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Isolation, Identification And Management Of Fungal Pathogen Responsible For Chilli Leaf Spot Disease

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Abstract: Chilli (*Capsicum annuum L.*) is an economically important crop in the world. Chilli is cultivated in almost 38.4 thousand hectares area of Pakistan and it is used three times in meal daily in fresh form or dried powder. Many biotic and abiotic factors caused reduction in its yield. In a-biotic factors the most devastating pathogens are fungi. Leaf spot is an important yield-reducing fungal disease of chilli caused by *Alternaria alternata*. In the current experiment the management of *A. alternata* was done in both in-*vitro* and *in-vivo* conditions by using various biocontrol agents i.e. META (*Metarahizum*), *Trichoderma harzianum,* LEC (*Lecanicillium lecani*), Siderophore *producing* by *Pseudomonas* bacteria. In in-*vivo* the research work was designed under CRD in pot trials and field trial under RCBD to check the effectiveness of various bioconrol agents and six cultivated verities (CH121, 900F1, Red wing, SAYBAN, AAHP-I, Priya chilli) against *A. alternata.* Isolation, identification, and purification of the pathogen causing leaf spot of chilli was carried out after the sampling of spots effected leaves from disease infested field of horticulture department and collected from the chak 597 TDA. For this purpose, all the verities were inoculated with *A. alternata* inoculum by spraying and drenching method and the results were assessed on the basis of development of the disease. And in the lab experiment the results were assessed by checking the development of inhibition zone. By using SPSS 19 software for statistical analysis the data were analyzed. Under in-*vitro T. harzianum* and *Metarhizium* were found more efficient in suppressing the growth of *A. Alternaria* while in field and pots only the former was efficient to check the disease.

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Key word: Chilli (*Capsicum annuum L.*), Leaf spot, biocontrol agents

Introduction

Chilli (*Capsicum annum* L.) is an important crop in Pakistan which belongs to family *solanaceous* (Sahi, 2010). Chilli belongs to kingdom *Plantae* order *Solanales* and genera *Capsicum*. Chilli consists of 90 genera of *solanasae* family. Thirty known species belongs to genus *Capsicum* like that *C. annum, C. chinense, C. beccatum, C. pubescent and C. frutescence* are grown in diverse parts of the world and also grow in kitchen gardening. After potato and tomato it is third most important vegetable crop of Pakistan. It has bushy appearance and height of chilli ranges from 60 to 80cm. Warm and moist climate conditions are good for the growth of chilli plants. On plant stem of chilli, there is mostly hairy growth. In different directions plant has tap root system from where many lateral roots originate. The fruit of *Capsicum annum* has different names, such as pepper, chilli or chilli pepper depending upon the nature of fruits and place. It grows in tropics, subtropics and also in temperate regions of the world (Hussain and Abid, 2011).

China, Mexico, Pakistan, India are top chilli producing countries. In the world chillies and peppers (green and dry) were harvested on approximately 1688082 and 1937370 hectares in 2014-15 with a total yield of 3818768 and 32324345 tones. India are produced around 1014.60 million tons of chilli with a total area of 654 million ha and a productivity of 1551 kg/ha, in 2005-06. Chilli is a major crop of Pakistan. In Pakistan total agricultural cropped area is 23.40 million ha. However only 0.386 million hectare land is under cultivation for vegetables. In Sindh province, of Pakistan it is considered an important cash crop (Khan *et al.,* 2014). In Asia Taluka, Kunri are located in the district of Umer Kot, of Pakistan, which is known as largest belt of chilli production. This region was known as the capital of the world for the production of chilli (FAO, 2010).

There are several factors which determine that per hectare yield is low. These factors include low yielding varieties, fertilizer overdoses, improper use of organic fertilizers, unfavorable environmental conditions. Many other factors that also effect of this

crop biotic and abiotic diseases cause in chilli. Fungi, bacteria, viruses and nematodes cause diseases in chillies (Hemannavar *et al*., 2009). Chilli is suffered from many diseases such as wilt, anthracnose, leaf spot, viruses and several insect pests can cause disease in chilli fruits (Airaki *et al*., 2012).

A pathogenic fungus is *Alternaria alternata.* Throughout the world *Alternaria is* caused disease in many plants. Symptoms of this disease is caused by *alternaria* and development in 1997. *Alternaria* is a genus of Ascomycota fungi. *Alternaria* species are the leading plant pathogens causing diseases. The Genus *Alternaria Nees.* Ex Fr. Associate to the sub-division *Deuteromycotina* class *Hyphomycetes*, family *Dematiaceae* (Woudenberg *et al.,* 2013). The genus is spread all over the world and caused disease in crops (Bochalya *et al.,* 2012).

This pathogen is affected leaves, stems, flowers and fruits. It is also affected ornamental plants. Spot on fruits and leaf caused by *Alternaria* pathogen most destructive disease. Leaf spot disease is caused in chilli at mature and immature stage (Roberts *et al*., 2009). Besides anthracnose infection, *Altrnaria alternata* also infects the chilli fruits. This pathogen is caused disease in some important host that cauliflower, broccoli, carrots, potatoes, apple, Chinese cabbage, and in tomatoes (Quresh *et al*., 2015).

Alternaria pathogen attacks on the aerial parts of host. Small circular, dark spots symptoms of *Alternaria* infections are produced. These spots size is ½ inch and are these spots are usually gray and black in color. Around the spots concentric rings are developed and pathogen growth rate is uniform due to environmental conditions. Lesion are developed on plants parts that appear in a specific pattern (Spalding and King, 1999). Spores are appeared on leaf surface of the infected plant. Effected area is covered by fungus spores. Pathogen directly penetrates into the host by stomata, wounds and other open cells. By spores and mycelia, pathogen are survived on host plant (Anwar and Arshad, 2010). *Alternaria* pathogen spreads by plant residues and infected seeds. If fungus is seed borne then should appear at seedling stage which is observed in case of *Alternaria*. Leaf spot is most destructive disease of chilli. Spores of this pathogen cause allergies and asthma symptoms in human (Khan *et al*., 2014). Its primary infections cause more damages in crops. Pathogen is found in dead organic matter in soil. Spores splashed by air and water. Fungus pathogens found everywhere in moist situation of the environment. Abiotic condition spots sometimes are resemble from *Alternaria* pathogen. Secondary infections also caused more damage and toxic produce in chilli crop. . This pathogen is saprophytic pollutants. Spores of this pathogen are multi celled club shaped. Spores are singly produced in long chain. Conidiophores formed in chain and branching. Chlorosis produced in vascular system of the plants when pathogen attacked. Pathogens are grown at the 25-28°C temperature which favorable conditions for *Alternaria* (Salo, 2006).

Before management of leaf spot disease symptoms of the disease should be identified based on the reports of this pathogen. Usually, disease free seed is used for cultivation ad if there are chances of seed borne pathogens then it must be treated with suitable fungicide. Moisture on the plant surface favors the disease development while during wind there are less chances of disease due to lack of surface moisture. If we keep plant free of injuries and insect, there are very less chances of disease. Disease is reduced by the weed control and crop residue destruction. Incidence of some *Alternaria* species are reduced by the ultraviolet light exposure. Free pathogenic plant stock material should be used. There are number of fungicides which are used against *alternaria.* Chlorothalonil, captan, fludioxonil, imazalil, iprodione, maneb, magalncozeb, thiram, and selected copper. Leaf spot disease are also managed from bio-control, plants extract, and chemical management (Narain *et al.,* 2000).

Following objectives were followed to overcome the problems

1. Isolation, identification, and purification of fungus from diseased samples of chilli.
2. Assessment of four bio-control agents against *Alternaria* leaf spot disease of chilli.

MATERIALS AND METHODS

Experiment was conducted at research area of Plant Pathology Department, University of Agriculture Faisalabad. Six genotype seed of chilli were collected from AARI, Faisalabad from vegetable department. First seeds were sown in beds form and after that nursery was shift in pots and in field block.

Table 1. Shows the genotypes were used in experimentation.

Sr. #	1	2	3	4	5	6
Genotype	CH-121	Chilli 900F1	Red wing	SAYBAN	AAHP-I	Priya chilli

Transplantation of chilli seedling in pots trial and field

After preparation of field beds and filling of pots transplantation of done nursery were after 35 days of sowing in beds form. In field plants were sown with 90cm plant to plant distance and 60cm row to row distance. Each genotype and treatment had three replications. Total 15 plants were in one replication also in field and in pots. Total 90 plants were in pots and in field. Different agronomic practices such as fertilization, Irrigation and eradication of weed were done regularly for better crop growth. The layout of experiment was Randomized Complete Block Design (RBCD) which show in fig 1.

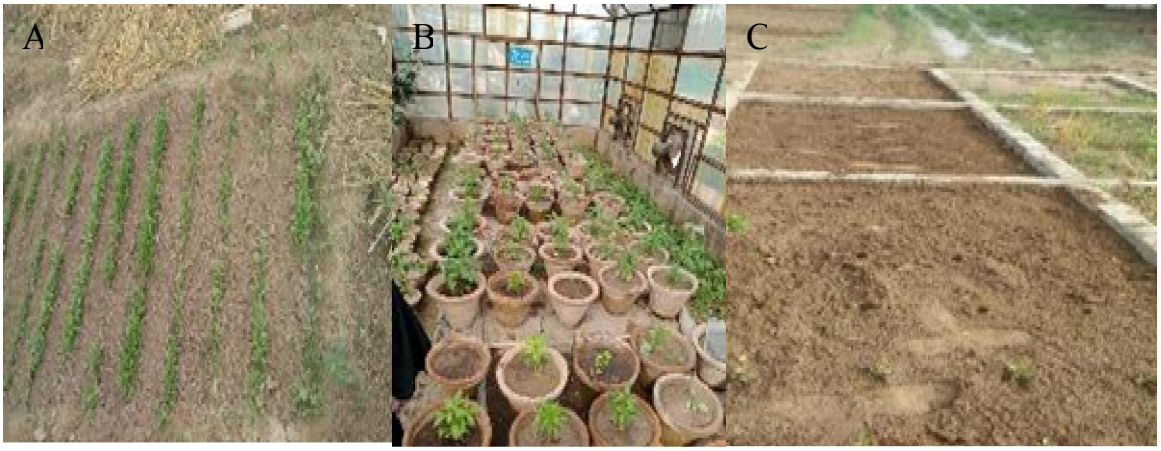

Fig 1. (A)Genotype grow in beds (B) Nursery transplant in pots (C) Nursery transplant in field

collections of diseased plant samples

Diseased chilli plants showing typical symptoms of leaf spot and marginal necrosis were collected from research field areas of University of Agriculture Faisalabad from horticulture field and from Tehsil kotaddu chak 597 TDA. Samples of those infected plants were brought to the Lab of Plant Pathology for pathogen isolation from infected leave parts. Samples were kept in plastic bag. Bags were tags with name for identifying samples of chilli. Samples were consisted on leaves. Some leaves were more infected with pathogen which show in fig 1.2.

