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Antifungal Efficacy and Growth Promotion by *Trichoderma virens* TRI 37 and *Bacillus amyloloiquefaciens* (VB7) against *Macrophomina phaseolina* - the Maize Charcoal Rot Pathogen

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A B S T R A C T

Charcoal rot caused by *Macrophomina phaseolina* is a serious and destructive disease in maize. In the present study, the biocontrol agents *Trichoderma virens* TRI 37 and *Bacillus amyloloiquefaciens* (VB7) were screened against *M. phaseolina* under *in vitro*, in which *T. virens* TRI 37 effectively inhibited the mycelial growth of pathogen to about 61.11% followed by *B. amyloliquefaciens* produced zone of inhibition and percent inhibition of mycelia growth with2.3 cm and 48.44% respectively. Different antimicrobial compounds produced by effective antagonists were analyzed. Agar well diffusion assay with the secondary metabolites of *T. virens* TRI37 and *B. amyloliquefaciens* (VB7) isolates were screened against *M. phaseolina* under *in vitro* in which *B. amyloliquefaciens* (VB7) produced zone of inhibition and percent inhibition of mycelial growth about 1.5cm and 87.11% followed by *T. virens* TRI 37 produced zone of inhibition and percent inhibition of mycelial growth about 1.16cm and 76% respectively. The fungal antagonists *T. virens* TRI 37 produced microbial non-volatile organic compounds (MNVOC) such as alanine-beta-naphthylamide, pyrazine, propanoic acid, octadecatrienoic acid, glutamine, hexadecenoic acid and decane. Similarly, the bacterial antagonists produced the microbial non-volatile organic compounds such as stigmasterol, nonadecane, cyclopropanol, piperazinedione, docosene, galactopyranoside, oleanane and ditertbutylphenol. Maize seeds were treated with fungal (10^8 conidia per ml) and bacterial antagonists (10^8 cells per ml) and sown under *in vitro* to assess the growth parameters of maize seedlings. The combination of *T. virens* TRI 37 and *B. amyloliquefaciens* (VB7) effectively increased the shoot length (17 cm), root length (22 cm) and stem girth (4 cm) followed by *B. amyloliquefaciens* (VB7) shoot length (15.5 cm), root length (17 cm) and stem girth (5.5 cm) as against control with shoot length (13 cm), root length (16 cm) and stem girth (2 cm).

Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro climatic conditions. Globally maize is known as "Queen of cereals" because it has the highest genetic yield potential among the cereals.

In India maize is the third most important food crops after rice and wheat and also it is referred as corn in North America originated in central Mexico. It is a superior cereal crop regarding the total production globally and productivity. It was introduced to Europe in the sixteenth century and it spread to Africa and Asia. Maize has been cultivated in an area of 117 M ha^{-1} with production of 967 MT and productivity of 5.5 Mt ha^{-1}. In India it has been grown in an area of 86.73 lakh ha with production of 222.5 lakh tones and average productivity of 2566 kg ha^{-1} (INDIA AGRISTAT, 2019). The charcoal rot pathogen is both seed and soil inhabitant in nature. In general, control of soil borne diseases is very difficult. However, the charcoal rot pathogen is managed through seed treatment and soil drenching with fungicides. Continuous use of fungicides has resulted in development of resistance in pathogen *M. phaseolina* resulting in considerable yield loss (Singh *et al.*, 1973).

Shoda, 2000 found that the biocontrol agents present in the maize rhizosphere were strongly antagonistic to *M. phaseolina*. Plant growth promoting rhizobacteria (PGPR) promotes plant growth directly or indirectly through biological control of the pathogen. Production of phytohormones and by antagonistic activities such as antibiosis, hyperparasitism and competition for nutrients and site. *Bacillus* sp. producing antifungal antibiotics, and exhibiting plant growth promoting traits like nitrogen fixation, phosphate solubilization and production of organic acids was reported by Pal *et al.*, 2001.

The current study was carried out to understand the effect of novel fungal and bacterial antagonists of *Trichoderma* and *Bacillus* on growth parameters of maize in protray experiments.

