the third largest oil seed producer in Gujarat. Groundnuts are subjected to multiple soil-borne fungal pathogens. In spite of having other beneficial effects groundnut is not much exported to other countries due to loss product yield and poor quality of crop and being contaminated with soil-borne fungal pathogens like *Fusarium*. (Lamprecht et. al. 2011) *Fusarium* a is a genus of filamentous fungus widely distributed throughout the tropical regions of the world with major species ranging from endophytic to phytopathogenic fungal strains. Causing infections to vegetables, grains, seeds, and evenly plant yield. Phytopathogenic species of *Fusarium* include some important plant pathogens associated with wilt and root rot in over 100 crops. *Fusarium oxysporum* and *Fusarium solani* are the two fungal phytopathogenic strains mainly isolated from soil that resides as conidia, mycelium, or chlamydospore. *Fusarium* mainly attacks numerous plants that are important to human life and animal nutrition. It infects plant parts causing reduced economical yield and poor product quality. Nowadays these plant diseases can be controlled by exploring the biocontrol agents that may inhibit the growth of pathogens. Antagonism refers to the suppression of normal growth and its pathogenicity. Several fungal isolates such as *Trichoderma harzianum* which is found in normal rhizosphere soil region. Besides having a significant role in bioremediation it is a biocontrol agent against phytopathogens like Fusarium species. The main aim of this study is to reduce plant diseases by exploring of potent antagonist *Trichoderma* strain.

Methods and material:

Various soil samples were collected from different areas of Saurashtra.

↓

0.1ml serially diluted soil sample was spread on Potato Dextrose Agar plates and DCPA plated incubated at 28C for 5 days for fungal isolation(Waksman,1922) (Ratna et al., 2015).

↓

The fungal isolated were identified through Lactophenol Cotton Blue staining at a 40X light microscope (Aneja 2002).

↓

Physicochemical soil analysis was performed to evaluate the pH, organic carbon, and Nitrogen contents of the soil (Ameen et al.,2016).

↓

Followed by invitro antagonistic activity against Trichoderma species to check the potency and pathogenicity of fungal isolates.

↓

Seed treatment by soaking method for pot culture assay(Rudresh et al., 2005)(Karthick et. al. 2010).

Observations and results:

Conclusion:

Fusarium being pathogenic causes various fungal diseases in groundnut which suppress the plant yield and efficiency of crops as well as soil quality. *Trichoderma* species were able effective in controlling the growth of