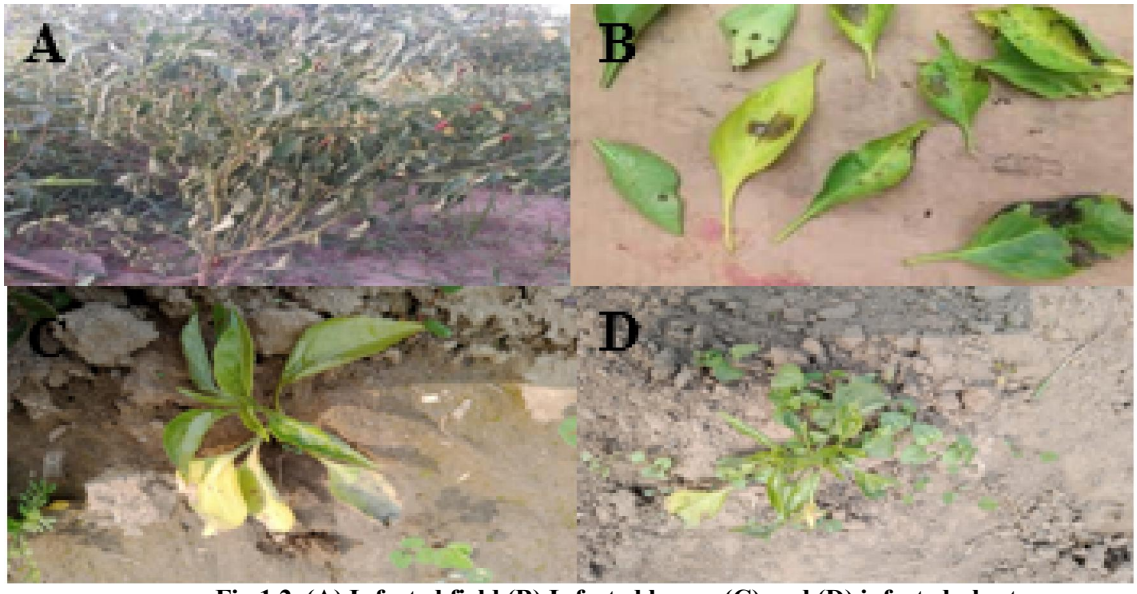

Fig 1.2. (A) Infected field (B) Infected leaves (C) and (D) infected plants.

Media Preparation and sterilization

Every fungus needs a specific growth media for their growth. Leaf spot fungus *Alternaria alternata* being facultative pathogen can grow well on artificial media like Potato Dextrose Agar medium on which fungus shows well performance. PDA medium was prepared and autoclave at 121°C at 15 psi for 20 min for their sterilization. In sterilized Petri plates medium was poured and was let in laminar flow to solidify. Since we were isolating *Alternaria* spp from plant leaves samples. Bacterial growth could be prevented by addition of some antibiotic Ampiciline, Kanamycine or chloremphenicol was added at the rate of 10mg/L.

Table 1.2: Antibiotics used in the work whereas the concentration of working and stock solution is given

Name of Antibiotic	Working concentration	Stock solution
Ampiciline	100 mg/l	1gram in 10 ml water
Kanamycine	50 mg/l	1gram in 20 ml water
Chloramphenicol	34 mg/l	340 mg in10 ml Ethyle alcohol

Isolation of *A. alternata* from infected samples

In the laminar flow, diseased plant samples along with all the required apparatus such as media plates, distilled water, blade and scissor, were placed at the time of isolation. Tap water was used to wash the samples gently and then air dried properly. The infected leaves including some healthy plant portions of diseased samples were cut into small pieces of 1-2cm. Surface sterilization of these samples was done by disinfecting them with 70 % Ethanol and then dipped them twice in autoclaved distilled water. After that, for the purpose of drying and soaking samples were blotted on sterilized filter paper. These disinfected samples were then placed on nutrient media (PDA) containing Petri plates. Four to five samples were placed on each media plate at equal distance. Cultured plates were placed in an incubator at controlled temperature of 25-30 ºC for 5-7 days so that further growth of fungi occurs in plates. In this way *Alternaria* fungus colonies were recovered properly.

Purification and Identification of fungus

Different fungal colonies were appeared on the plates after seven days. Hyphal tip and mycelium transfer method was used to purify the fungal pathogen. Blackish cottony mycelium or spore of fungus was picked very carefully with the help of inoculating needle and placed on another sterilized petri plate that contained PDA media (Fig 1.3).

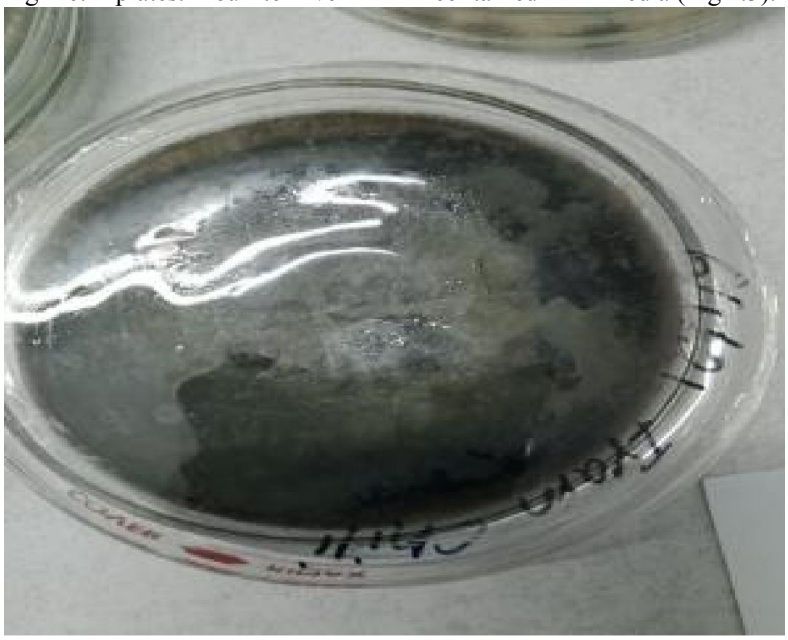

Fig. 1.3: Purified culture of *A. alternata* associated with leaf spot disease in chilli on PDA media

Slide preparation

Slide preparation was done after mass culturing for further analyses. *Alternaria alternate* identification was done on the basis of available literature on morphology such as colony color and size, spore shape, structure and growth pattern.

Spore collection

Spore suspension was made from one month old pure culture of *Alternaria alternate* isolates. Spores were collected by pouring 10ml of double distilled autoclaved cold water in each petri plate of pure cultured fungus and then scrapping the mycelium surface gently with the help of sterilized spatula to mix it in distilled water under a laminar flow. When a suspension of spore was made then at the end, it was passed through three to four layers of muslin cloth very carefully. Spores were stored in falcon tubes and kept in freezer at $4C^{0.}$

Staining of spores

For identifying pathogen and physiological difference, staining of spores was done after collecting spores of each isolate. Lacto phenol tryphan blue stain was used to stain the spores.

Isolation of DNA from *A. alternata* pure colonies

DNA of *Alternaria alternate* was isolated by using CTAB method. Fresh fungal mycellial mass of 200mg of fresh fungal culture was taken from PDA was grinded with liquid nitrogen in autoclaved pestle and morter. After grinding, 500 ml of TES buffer was added to the grinded product. Then 60 -100ml of buffer aided with proteinase K was added to the same tube to digest the proteins and mixture was vortex for short time. This step was more important for DNA extraction because without using proteinase K, final pellet of DNA was mixed with proteins, and was showing bulky white pellet, gel electrophoretic assay show no bands of DNA. Mixture was incubated for 60 minutes at 65C and was mixed gently after every 10 minutes. Add 1/10 volume of 10%CTAB after incubation and was incubate it for another 10 minutes at 65C.Now equal volume of phenol chloroform Isoamyle alcohol was added. As result two phase of liquid was formed and mixed it gently and then it was incubating at 0C for 30 minutes. It was centrifuge on 4C for 10 minutes at 13000 rpm. Equal volume of 5M sodium acetate was added, mixed gently, and was placed on ice for 40-60 minutes and then centrifuge at 13000 rpm for 10 minutes. A small protein pellet was formed at the base, supernatant was transfer into new eppendorf tube and will be put 0.50 volume of chilled isopropanol to precipitate DNA. At 13000 rpm it was centrifuge for 5minutes. A small pellet was appearing at base of eppendorf tube, then pellet was washed with 70% cold ethanol three times and the pellet was dry overnight. Pellet was dissolved in 30ul double distilled autoclave water or TE buffer. For RNA digestion 5ul RNase was added and was incubated for 10-20minutes at 37^0C.

Isolation of DNA from 6 genotypes of chilli

Plant material like young leaflets of each variety of Chilli were taken. DNA isolation was performed with the same procedure as was used for DNA isolation from the fungal samples.

Agarose Gel Electrophoresis

Electrophoresis is used to determine the molecular weight of DNA and by comparing it with standard or known markers we can assess the size of DNA isolated from our samples. The gel was run at40-50Volts for30 minutes before visualizing it. To achieve optimal separation for a specific range of sizes, 1-2 % concentration of gel was used.

The gel was placed into the tank carefully and pour TAE 1X buffer into the tank. Then add 1ul loading dye in Polymerase chain reaction tubes. Then add 6ul of DNA samples in each PCR tube. Total 6 µl DNA was loaded in the wells of the gel. 6µl of marker DNA was added in the first well of gel. For each sample, tips changed. The power supply was connected with tank. Voltage was set at-150voltage. The bubble was produced with passage of current and samples migrate towards Positive electrode. To ensure the dyes are migrating watch the gel. For half an hour run until dye move most of the way through gel. It was monitored the progress of DNA by Ulrta-violet light on the gel as it was running by placing it over UV tray with all cares.