Materials and Methods

Isolation and pathogenicity of charcoal rot pathogen

The maize plants with charcoal rot disease were collected from the field of Department of Millets, TNAU, Coimbatore. The pathogen *Macrophomina phaseolina* was isolated from the basal stem portion of the charcoal rot infected maize plants. The small portion of the infected stem bits were cut with a sterile scalpel and surface sterilized in 70 per cent ethanol for 1 minute.

The infected portions were transferred into sterile distilled water for 1 minute. After surface sterilization, the infected portions were blotted on a sterile filter paper to absorb moisture, and placed on a Potato Dextrose Agar medium (PDA) and kept for incubation at 28 ± 2˚C. Mycelial growth was observed after 2 days of incubation and a small portion of fungal mycelium was transferred to the sterile petri plate with PDA medium to obtain pure culture of *Macrophomina phaseolina*

Pathogenicity

The pathogen was multiplied in sand maize medium (Riker and Riker, 1936). Sand and ground maize seeds were mixed in the rate of 19:1 and moistened with 200 ml of water per 500g. The sand maize medium was steam sterilized at 120 lb pressure in autoclave for 2 hours for two times on alternate days. *M. phaseolina* was grown on PDA for seven days and mycelial disc (9mm) was inoculated into the sand maize medium and incubated at room temperature 28 ± 2˚C for 15 days.

The potting mixture was prepared with garden soil, sand and FYM in the ratio of 1:1:1 and was sterilized in an autoclave at 120 lb pressure for 2 hours. Then the pots were filled with five kilograms of sterilized soil. *M. phaseolina* inoculum multiplied in the sand maize medium was added at the rate of five percent (w/w) to the soil in the pots and mixed well. The pots were seeded with susceptible maize inbred (CM-501) at 3 seeds per pot and kept in glasshouse for further observation. Similarly, uninoculated control was also maintained. Observations were taken regularly for the appearance of symptom development. After symptom development, re-isolation was done and compared with the original culture for confirmation of the Koch postulates.

Morphological and Molecular characterization of *M. phaseolina*

The morphological variations in mycelial characters, number of sclerotial production, size of sclerotia were documented by culturing the pathogen in potato dextrose agar (PDA) medium and were observed in Phase contrast microscope model LEICA DM30000 (DST-FIST Lab), to record the spore morphologies *viz.*, length, width, colour and shape of the sclerotium.

To confirm it to the species level, DNA was extracted from *Macrophomina* (Chakrabarthy *et al.*, 2010). Amplification of Internal Transcribed Spacer (ITS) regions for the isolates of *Macrophomina* sp. was carried out using PCR with conserved primers ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) (White *et al.*, 1990) to get the amplicon size of 560 bp. PCR amplifications were performed in a thermal cycler (Eppendorf Master Cycler, German) and initial denaturation was executed at 2 minutes at 95℃, followed by 40 cycles of denaturation 95℃ for 1 minute, annealing at 58℃ for 1 minute, extension at 72℃ for 1 minute and finally extension at 72℃ for 5 minutes. Gel electrophoresis was done by taking 10 µl of PCR products and 1.2 per cent of agarose gel in TAE buffer solution (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA) at 80 v for 5 minutes at 25℃. Ethidium bromide (0.5 µg/ml) was used to stain the gel, and the PCR products were viewed using UV light and photographed using gel documentation system (Alpha Innotech. Corporation, San Leandro, California). Molecular marker (DNA Ladder Mix, 1kb,) was used to determine the size of amplicon.