PCR was performed via ITS rDNA primers. Reaction was performed according to Gonzalez *et al.* (2008). PCR amplification was done by using primer set ITS1 Forward primer (TCCGTAGGTGAACCTGCGG) and ITS Reverse primer (TCCTCCGCTT ATTGATATGC). Amplification was done in PCR tubes and mixture contain following recipes. The PCR reaction was done in a master gradient thermal cycler (LABNET, NJ, USA). 30 cycles were completed after denaturing and before giving extension temperature. The amplified PCR amplicons were confirmed through gel electrophoresis using 1.2% agarose gel. UV light was photographed with a gel documentary system (GDS) to visualize DNA.

Inoculum application in pots trial and in field

Chilli nursery was transplanted in pots in the green house of plant pathology experimental fields of UAF. Six varieties were sown with three replications. In the laminar flow spore suspension was made by mixing distilled water in the pure cultured plate and scrapping mycelium from surface of colony gently. With the help of hypodermic needle stem of chilli plant was inoculated with fungal inoculum after one month of transplantation. All the plants were equally treated with spore suspension in the field. Inoculum was applied in field experiment. Inoculum was of *Alternaria alternata.* In field block inoculum was apply in spray form on leaves without negative control plants.

Assessment of disease severity

After 10 days of inoculation symptoms of leaf spot were scored. Development of disease was recorded and monitored. Disease was appeared at seedling stage and flowering stage. At both the stages disease was recorded. A disease rating scale was used for comparison to record disease severity. Disease grading was done according to (Wongpia and Lomthaisong, 2010) scale.

On the basis of visual symptoms of leaf spot number of leaves disease was recorded

1. 0 spot not infected by leaf spot
2. Infected 10% leaves by leaf spot disease symptoms 3-1-10%
3. 25% infected by leaf spot disease on leaves 5-11-25%
4. 50% infected by leaf spot disease on leaves 7-26-50%

In *vitro* suppressed growth of *Alternaria alternata* by using BCA (Bio-Control Agents) and in *vivo* BCA apply in pots and in field by spraying method.

- **Collection of Bio-control agents (BCA):** The untainted culture of 4 type's different antagonistic organism *Trichoderma harzianum, Metarhizium* (META), *Lecanic illiumlecanii* (LEC) and *Siderophore* act as *pseudomonas aerugnosa* bacteria (SID) were collected from the Soil and Environmental Science Department of University of Agriculture, Faisalabad (UAF).
- **Preparation of liquid broth (LB) media:** SID bacteria were grown on LB media which is generally used for growth of bacteria.

Multiplication of BCAs

The pure culture was multiplied in petri plates, *Tricoderma,* META, LEC, were multiplied on PDA plates. For the multiplication of SID bacteria LB media was prepared. *Tricoderma*, META, LEC, were sub cultured again by single spore culture method on PDA at 25±2C° for culturing of SID bacteria streaking was done from pure culture and placed in incubator at 25±2C°.

Dual culture technique

Fungal antagonists were evaluated by inoculating the pathogen at one side of petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4cm gap. For this activity growing cultures were used. After required period of incubation after control plate reached 90mm diameter, the radial growth of pathogen was measured.

Biological Control in *vitro*

BCA *Trichoderma*, META, LEC, were fungus. These were used as BCA in lab but SID was a bacteria use as biocontrol against *Alternaria alternata* pathogen which cause disease leaf spot in chilli crop. Fungus BCA were grown on PDA (potato dextrose agar) media. After 14 days pure colony of fungal and one bacteria BCA colony were grown. Then evaluated by inoculating needle pathogen at one side of the petri plate and antagonist (BCA) at exactly opposite side of the same plate by leaving about 4cm gap by using dual culture or inhibition technique. Diameter of mycelial growth of the fungus was observed after 21 days of incubation. The percentage inhibition of the growth of the fungus at different concentrations over control was calculated which show in fig 1.4.

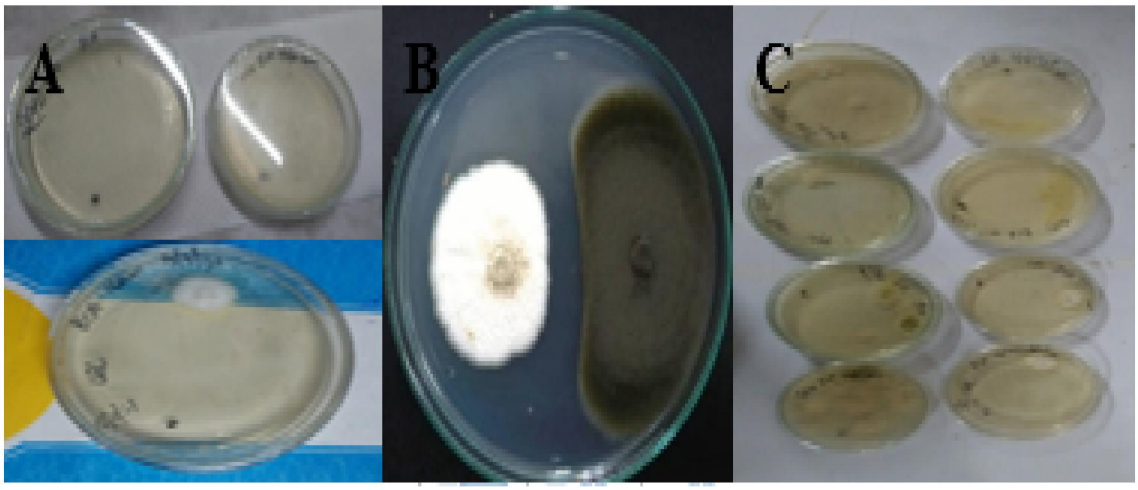

Fig 1.4. (A) For suppress growth of pathogen dual culture assay (B) one side growth of BCA and other side pathogen growth (C) All plates with BCA for *A. alternata* inhibition growth

Biological Control in *vivo*

Spores were collected from 10 days old pure culture plate distilled autoclaved and chilled at 4C water was used and water was added in the petri plate containing *Trichoderma harziantum,* META, LEC, culture on and scratch it with needle softly and then filter with 4 folding of muslin cloth. Hemocytometer was used for counting spores then spore suspension was prepared. LB broth media was prepared for SID bacteria large piece of suspension was taken from two days old culture plate shaking was done 48 hours in a shaker.

BCA apply in field and in pots

Spore suspension of *Trichoderma,* META, LEC, was applied after 10 days of fungal inoculation when indications of the disease appear on the plants. 5ml of spore suspension10^3 spores/ml was given with the help of needle syringe on leaves of the plants in such a way equally and simple treatment for all plants. Soil drenching method was used for the application of SID culture 5ml apply on plants. Foliar application was also done with the help of sprayer as layout design of pots and in field.

Data recording

- Disease severity will be calculated by the following formulas given below

$$\text{Disease Severity \%} = \frac{\text{No. of infected leaves}}{\text{No of tota leaves}} \times 100$$

The data were recorded for following traits like FRW (Fresh root weight), FSW (Fresh shoot weight), plant height, FFW (Fresh fruit weight), DFW (dry fruit weight) and infected leaves per plants in pots and filed conditions

Statistical Analysis

Finally, all observations related to the plant were registered, and the data were subjected to RCBD statistical analysis using SPSS 19. Statistical software.

Results and discussion

Isolation and identification of Pathogen from chilli leaves disease sample

Diseased and healthy samples were collected from vegetable area of horticulture department university of Agriculture Faisalabad and collect from Tehsil kotaddu chak 597 TDA. Collected samples were visually examined for disease symptoms in fruits, and leaves. Selected samples were washed in running tap water, immersed in 1% sodium hypochlorite for 10 minutes and dried on sterile filter papers. Diseased tissues were cut into thin slices containing healthy and disease portion. Placed on PDA media in petri plates. The petri plates were placed in incubator at 28C for 7 days (Sariah *et al.,* 1989). Isolation and establishment of single spore culture was carried out by following procedure described. Which show in fig 8 (Than *et al.,* 2008).

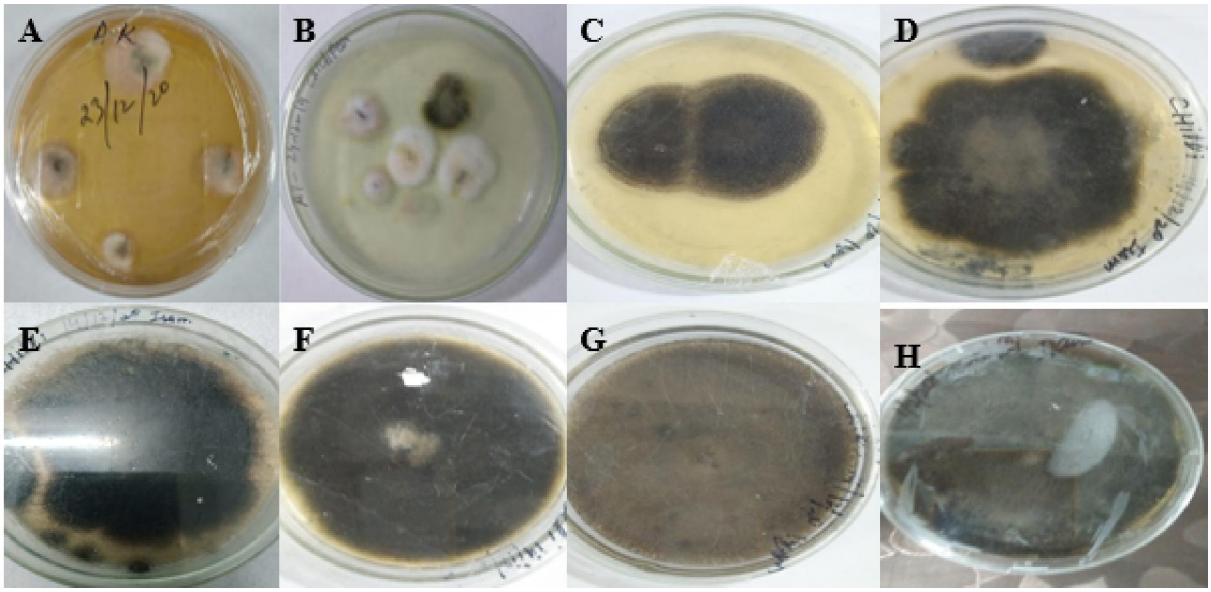

Fig 2: Leaves samples of chilli on PDA media plates for isolation of *A. alternata*

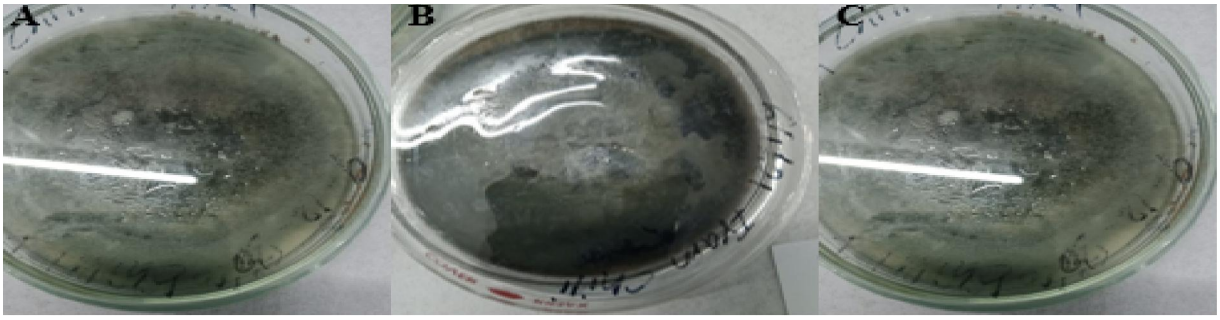

Fig 2.1: Growth rate and morphological variation of pathogen and colony show black color

The microscopic morphological characters such as size and shape of conidia, existence of setae and colony character such as color, growth rate and texture, was used for the identification of *A. alternate* (Smith and Black., 1996). *A. alternata* was identified following the method of lacto phenol cotton blue mounting. It stains the fungal cytoplasm and provides a light blue background beside which the walls of hyphae can easily observed. A drop of lacto phenol cotton blue was placed on a slide. A small tuft of fungus preferably with spores and fruiting bodies were transferred into the drop using flamed sterilized needle. The material was tested and mixed gently. A cover slip was placed on the top of the preparation by applying pressure or gently heating or by addition of more lacto phenol cotton blue. The excess amount around the cover was removed with 70% ethanol with the help of blotting paper. And check under microscope for identified pathogen which show in fig 2.2, 2.3 and 2.4.

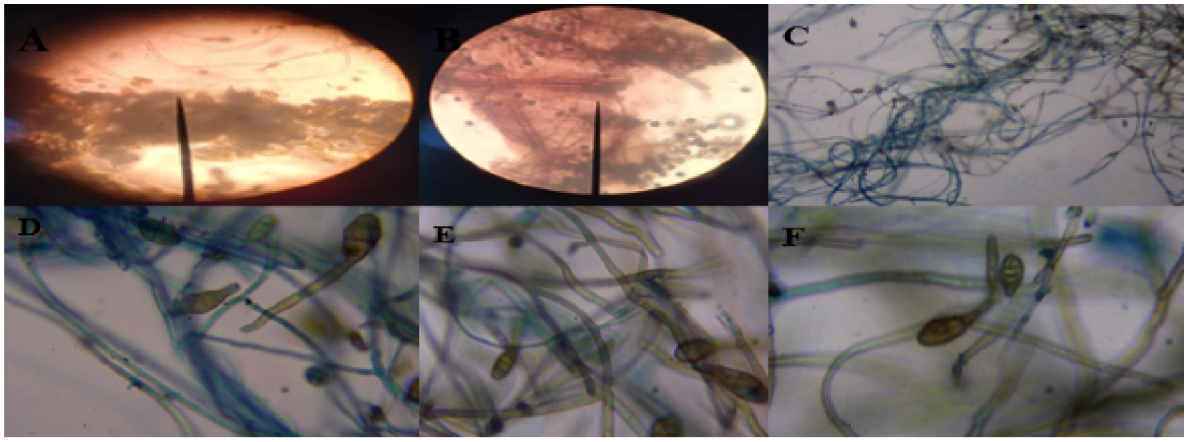

Fig 2.2: Identification of pathogen under microscope

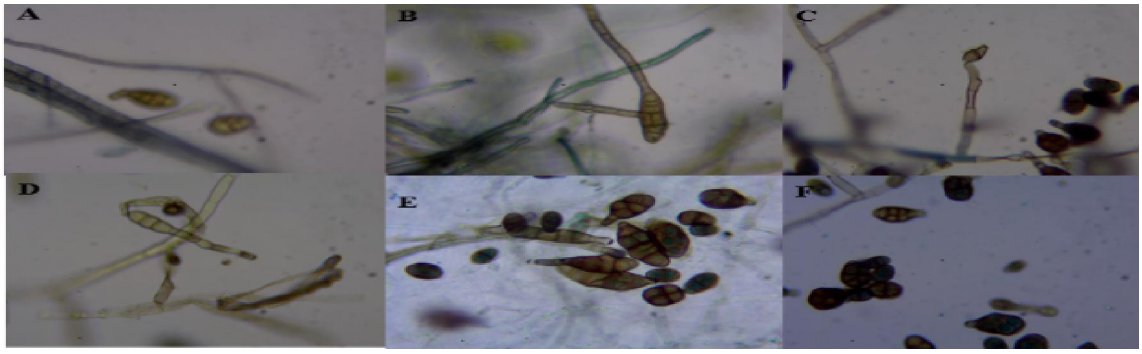

Fig 2.3: Identified by lacto phenol cotton blue of pathogen (A) (B) (C) Show mycelia and hyphae (D) (E) (F) Show spores of pathogen

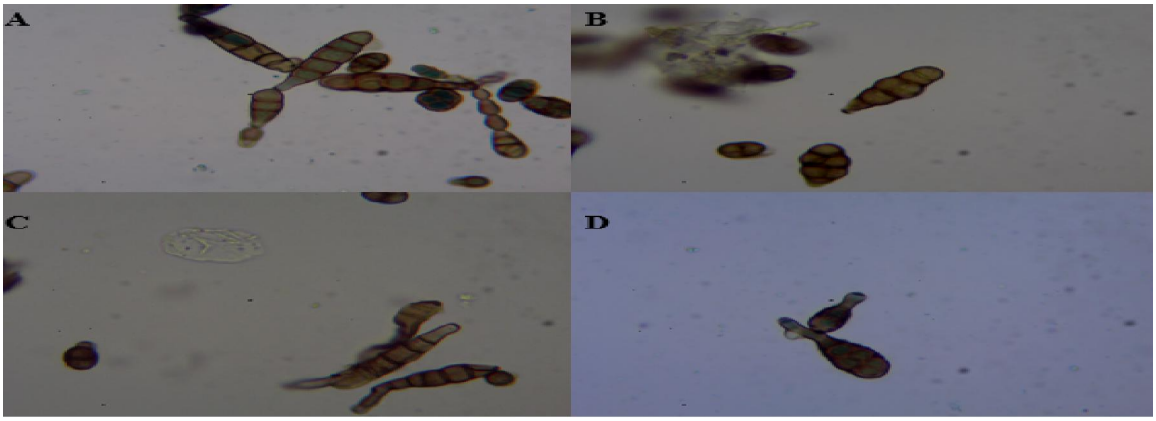

Fig 2.4. Spores of pathogen and dye by lacto phenol

Isolation of DNA from plant samples and *A. alternata* sample

DNA of *A. alternata* was isolated by using the CTAB method. Fresh fungal mycelial mass of 200mg of fresh fungal culture was taken from PDA was grinded with liquid nitrogen in autoclaved pestle and mortar. After grinding, 500 ml of TES buffer was added to the grinded product. Then 60 -100ml of buffer aided with proteinase K was added to the same tube to digest the proteins and the mixture was vortex for short time. This step was more important for DNA

extraction because without using proteinase K, the final pellet of DNA was mixed with proteins, and was showing bulky white pellet, gel electrophoretic assay show no bands of DNA Mixture was incubated for 60 minutes at 65°C and was mixed gently after every 10 minutes. Add 1/10 volume of 10% CTAB after incubation and incubated for another10 minute at 65°C. And then it was incubating at 0°C for 30 minutes. It was centrifuge at 4°C for 10 minutes at 13000 rpm. An equal volume of 5M sodium acetate was added, mixed gently, and was placed on ice for 40-60 minutes and then centrifuged at 13000 rpm for 10 minutes. A small protein pellet was formed at the base; the supernatant was transferred into a new Eppendorf tube and put 0.50 volume of chilled isopropanol to precipitate DNA. At 13000rpm it was centrifuge for 5 minutes. A small pellet was appearing at the base of Eppendorf tube, then the pellet was washed with 70% cold ethanol three times and the pellet was dry overnight. Pellet was dissolved in 30ul double-distilled autoclaved water or TE buffer. For RNA digestion 5ul RNase was added and was incubated for 10-20minutes at 37°C. The results are shown in the Fig. 3, 3.1, and 3.2.

Visualization of DNA bands on the Agarose Gel

DNA bands were visualized under UV light. For this purpose used /transilluminator. For this the gloves and mask were necessary. Carefully carried gel and put into transilluminator by opening the plastic cover. Closed the plastic cover which protects from UV. Turn on the UV transilluminator and bands were observed and also take the picture.