Fungal and bacterial isolates

Fungal antagonist like *T. asperellum* TRI 15 (KX533985), *T. harzianum* TRI 36 (KX533990), *T. virens* TRI 37 (KU666466) and *T. koningiopsis* TRI 41 (MF423101) and bacterial antagonist like *Bacillus licheniformis* (MG241257), *B. amyloliquefaciens* (VB7) (MG241252), *B. sonarensis* (MG241231), *B. subtilis* (MG241251) were obtained from the culture collection center of Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Efficacy of antagonism of fungal and bacterial isolates against *M. phaseolina* under *in vitro*

The antifungal efficacy of four fungal and four bacterial antagonists were tested by dual culture technique (Dennis and Webster, 1971) against *M. phaseolina* in PDA medium. A mycelial disc of *M. phaseolina* (9 mm dia.) from the actively growing four days old culture of the pathogen was placed at one end and a 9 mm mycelial disc of the mycelium from the actively growing *Trichoderma* spp. was placed at the opposite end of the petri plate, whereas in case of bacteria, a mycelial disc of the pathogen (9 mm dia.) was placed at the one end of the petri plate and the bacterial antagonists were streaked 1.0 cm away from

the periphery of the plate from the opposite side. The plates were incubated at 28±2℃ for 5 days. Efficacy of the antagonistic organisms against the damping off pathogen was rated based on the inhibition zone observed. Per cent inhibition over control was calculated by using the following formula:

$$PI = \frac{C - T}{C} \times 100$$

Where,

C- Mycelial growth of the pathogen in Control

T- Mycelial growth of the pathogen in Treatment

PI- Per cent Inhibition

Extraction of secondary metabolites from antagonistic fungi

Based on the in vitro screening of the fungal and bacterial antagonisits, the effective antagonisits viz., *Trichoderma virens* and *B. amyloliquefaciens* were further checked for the presence of various secondary metabolites to identify their antifungal activity.

The secondary metabolites of antagonistic fungi were extracted as per the protocol described by Prapagdee *et al.,* (2012). *Trichoderma virens* was grown in potato dextrose broth and incubated at 28±2℃ for 8 days. The supernatant was collected after 8 days by centrifugation at 5,000 rpm for 30 min at 28 ± 2℃. Then supernatant was adjusted to acidic pH 2.0 by adding concentrated HCL and the mixture was stirred at 100 rpm in an orbital shaker for 8 hours. Antifungal compounds in supernatant or culture broth was extracted by adding equal volume of ethyl acetate and shaken vigorously for 1-2 hours. Culture broth was extracted twice with ethyl acetate for complete extraction. The solvent fraction that contained antifungal compounds were combined and concentrated by evaporation in the rotary flask evaporator (Equitron Roteva) maintained at 60℃ at 80 rpm till the compounds were condensed. The concentrated crude extract of the extracellular antifungal compounds was dissolved in 1 ml of HPLC grade methanol and used to assay for the *in vitro* antifungal activity and GC/MS analysis.

Extraction of secondary metabolites from antagonistic bacteria

B. amyloliquefaciens was inoculated in nutrient broth and incubated at 28℃ for six days. The supernatant was collected by centrifugation at 10000 rpm for 15 min at 4℃. Then the pH of the supernatant was adjusted to 2.0 by adding 1N HCL. Equal amount of ethyl acetate was added to the supernatant to extract the antifungal compounds and the mix was kept under shaking condition for overnight. Culture broth was extracted twice with ethyl acetate and shaken vigorously for complete extraction. The ethyl acetate fraction containing antifungal compounds were pooled and concentrated by evaporation in the vacuum flask rotary evaporator maintained at 55℃ at 80 rpm till the compounds get condensed. The concentrated crude extract of the extracellular antifungal compounds was dissolved in 1 ml of HPLC grade methanol and used to assay for the *in vitro* antifungal activity and GC/MS analysis.

Bio assay of secondary metabolites of fungal and bacterial antagonists against *M. phaseolina* by agar well diffusion method

The PDA medium (15 ml) was poured into sterile Petri plate. After solidification of the medium, the sterilized cork borer of 9mm diameter was used to excise the medium on all four sides of the plate by leaving 1 cm away

from the periphery. The 9 mm agar from the excised areas was removed using the sterile inoculation needle.

Actively growing seven days old culture of *M. phaseolina* on PDA medium was excised and placed in the center of the sterile PDA plate. Extracted crude secondary metabolites from *T. virens* and *B. amyloliquefaciens* were poured into the wells at the rate of 75 µl per well separately into different plates and incubated for 96 h at 28±2℃ (Islam *et al.*, 2012).

The mycelial growth and the zone of inhibition were recorded after incubation. Area of inhibition was measured by tracing the surface area of inhibition in a tracing paper and then plotted on to the graph sheet and the zone of inhibition was measured. Each different dose was replicated three times. Each replication consists of 10 Petri plates per replication.

The experiment was repeated twice for the confirmation. Sterile water and methanol control were also maintained to assess the effect of crude antibiotics.

Effect of *Trichoderma virens* and *Bacillus amyloliquefaciens* on growth parameters of maize seedlings

The maize seeds (COH(M)6) were treated with *T. virens* and *B. amyloliquefaciens* as per the following treatment schedule. The treated seeds were sown in portrays containing sterilized vermicompost: sand: soil (1:1:1). *Macrophomina* incoulum was prepared in sand maize medium and was used at the rate of 1% to the coco peat of protray for the treatments T_4, T_5, T_6 and T_8. Ten replications were maintained for each treatment with 25 seedlings per replication. The plants were grown for about 35 days. The growth parameters such as root length shoot length and stem girth were recorded.

Statistical analysis

All the experiments were analyzed independently. The treatment means were compared by Duncan's Multiple Range Test (DMRT**)** (Gomez and Gomez, 1984**).** The package used for analysis was SPSS version 16.0 developed by IBM Corporation.

Results and Discussion

Isolation and characterization of pathogen

The charcoal rot pathogen was isolated from different locations of Tamil Nadu and cultured on PDA. The pathogen produced a black coloured mycelium. Sclerotium production was observed after 10 days of incubation. The pathogen produced coloured septate mycelium. Based on the morphological characters, the pathogen was confirmed as *Macrophomina* sp. (Table 1)

For all the four isolates (MP CBE, MP ANT, MP PDU and MP ODM), an expected amplicon of 560bp were obtained. The amplified partial PCR products of four isolates of *Macrophomina* were sequenced. The DNA homology search was performed using nucleotide BLAST program through internet server at the National Center for Biotechnology Information (NCBI), USA. The comparison of sequence was made with sequence from the GenBank database to determine the species level homology. They were submitted in the NCBI data base, and were assigned with the accession numbers *viz.*, MN636185, MN636186, MK791241 and MK791245.

In vitro screening of bacterial and fungal strains against *M. phaseolina*

Four different isolates of *Trichoderma* and four different isolates of *Bacillus* were screened for *in vitro* antagonism against *M.*

phaseolina by dual culture technique. The *in vitro* efficacy of antagonism of *T. virens* TRI 37 revealed that the myclelial growth of *M. phaseolina* was suppressed with 61.10 per cent over control and differed significantly with isolates *T. harzianum, T. asperellum* and *T. koningiopsis* which inhibit the mycelial growth of *M. phaseolina* to extent of 51.85, 49.90 and 49.25 per cent over control. The hyperparasitized mycelium of *M. phaseolina* by *T. virens* TRI 37 was also observed under field emission scanning electron microscope. The hyphal coiling and wall alterations along with the rapid collapse and loss of cell turgor of *M. phaseolina* observed under SEM investigation. Likewise, the bacterial antagonists also screened against *M. phaseolina*. The *Bacillus amyloliquefaciens* inhibit the mycelial growth of the pathogen to extend of 48.14 per cent over control with 2.33 cm inhibition zone. It was followed by *B. lichiniformis, B. subtillis* and *B. sonarensis* with inhibition of 40.73, 31.47 and 25.92 per cent respectively. The effective isolates were used for further studies (Table 2 &3).

Characterization of compounds produced by antagonistic organisms

Among the four fungal and four bacterial antagonists tested against *M. phaseolina*, the effective fungal antagonists of *T. virens* (TRI 37) and bacterial antagonists of *B. amyloliquefaciens* (VB7) were analyzed for the presence of antifungal non-volatile organic compounds through GC-MS analysis.