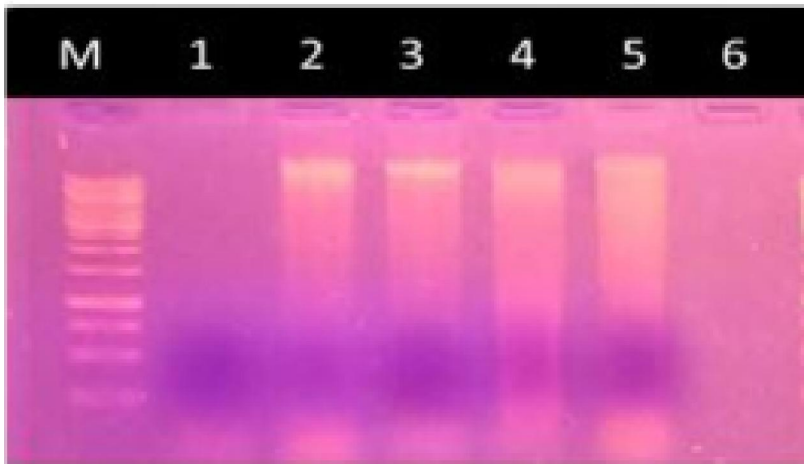

Fig 3: DNA bands of plant sample of chilli on gel, M is 1 Kb marker while 1-4 are the DNA samples of chilli genotypes

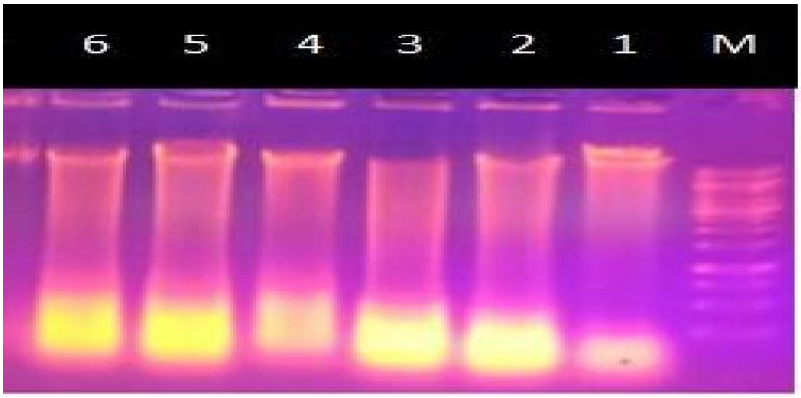

Fig 3.1: DNA bands of plant sample of chilli on gel, M is 1 Kb marker while 1-14 are the DNA samples of chilli genotypes (V1 to V6)

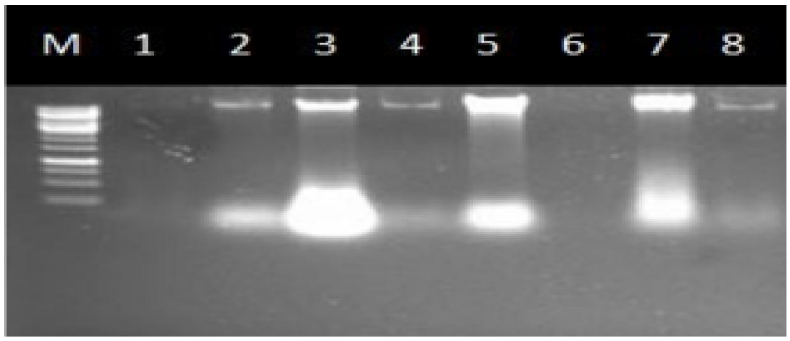

Fig 3.2: DNA bands of plant sample of chilli on gel, M is 1 Kb marker while 1-14 are the DNA samples of chilli genotypes (V1 to V6) and pathogen

Polymerase chain reaction for ITS region of *A. alternata*

PCR was performed by using ITS1 and ITS4 primers. The reaction was performed according to Gonzalez *et al.* (2008). PCR amplification was done by using primer set ITS1 Forward primer (TCCGTAGGTGAACCTGCGG) and ITS4 Reverse primer (TCCTCCGCTTATTGATATGC). Amplification was done in PCR tubes with total reaction mixture of 25-30 µl. The PCR reaction was done in a master gradient thermal cycler (LABNET, NJ, USA). The 30 cycles were completed after denaturing and before giving extension temperature. The amplified PCR amplicons were confirmed through gel electrophoresis using 1 % agarose gel. UV light was used to visualize the gel and after completion was photographed with a gel documentary system (GDS) to visualized DNA. Following results were obtained which show in (Fig 3.3).

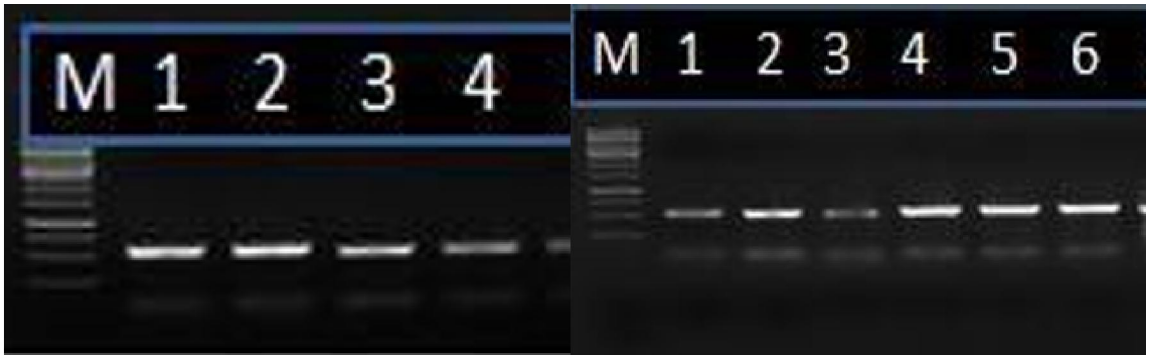

Fig. 3.3. Line 1: M is 1 kb marker of Plant DNA and patgoen DNA bands Antagonistic test of BCA against pathogen:

Table 2. Completely Randomized analysis of variance for in vitro treatments to manage *Alternaria* spp. ANOVA for growth inhibition (*Alternaria alternata*) by bio-control agents over control

Source	DF	SS	MS	F	P
V001	8	128.601	16.0751**	129	0
Error	18	2.252	0.1251		
Total	26	130.852			

Grand Mean 4.8407 **CV** 7.31

Table 2.1. Application of different bio control agents to manage *Alternaria* in *vitro*

Treatment	Mean	SE	Significance
Alternaria+Tricho	2.19	0.1595	F
Alternaria+Meta	3.14	0.0684	E
Alternaria+LEC	4.06	0.1484	D
Alternaria+SID	4.26	0.0115	D
Tricho (Control)	8.00	0.1233	B
Meta (Control)	5.70	0.2082	C
LEC (Control)	4.40	0.2646	D
SID (Control)	2.83	0.2404	E
Alternaria (Control)	8.97	0.3697	A

LSD: 0.6067

In-vitro evaluation of bio-control microorganism against *A. alternata*

Different antagonistic fungi one bacteria such as *Trichoderma harzianum, Metarhizium* (META), *Lecanicillium lecanii* (LEC), SID *Siderophore* material that remove *by pseudomonas aeruginosa* bacteria effect on mycelial growth of *A. alternate* is given in fig.4. With the variable response of test fungus all of antagonistic fungi showed significant results. Results indicated that growth of *A. alternate* was significantly reduced by all of the antagonistic fungi as compared to control. In vitro test of bio-control microorganisms with *A. alternata* showed that *Trichoderma harzianum* had more mycelial growth inhibition of pathogen 81%. *Metarhizum* was proved to be second best BCAs which inhibit the growth 59% of pathogen. *Lecanicilium Lecanii* was proved to be third BCAs which inhibit the growth 47% of pathogen. *Pseudomonas* was proved to be fourth BCAs which inhibit the growth 29% of pathogen. As control growth of the *A. alternata* pathogen was showed by means values 8.97 respectively. Maximum inhibit of pathogen growth was by *Trichoderma* and *Metarhizium* control and minimum by inhibit by SID *Siderophore* material that remove *by pseudomonas* bacteria.

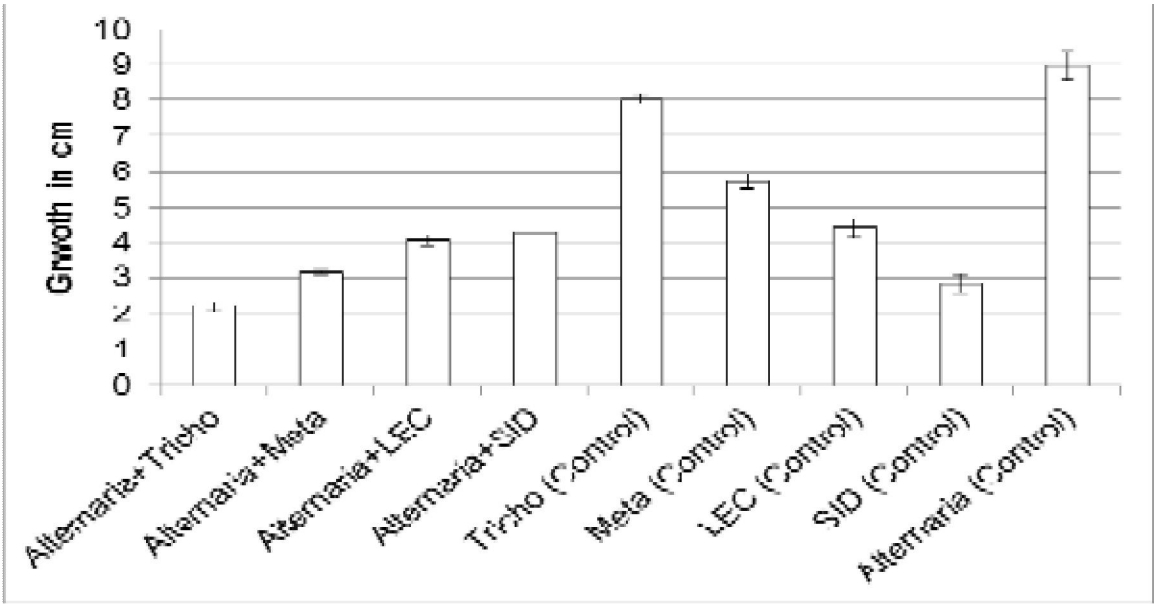

Fig 4: The bar graph shows the PGI (percent growth inhibition) of *Alternaria alternata* through four Bio-control agents. Positive and negative controls are also shown in the graph.

Response of seedling attributes under natural inoculum conditions

The experiments were conducted in in pots and field block for assessment of plant growth parameters of different varieties. Seedlings of six different varieties CH121, 900F1, Red wing, SAYBAN, AAHP-I, and Priya chilli were transplanted under natural inoculum conditions in the field block and pots. After some time period following parameters were recorded and analyzed.

Fresh root weight

Analysis of Variance Table for fresh root weight of pot experiment showed that there is a significant relation between treated plant, varieties and their interaction effect. The Analysis of Variance Table for field experiments showed that there is significant relation between treated plants and non-significant were observed for verities and interaction effect of verities and treated plants (Table 3 and Table 3.1).