The secondary metabolites and extracellular antifungal non-volatile organic compounds from *T. virens* (TRI 37) had antimicrobial biomolecules such as Alanine-beta-naphthylamide, Pyrazine, Propanoic acid, Octadecatrienoic acid, Glutamine, Hexadecenoic acid and Decane. The secondary metabolite and extra cellular antifungal non-volatile organic compounds from *B. amyloliquefaciens* (VB7) had antimicrobial molecules such as Stigmasterol, Nonadecane, Cyclopropanol, Piperazinedione, Docosene, Galactopyranoside, Oleanane, Ditertbutylphenol.

Effect of novel fungal and bacterial antagonists on growth parameters of maize seedlings

The effect of *Trichoderma* spp. and *Bacillus* spp. on root length, shoot length and stem girth in (Vermicompost: soil: sand) medium were presented in (Table.4)

Root length

Among the eight treatments, the root length was found to be more in the consortium of the effective antagonists *T. virens* TRI 37 and *B. amyloloiquefaciens* (VB7) (22 cm) followed by *B. amyloloiquefaciens* (VB7) (19 cm) seed treated plants grown in vermicompost: sand: soil medium as against control (16 cm)

Shoot length

Among the eight treatments, the shoot length was found to be more in the consortium of effective antagonists *T. virens* TRI 37 and *B. amyloloiquefaciens* (17 cm) (VB7) followed by *T. virens* TRI 37 (16.5 cm) seed treated plants grown in vermicompost: sand: soil medium as against control (13 cm).

Root weight

Among the eight treatments, the root weight was more in the maize seeds were treated with the conidial suspension of *T. virens* TRI 37 (6.5 cm) followed by consortium of effective antagonists *T. virens* TRI 37 and *B. amyloloiquefaciens* (VB7) (5.5 cm) as against control (5 cm). seed treated plants grown in vermicompost: sand: soil medium.

Table.1 Treatment details

Treatment details	
T_1	Seed treatment with conidial suspension of *T. virens* TRI 37 @ 10^8 conidia per ml
T_2	Seed treatment with bacterial suspension of *B. amyloliquefaciens* (VB7) @ 10^8 cells per ml.
T_3	Seed treatment with conidial suspension of *T. virens* TRI 37 37 @ 10^8 conidia per ml + Seed treatment with bacterial suspensions of *B. amyloliquefaciens* (VB7) 10^8 cells per ml
T_4	Seed treatment with conidial suspension of *T. virens* TRI 37 @ 10^8 conidia per ml + application of *M. phaseolina inoculum* @ 1% to coco peat
T_5	Seed treatment with bacterial suspension of *B. amyloliquefaciens* (VB7) 10^8 cells per ml + application of *M. phaseolina inoculum* @ 1% to coco peat
T_6	Seed treatment with conidial suspension of *T. virens* TRI 37 @ 10^8 conidia per ml + Seed treatment with bacterial suspensions of *B. amyloliquefaciens* (VB7) 10^8 cells per ml + application of *M. phaseolina inoculum* @ 1% to coco peat
T_7	Untreated control
T_8	Inoculated control

Table.2 Cultural charecters of charcoal rot of pathogen (*M. phaseolina*) of Maize

Characters	Charcoal rot
Radial mycelial growth	**Fast**
Days to cover 90mm Petri plate	**5.00**
Sclerotial formation (days after inoculation)	**3.00**
Sclerotial size (µm)	**56.86**
Sclerotial intensity (7 days after inoculation)	**Abundant**
Colony colour	**Light grey and whitish centre**
Colony appearance	**Linear**
Shape	**Oblong**

Table.3 Antifungal activity of Fungal antagonists against *Macrophomina phaseolina* by dual plate technique