Moreover, Performance of different treatments and varieties for FRW under field and pot experiments showed clear significant differences (Table 3.2)

The comparison of treatments for field experiment that LEC showed maximum results and minimum were observed in positive control While under pot experiment, Meta gives highest fresh root weight and minimum were observed in positive control same as in field experiment. Furthermore for verities, 900F1 showed maximum fresh root weight for field experiment. And minimum were observed in Priya Chilli. Similarly in pot experiment, all variety showed maximum fresh root weight. These are all non-significant because no relation between them and not any effect each other (Table 3.2).

Table 3. Analysis of Variance Table for FRW under pot experiment

Source	DF	SS	MS	F	P
Replication	2	0.96	0.4815		
Treatment	5	150.96	30.1926**	4.75	0.0009
Varieties	5	6.63	1.3259^{NS}	0.21	0.9578
Treatment×varieties	25	402.37	16.0948**	2.53	0.0012
Error	70	445.04	6.3577		
Total	107	1005.96			
Grand Mean = 6.0185		**CV**=41.8			

Table 3.1 Analysis of Variance Table for FRW under field experiment

Source	DF	SS	MS	F	P
Blocks	2	1.463	0.7315		
Treatment	5	135.185	27.037**	8.09	0
Varieties	5	51.407	10.2815^{NS}	3.08	0.0144
Treatment*varieties	25	173.593	6.9437^{NS}	2.08	0.0089
Error	70	233.87	3.341		
Total	107	595.519			
Grand Mean=4.7963			**CV=** 38.11		

Table 3.2. Performance of different treatments and varieties for FRW under field and pot experiments

Experiments	**Field**		**Pot**	
Treatments				
Trichoderma	4.39	BC	6.22	B
Meta	4.44	BC	8.11	A
LEC	7.11	A	6.17	B
SID	4.78	B	6.28	B
+ve control	3.44	C	4.33	C
-ve control	4.61	BC	5.00	BC
LSD value	1.22		1.68	
Varieties				
CH-121	4.28	C	6.28	A
900F1	5.89	A	5.67	A
Red wing	4.67	BC	6.22	A
SAYBAN	4.39	BC	5.83	A
AAHP-I	5.56	AB	6.28	A
Priya Chilli	4.01	C	5.83	A
LSD value	1.22		1.68	

Fresh Shoot Weight

Analysis of Variance Table for fresh shoot weight of pot and field block experiment showed that there is a significant relation between all treated plant the interaction effect of verities and treated plant except the verities which showed non-significant results (Table 4 and Table 4.1). Moreover, Performance of different treatments for FSW under field and pot experiments showed clear significant differences (Table 4.2). The comparison of treatments for field experiment showed that LEC control gives maximum results and minimum were observed in META and SID. While under pot experiment, negative control gives highest fresh shoot weight and minimum were observed in LEC treatment. Furthermore, for verities, all showed maximum fresh shoot weight for field experiment and for pots experiment. There are non-significant relation between them (Table 4.2).

Table 4. Analysis of Variance Table for FSW under pot experiment

Source	DF	SS	MS	F	P
Replication	2	325.2	162.62		
Treatment	5	1960.2	392.031**	5.42	0.0003
Varieties	5	224.2	44.831 ^{NS}	0.62	0.6852
Treatment×varieties	25	3622.9	144.91 ^{NS}	2	0.0122
Error	70	5064.8	72.354		
Total	107	11197.2			

Grand Mean 25.769 **CV** 33.01

Table 4.1. Analysis of Variance Table for FSW under field experiment

Source	DF	SS	MS	F	P
Blocks	2	156.9	78.454		
Treatment	5	4490.2	898.031**	4.95	0.0006
Varieties	5	560.6	112.12 ^{NS}	0.62	0.686
Treatment*varieties	25	7503.3	300.14 ^{NS}	1.66	0.0516
Error	70	12688.4	181.263		
Total	107	25399.4			

Grand Mean 27.12 **CV** 49.64

Table 4.2. Performance of different treatments and varieties for FSW under field and pot experiments

Experiments	**Field**		**Pot**	
Treatments				
Trichoderma	25.44	BC	23.28	BC
Meta	19.83	C	27.72	AB
LEC	38.28	A	20.11	C
SID	19.83	C	22.50	BC
+ve control	28.44	BC	28.11	AB
-ve control	30.89	AB	32.89	A
LSD value	8.95		5.66	
Varieties				
CH-121	23.33	A	22.89	A
900F1	26.06	A	25.28	A
Red wing	31.00	A	26.22	A
SAYBAN	27.06	A	26.00	A
AAHP-I	27.83	A	27.28	A
Priya Chilli	27.44	A	26.94	A
LSD value	8.95		5.66	

Fresh fruit weight

Analysis of Variance Table for fresh fruit weight of pot experiment showed that there is a non-significant relation between all treated plant and the interaction effect of verities and treated plant with the verities which showed non-significant results (Table 5). Analysis of Variance Table for fresh fruit weight of field block experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant except the verities which showed non-significant results (Table 5.1). Moreover, Performance of different treatments and varieties for FFW under field and pot experiments showed clear significant differences (Table 5.2). The comparison of treatments for field experiment showed that *Trichoderma* gives maximum results and minimum were observed in positive control. While under pot experiment, negative control gives highest fresh fruit weight and minimum were observed in META, LEC, and SID. Furthermore for verities, CH-121 showed maximum fresh fruit weight for field experiment. And minimum were observed in SAYBAN. Similarly in pot experiment, CH-121 and Priya chili variety showed maximum fresh fruit weight and minimum was in SAYBAN (Table 5.2).

Table 5. Analysis of Variance Table for FFW under pot experiment

Source	DF	SS	MS	F	P
Replication	2	5.241	2.6204		
Treatment	5	41.63	8.326 ^{NS}	1.4	0.2347
Varieties	5	77.296	15.459 ^{NS}	2.6	0.0324
Treatment×varieties	25	225.37	9.0148 ^{NS}	1.52	0.0889
Error	70	416.093	5.9442		
Total	107	765.63			

Grand Mean 6.8519 **CV** 35.58

Table 5.1. Analysis of Variance Table for FFW under field experiment

Source	DF	SS	MS	F	P
Blocks	2	17.85	8.9259		
Treatment	5	252.6	50.5204**	6.77	0
Varieties	5	102.05	20.4093 ^{NS}	2.74	0.0258
Treatment*varieties	25	405.45	16.2181**	2.17	0.0059
Error	70	522.15	7.4593		
Total	107	1300.1			

Grand Mean 8.213 **CV** 33.25

Table 5.2. Performance of different treatments and varieties for FFW under field and pot experiments

Experiments	**Field**		**Pot**	
Treatments				
Trichoderma	10.72	A	6.94	AB
Meta	8.56	BC	6.33	B
LEC	6.89	CD	6.50	B
SID	9.06	AB	6.44	B
+ve control	5.94	D	6.72	AB
-ve control	8.11	BC	8.17	A
LSD value	1.82		1.62	
Varieties				
CH-121	9.56	A	8.06	A
900F1	8.89	AB	6.44	AB
Red wing	7.67	BC	6.83	AB
SAYBAN	6.72	C	5.44	B
AAHP-I	8.89	AB	6.67	AB
Priya CHilli	7.56	BC	7.67	A
LSD value	1.82		1.62	

Dry Fruit Weight

Analysis of Variance Table for dry fruit weight of pot experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant with the verities which showed non-significant results (Table 6). Analysis of Variance Table for dry fruit weight of field block experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant significant except the verities which showed non-significant results (Table 6.1). Moreover, Performance of different treatments and varieties for DFW under field and pot experiments showed clear significant differences (Table 6.2). The comparison of treatments for field experiment showed that Trichoderma gives maximum results and minimum were observed in LEC and positive control. While under pot experiment, negative control gives highest dry fruit weight and minimum were observed in META, LEC, and SID. Furthermore for verities, CH-121 showed maximum dry fruit weight for field experiment. And minimum were observed in SAYBAN. Similarly in pot experiment, CH-121 and Priya chili variety showed maximum dry fruit weight and minimum was in SAYBAN (Table 6.2).

Table 6. Analysis of Variance Table for DFW under pot experiment

Source	DF	SS	MS	F	P
Replication	2	7.186	3.5928		
Treatment	5	119.105	23.821**	4.35	0.0017
Varieties	5	72.099	14.4198 ^{NS}	2.63	0.0307
Treatment×varieties	25	193.862	7.7545 ^{NS}	1.42	0.1295
Error	70	383.354	5.4765		
Total	107	775.605			

Grand Mean 5.4837 **CV** 42.68

Table 6.1. Analysis of Variance Table for DFW under field experiment

Source	DF	SS	MS	F	P
Treatment	5	186.9	37.3796**	5.89	0.0001
Varieties	5	90.35	18.0704 ^{NS}	2.85	0.0211
Treatment*varieties	25	360.05	14.4019**	2.27	0.0037
Error	72	456.96	6.3467		
Total	107	1094.26			

Grand Mean 6.4939 **CV** 38.79

Table 6.2. Performance of different treatments and varieties for DFW under field and pot experiments

Experiments	**Field**		**Pot**	
Treatments				
Trichoderma	8.79	A	5.37	BC
Meta	6.44	BC	4.43	C
LEC	5.18	C	4.40	C
SID	6.29	BC	5.20	BC
+ve control	4.89	C	6.05	AB
-ve control	7.37	AB	7.46	A
LSD value	1.67		1.56	
Varieties				
CH-121	7.80	A	6.79	A
900F1	6.72	ABC	5.00	BC
Red wing	6.03	BC	5.63	ABC
SAYBAN	5.08	C	4.12	C
AAHP-I	7.36	AB	5.43	ABC
Priya CHilli	5.96	BC	5.93	AB
LSD value	1.67		1.56	

Fruit Yield

Analysis of Variance Table for number of fruits yield of pot experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant with the verities which showed non-significant results (Table 7). Analysis of Variance Table for fruits yield of field block experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant significant except the verities which showed non-significant results (Table 7.1). Moreover, Performance of different treatments and varieties for fruits yield under field and pot experiments showed clear significant differences (Table 7.2). The comparison of treatments for field experiment showed that Trichoderma, META, LEC, SID, and negative control gives maximum results and minimum were observed in positive control. While under pot experiment, LEC, SID and negative control gives highest fruits yield and minimum were observed in Trichoderma, META, and positive control Furthermore for verities, showed non-significant in pots and field block because no interaction show between them for fruits yield (Table 7.2).

Table 7. Analysis of Variance Table for FY under pot experiment

Source	DF	SS	MS	F	P
Replication	2	11.13	5.5648		
Treatment	5	127.185	25.437**	7.5	0
Varieties	5	6.741	1.3481 ^{NS}	0.4	0.8491
Treatment×varieties	25	97.593	3.9037 ^{NS}	1.15	0.3158
Error	70	237.537	3.3934		
Total	107	480.185			

Grand Mean 5.1296 **CV** 35.91

Table 7.1. Analysis of Variance Table for FY under field experiment

Source	DF	SS	MS	F	P
Blocks	2	15.056	7.5278		
Treatment	5	183.75	36.75**	8.45	0
Varieties	5	12.417	2.4833 ^{NS}	0.57	0.7217
Treatment*varieties	25	283.417	11.3367**	2.61	0.0009
Error	70	304.278	4.3468		
Total	107	798.917			

Grand Mean 5.8611 **CV** 35.57

Table 7.2. Performance of different treatments and varieties for FY under field and pot experiments

Experiments	**Field**		**Pot**	
Treatments				
Trichoderma	7.11	A	4.00	B
Meta	6.67	A	4.17	B
LEC	5.78	A	5.72	A
SID	6.50	A	6.78	A
+ve control	3.11	B	4.11	B
-ve control	6.00	A	6.00	A
LSD value	1.39		1.22	
Varieties				
CH-121	6.17	A	5.44	A
900F1	5.39	A	5.11	A
Red wing	5.72	A	5.11	A
SAYBAN	5.50	A	4.83	A
AAHP-I	6.17	A	5.44	A
Priya Chilli	6.22	A	4.83	A
LSD value	1.39		1.22	

Number of Infected leaves

Analysis of Variance Table for number of infected leaves of pot experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant which show significant results but verities which showed non-significant results (Table 8). Analysis of Variance Table for number of infected leaves per plants of field block experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plants with verities which showed non-significant results (Table 8.1). Moreover, Performance of different treatments and varieties for infected leaves per plants under field and pot experiments showed clear significant differences (Table 2). The comparison of treatments for field experiment showed that positive control gives maximum results and minimum were observed in SID. While under pot experiment, positive control gives highest infected leaves per plants and minimum were observed in META, LEC, and SID and in negative control. Furthermore for verities, AAHP-I showed maximum infected leaves per plants for field experiment. And minimum were observed in 900F1. Similarly in pot experiment, AAHPI and variety showed maximum infected leaves per plants and minimum was in 900F1. (Table 8.2).

Table 8. Analysis of Variance Table for NOIL under pot experiment

Source	DF	SS	MS	F	P
Replicati	2	5.574	2.787		
Treatment	5	185.491	37.0981**	17.26	0
Varieties	5	11.157	2.2315 ^{NS}	1.04	0.4021
Treatment×varieties	25	128.343	5.1337**	2.39	0.0023
Error	70	150.426	2.1489		
Total	107	480.991			

Grand Mean 2.4907 **CV** 58.86

Table 8.1. Analysis of Variance Table for NOIL under field experiment

Source	DF	SS	MS	F	P
Blocks	2	16.074	8.037		
Treatment	5	275.713	55.1426**	13.96	0
Varieties	5	23.157	4.6315 ^{NS}	1.17	0.3316
Treatment*varieties	25	164.565	6.5826 ^{NS}	1.67	0.0496
Error	70	276.593	3.9513		
Total	107	756.102			

Grand Mean 3.213 **CV** 61.87

Table 8.2. Performance of different treatments and varieties for NOIL under field and pot experiments

Experiments	**Field**		**Pot**	
Treatments				
Trichoderma	3.06	BC	2.06	B
Meta	2.78	BCD	1.89	B
LEC	3.56	B	1.61	B
SID	1.50	D	1.78	B
+ve control	6.44	A	5.39	A
-ve control	1.94	CD	2.22	B
LSD value	1.32		0.97	
Varieties				
CH-121	3.28	AB	2.56	AB
900F1	2.50	B	2.11	B
Red wing	3.56	AB	2.56	AB
SAYBAN	3.39	AB	2.22	AB
AAHP-I	3.83	A	3.11	A
Priya CHilli	2.72	AB	2.39	AB
LSD value	1.32		0.97	

Plant Height

Analysis of Variance Table for Plant height of pot experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant significant the verities which showed non-significant results (Table 9). Analysis of Variance Table for plant height of field block experiment showed that there is a significant relation between all treated plant and the interaction effect of verities and treated plant with the verities which showed non-significant results (Table 9.1).

Moreover, Performance of different treatments and varieties for plant height under field and pot experiments showed clear significant differences (Table 9.2). The comparison of treatments for field experiment showed that negative control gives maximum results and minimum were observed in Trichoderma. While under pot experiment, negative control gives highest plant height and minimum were observed in LEC. Furthermore, for verities, AAHP-I showed maximum Plant height for field experiment. And minimum were observed in CH-121,900F1, SAYBAN. Similarly in pot experiment, 900F1 variety showed maximum plant height and minimum was in CH-121 (Table 9.2).

Table 9. Analysis of Variance Table for PH under pot experiment

Source	DF	SS	MS	F	P
Replication	2	74	37.021		
Treatment	5	3373.3	674.665**	9.34	0
Varieties	5	562.1	112.426 ^{NS}	1.56	0.1837
Treatment×varieties	25	4676.8	187.071**	2.59	0.001
Error	70	5055.3	72.218		
Total	107	13741.6			

Grand Mean 44.958 **CV** 18.9

Table 9.1. Analysis of Variance Table for PH under field experiment

Source	DF	SS	MS	F	P
Blocks	2	1.72	0.861		
Treatment	5	2494.78	498.956**	12.16	0
Varieties	5	451	90.2 ^{NS}	2.2	0.0641
Treatment*varieties	25	1830.22	73.209 ^{NS}	1.78	0.0307
Error	70	2872.94	41.042		
Total	107	7650.67			

Grand Mean 49.556 **CV** 12.93

Table 9.2. Performance of different treatments and varieties for PH under field and pot experiments

Experiments	**Field**		**Pot**	
Treatments				
Trichoderma	40.11	D	42.39	CD
Meta	50.11	BC	45.69	BC
LEC	53.94	AB	36.83	D
SID	48.67	C	41.22	CD
+ve control	49.56	C	50.00	AB
-ve control	54.94	A	53.61	A
LSD value	4.26		5.65	
Varieties				
CH-121	48.61	B	42.17	B
900F1	46.83	B	48.00	A
Red wing	50.78	AB	43.19	AB
SAYBAN	48.00	B	43.00	AB
AAHP-I	53.11	A	45.92	AB
Priya Chilli	50.00	AB	47.47	AB
LSD value	4.26		5.65	

Disease severity (%)

Table 10 Response of different varieties of chilli against *Alternaria* leaf spot

Scales	Disease Categories	Varieties	Disease Severity (%)	Response
1	0 %	–	–	Immune
2	1-10 %	–	–	Highly Resistant
3	11-20 %	CH-121	18.33	Resistant
4	11-20%	AAHP-I	20	Resistant
5	21-30%	Priya chilli	28.22	Moderately resistance
6	21-30 %	900F1	29	Moderately Resistant
7	31-50 %	SAYBAN	46.1	Susceptible
8	31-50%	Red Wing	49.2	Susceptible

CH-121 and AAHP-I resistance verities 900F1 and Priya chilli moderately resistance and Red wing, SAYBAN susceptible verities.

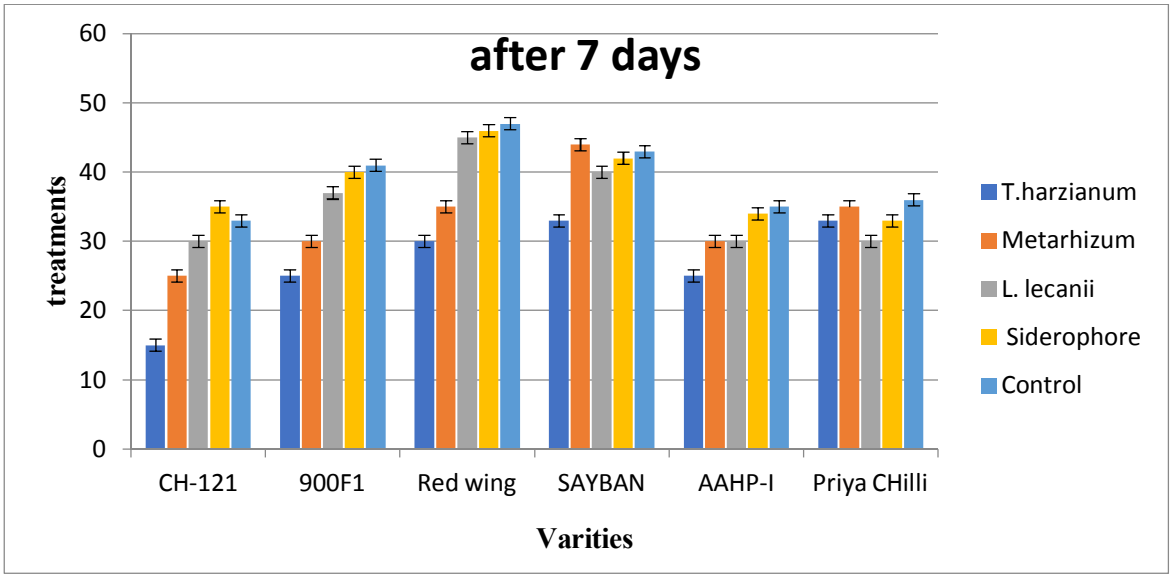

Fig 5: The bar graph shows the % disease severity after 7 days of inoculum apply

Figure 5 showed effect of various biocontrol agents on disease severity after 7 days of inculcation. The CH-121 verity under *T. hariznum* treatment showed minimum disease severity of about 15%. And maximum disease severity was observed in plants applied with siderophore of verity red wing followed by control.