S.No	Treatments	Growth of the pathogen (cm)	Growth of the antagonists (cm)	Days taken for complete Hyper parasitism	Overgrowth of antagonists on pathogen (cm)	Percent inhibition over control (%) **
1.	*T. virens* TRI 37	3.50[d]	5.16[a] (2.27)	9	2.00	61.10[a] (51.41)
2.	*T. harzianum* TRI 36	4.90[b]	3.83[c] (1.82)	12	2.00	51.85[b] (42.45)
3.	*T. asperellum* TRI 15	4.83[b]	4.00[b] (2.00)	14	4.16	49.90[c] (44.94)
4.	*T. koningiopsis* TRI 41	4.56[c]	3.83[c] (1.96)	7	2.50	49.25[c] (44.57)
5.	Untreated control	9.00[a]	0.00[e] (0.71)	0	0.00	0.00[d] (0.71)

Values are the means of three replications. **Values in the parenthesis are arc sign transformed values. *values in the parenthesis are square root transformed values. Means followed by a common letter are not significantly different at 5% level by DMRT.

Table.4 Antifungal activity of Bacterial antagonists against *Macrophomina phaseolina* by dual plate technique

S.No	Name of the antagonists (+)	Inhibition zone (cm)*	Mycelial growth of the pathogen (cm)*	Percent inhibition over control (%)**
1.	*B. amyloliquefaciens* (MG241252)	2.33[a] (1.53)	4.66[e] (2.16)	48.14[a] (43.96)
2.	*B. licheniformis* (MG2421257)	1.40[b] (1.18)	5.33[d] (2.31)	40.73[b] (39.76)
3.	*B. subtilis* (MG241251)	0.46[c] (0.68)	6.16[c] (2.48)	31.47[c] (34.12)
4.	*B. sonarensis* (MG241231)	0.36[d] (0.60)	6.66[b] (2.58)	25.92[d] (30.61)
5.	Untreated control	0.00[e] (0.71)	0.00[a] (0.71)	0.00[e] (0.71)

Values are the means of three replications. **Values in the parenthesis are arc sign transformed values. *values in the parenthesis are square root transformed values. Means followed by a common letter are not significantly different at 5% level by DMRT; (+) The number in the parentheses refers Accession numbers.

Table.5 Effect of root metabolites on Root length, shoot length, root weight and Shoot weight on Maize

S.No	Treatments	Root length (cm)	Shoot length (cm)	Root weight (cm)	Shoot weight (cm)	Stem girth (cm)
1.	ST with conidial suspension of *T. virens* TRI 37	19.0^{a}	16.5^{d}	6.5^{e}	7.5^{e}	3.0^{b}
2.	ST with bacterial suspension of *B. amyloliquefaciens*	17.0^{cd}	15.5^{g}	5.5^{cd}	7.0^{ad}	4.0^{c}
3.	ST with conidial suspension of *T. virens* TRI 37 + ST with bacterial suspension of *B. amyloliquefaciens*	22.0^{a}	17.0^{cd}	5.5^{ab}	6.5^{ab}	3.5^{b}
4.	ST with conidial suspension of *T. virens* TRI 37 in *M. phaseolina* inoculated soil	18.0^{cd}	17.0^{ef}	5.0^{bc}	6.5^{c}	2.5^{ef}
5.	ST with bacterial suspension of *B. amyloliquefaciens* in *M. phaseolina* inoculated soil	16.0^{ab}	11.0^{bc}	4.5^{abc}	4.5^{ab}	3^{c}
6.	ST with conidial suspension of *T. virens* TRI 37 + ST with bacterial suspensions of *B. amyloliquefaciens* in *M. phaseolina* inoculated soil	14.0^{bc}	13.5^{f}	6.5^{bc}	5.0^{bcf}	3^{ac}
7.	Healthy control	16.0^{d}	13.0^{a}	5.0^{d}	4.5^{d}	2^{bc}
8.	Inoculated control	15.0^{ab}	14.0^{b}	6.0^{bc}	6.5^{bcd}	2.5^{f}

*****values of means are ten replications and means followed by common letter are not significantly different at 5% level by DMRT.
ST – Seed Treatment.