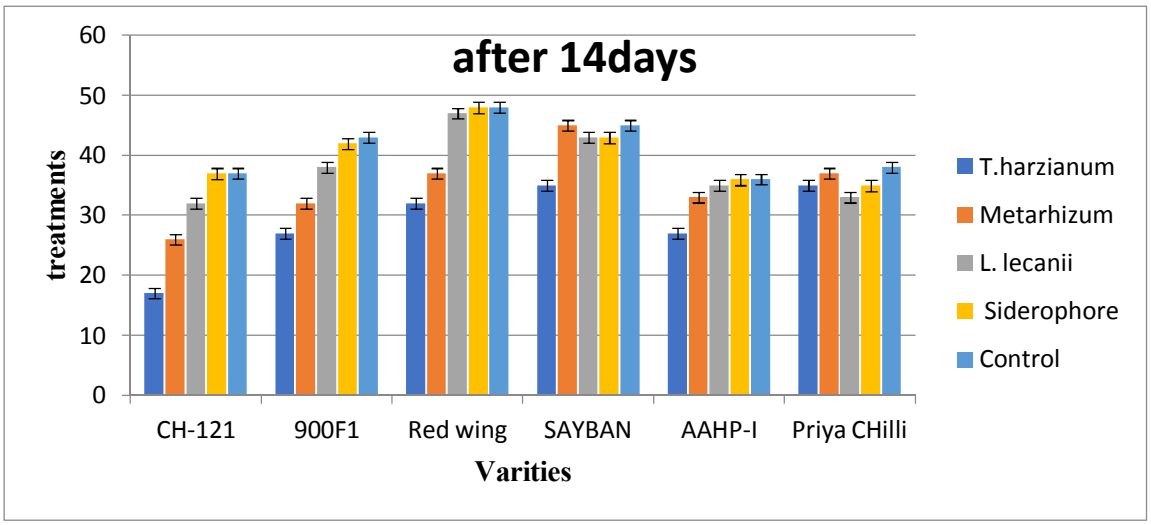

Fig 6: the bar graph shows the % disease severity after 14 days of inoculum apply

Figure 6 showed effect of various biocontrol agents on disease severity after 14 days of inculcation. The CH-121 verity under *T. hariznum* treatment showed minimum disease severity of about 15%. And maximum disease severity was observed in plants applied with *siderophore* of verity red wing followed by control.

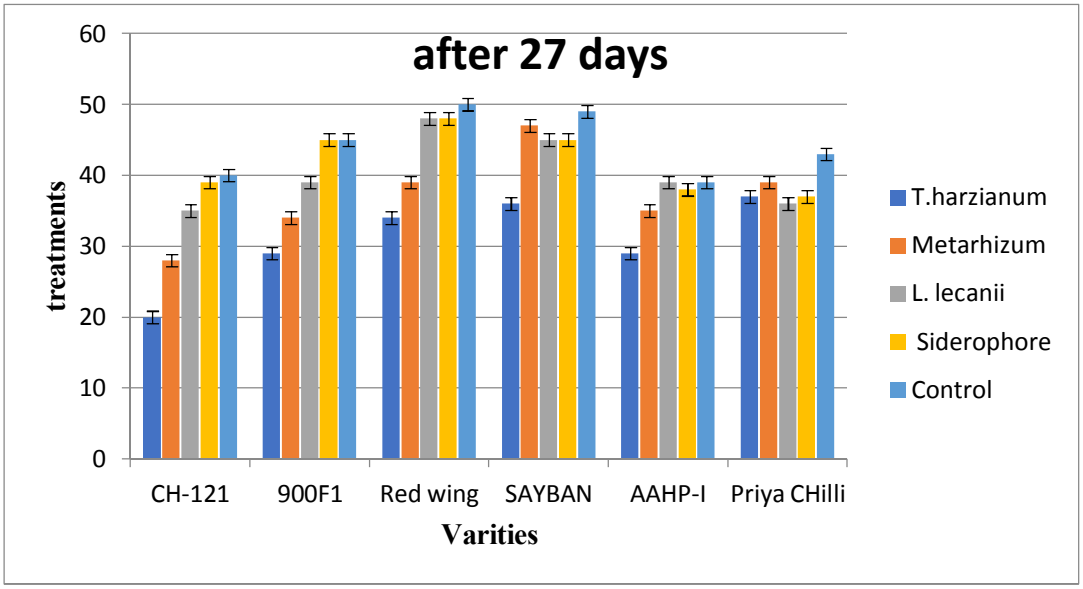

Fig 7: the bar graph shows the % disease severity after 27 days of inoculum apply

Figure 7 showed effect of various biocontrol agents on disease severity after 27 days of inculcation. The CH-121 verity under *T. hariznum* treatment showed minimum disease severity of about 15%. And maximum disease severity was observed in plants applied with siderophore of verity red wing and in plants applied with *metaharizum* of verity SAYBAN followed by control.

DISCUSSION

Chilli (*Capsicum annum* L) taxonomically belongs to family *Solanaceae* and is considered one of the chief and commercially important vegetable in the world, ranking second to potato and tomato. Chilli crop is susceptible to attack by several biotic and abiotic factors which is responsible for low yield of chilli. Among them, leaf spot disease belonging to *Alternaria alternata*. It is a major constraint towards yield, including vegetables. Due to the wide host range and adaptability of fungus in different soil

types, the control of leaf spot with especial reference to *Alternaria alternata* is challenging. The use of fungicides is the cheapest and efficient method of managing the plant diseases caused by fungus. Amendment of biocontrol has shown to increase plant growth. Biocontrol improve soil fertility and besides this it plays vital role in suppressing the soil borne pathogen population. Increasing in plant growth by application of biocontrol has been testified for different plans species (Windham *et al*., 2001).

The effect of different four of various biocontrol was assessed on plant growth parameters like Growth, Yield, Plant length (PL), number of fruits per plant, fresh shoot weight (FSW), fresh root weight (FRW), fresh fruit weight, dry fruit weight. Biocontrol addition in the soil and spray can enhance the chilli plant biomass. Performance of different treatments and varieties under field and pot experiments showed clear significant differences. Comparison of varieties for disease severity at different three dates indicated the minimum disease severity by SAYBAN (19.12) and CH-121 (20) at date 027-02-2021 followed by 900F1 (28.22), AAHP-1 (29), Red wing (44.1) and Priya chilli (47.2) respectively. At date 27-02-2021 the minimum disease severity which show in SAYBAN (20) and in CH-121 (20) followed by 900F1 (29) AAHP-I (29.22), Red wing (44.88) and Priya chilli (49.88) respectively.

In *vitro* significant result showed of BCA. *Trichoderma* which showed was maximum inhibit of the pathogen with 2.19 value other META inhibit the pathogen 3.14 third LEC 4.06 inhibit the pathogen by mean value fourth number SID inhibit the pathogen with mean value 4.26. Maximum fresh root weight in field experiment was recorded in verities 900F1 and Red wing with the treatment LEC (9.00) mean value and minimum fresh root weight was recorded in CH-121 with the treatment trichoderma was (2.00) and Red wing, Priya chilli with the treatment positive control was (2.33) value. Maximum fresh root weight in pots experiment was recorded in verities SAYBAN with the treatment *Metarhizum* (13.67) mean value and in SAYBAN verities with the LEC treatment and Red wing with the positive control minimum fresh root weight (2.67) was recorded. Maximum fresh shoot weight in field experiment was recorded in verities Priya chilli with the treatment LEC (56.67) value and minimum fresh shoot weight was recorded in priya chilli with the treatment metarhizium was (8.49). Maximum fresh shoot weight in pots experiment was recorded in verities SAYBAN with the treatment Metarhizum (45.67) value and minimum fresh shoot weight was recorded in SAYBAN with the treatment LEC was (10.67) value was recorded.

Maximum fresh fruit weight in field experiment was recorded in verities 900F1 with the treatment Trichoderma (16.00) value and minimum fresh fruit weight was recorded in red wing with the treatment positive control was (3.67) value was recorded. Maximum fresh fruit weight in pots experiment was recorded in verities CH-121 with the treatment trichoderma (13.67) value and minimum fresh fruit weight in SAYBAN with the treatment trichoderma (4.00) value was recorded. Maximum dry fruit weight in field experiment was recorded in verities 900F1 with the treatment Trichoderma (14.01) value and minimum dry fruit weight was recorded in SAYBAN with the treatment LEC was (4.67) value was recorded. Maximum dry fruit weight in pots experiment was recorded in verities CH-121 with the treatment trichoderma (11.84) value and minimum dry fruit weight in SAYBAN with the treatment trichoderma (3.00) value was recorded. Maximum growth in field experiment was recorded in verities red wing with the treatment metarhizium (14.98) value and minimum growth was recorded in all other. Maximum growth in pots experiment was recorded in verities SAYBAN with the treatment metarhizium (14.84) value and minimum growth in CH-121 with the treatment negative control (4.67) value was recorded.

Maximum fruit yield in field experiment was recorded in verities CH-121 with the treatment Sideropora (10.33) value and minimum fruit yield was recorded in 900F1 with the treatment positive control was (1.33) value was recorded. Maximum fruit yield in pots experiment was recorded in verities 900F1 with the treatment negative control (7.67) value and minimum fruit yield in 900F1, SAYBAN with the treatment trichoderma (2.67) and SAYBAN, priya chilli with the treatment Metarhizium and in 900F1 verities with the treatment positive control with (2.67) value was recorded. Maximum plants height in field experiment was recorded in verities priya chilli with the treatment LEC (63.cm) value and minimum plant height was recorded in CH-121 and 900F1 with the treatment trichoderma was (32cm) value was recorded. Maximum plant height in pots experiment was recorded in verities 900F1 with the treatment positive control (64.67cm) value and minimum plant height in 900F1 with the treatment LEC (25.15cm) value was recorded. Addition of biocontrol may accumulate beneficial microbes which could be help to enhance the plant growth in addition to fungus control through antagonistic effects of these microbes against fungus pathogen (Mukhtar, 2008). The current studies revealed that the biocontrol amendment in the soil can enhance the chilli plant biomass as well as reduces the fungus population or inhibit them and management of

disease to invade in the plant leaves by activating other beneficial organisms in the soil and leaves which is in the favor of some previous studies (Fermandes *et al.,* 2010). In lab experiment inhibit the pathogen best result showed *Trichoderma herzianum* control and disease severity record three times after inoculum apply CH-121 and SAYBAN resistance verities 900F1 and AAHP-I moderately resistance and Red wing, Priya chilli susceptible verities were record.

Conclusions

Present study concludes that Trichoderma herzianum gives significantly result showed in vitro and in vivo pots and field block experiment and somehow it contributed in enhancing overall plant growth. This bio-agent can be used as a promising biological control strategy against various diseases of vegetables crops caused by Alternaria spp.

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