Shoot weight

Among the eight treatments, the shoot weight was found to be more in the maize seeds were treated with the conidial suspension of *T. virens* TRI 37 (7.5 cm) followed by *B. amyloloiquefaciens* (VB7) (7 cm) as against control (4.5 cm). seed treated plants grown in vermicompost: sand: soil medium.

Stem girth

Among the eight treatments, the stem girth was found to be more in the maize seeds were treated with the bacterial suspensions of *B. amyloloiquefaciens* (VB7) (4 cm) followed by consortium of effective antagonists *T. virens* TRI 37 and *B. amyloloiquefaciens* (VB7) (3.5 cm) as against control (2 cm). seed treated plants grown in vermicompost: sand: soil medium

Maize charcoal rot caused by *Macrophomina phaseolina* is a serious threat to cultivation of Maize and other crops, which causes severe yield loss. In the present study, the maize charcoal rot pathogen *M. phaseolina* was isolated from infected maize stalk regions showing water-soaked lesions on the roots and later turns black (Kumar and Shekhar, 2005). The isolated pathogen was amplified with an amplicon size of 560 bp corresponding to 18S rRNA and sequence determined. Sequence of 18S rRNA of all the strains showed closest

homology, i.e., 98% similarity with the reference sequences deposited in Gene Bank and confirmed their identification as *M. phaseolina*. Similarly, the identification of fungi based on 18S rDNA is authenticated and well reported (Romanelli *et al.*, 2014).

Trichoderma spp. and *Bacillus* species are widely exploited as biocontrol agents because of their efficiency in impeding various plant pathogens with multifaceted approach. *Trichoderma* spp. inhibited mycelial growth and mycoparasitized the hyphae of *M. phaseolina.*
Crude metabolites of *Trichoderma* inhibited the mycelial growth of *R. solani* and *M. phaseolina*
(Asad *et al.*, 2015). Similarly, in the present study crude metabolites extracted from *T. virens* TRI 37 inhibited the mycelia growth of *M. phaseolina.* Besides, GC-MS analysis of the crude metabolite revealed the presence of various active biomolecules, which is endorsing the earlier reports for antifungal activity (Wang *et al.*, 2011). Vinod Kumar *et al.*, (2018), reported that, *B. amyloliquefaciens* VB7 was much effective in inhibiting mycelial growth and sclerotial production of *S. sclerotiorum*. Similarly, in the present investigation, bacterial antagonists *B. amyloliquefaciens* VB7 produced inhibition zone of 2.33 and significantly reduced the size and germination of sclerotia.

Mycoparasitism involves morphological changes, such as coiling and formation of appressorium- like structures, which serve to penetrate the host (McIntyre, 2004). The first contact between *Trichoderma* spp. and pathogen *M. phaseolina* occurred after 2 to 3 days of inoculations, followed by growth inhibition. Differential antagonistic activity has even been observed for various *Trichoderma* spp. which demonstrates semi-specificity in the interaction of *Trichoderma* with its host (Schirmbock, 1994) Similarly, in the present study, our results on light microscopic study revealed that *T. virens* TRI 37 showed effectively coiling on pathogen *M. phaseolina* at 7 DAI.

In protray experiments, the consortium of the effective antagonists *T. virens* TRI 37 and *B. amyloloiquefaciens* (VB7) treated seedlings had more root length, shoot length, stem girth when compared to other treatments. Similarly, with these findings (Kleifeld and Chet, 1992; Harman *et al.*, 2004) suggested that *T. harzianum* might affect plant growth and influence plant hormones and vitamins.

Bacillus species in the natural environment can produce novel beneficial metabolites that promote plant growth and yield (Sharma and kaur, 2010). *B. amyloloiquefaciens* (SQR9) enhanced growth promotion in maize plants (Zhang *et al.*, 2015). Soil application of *B. amyloloiquefaciens* promoted plant growth by the production of IAA (Idris *et al.*, 2007)

In conclusion the effective fungal and bacterial antagonists play a novel role in increasing antifungal activity and growth parameters to manage the maize charcoal rot under field conditions.

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