Biological Control of groundnut stem rot and collar rot pathogens under *in vitro* conditions

ABSTRACT

The experiment was conducted under laboratory conditions by using native isolates. These were tested against stem rot (*S. rolfsii)* and collar rot (*A. niger*) pathogens of groundnut under *in vitro* conditions by using dual culture technique. The fungal and bacterial bioagents which are inhibitory against these pathogens were identified by 18S rRNA (fungi) and 16S rRNA (bacteria) techniques and were compared with those from the GenBank using the BLAST program. Among fungal isolates *T. harzianum* (MBNRT-1) was superior in inhibition of *S. rolfsii* and *A. niger* and the per cent inhibitions were 70.5% in case *S. rolfsii* whereas; in *A. niger* the inhibition was 72.9 per cent. Among native bacterial isolates the isolate *B. amyloliquifaciens* (MBNRB-3) and is significantly superior over the other isolates in inhibiting the pathogens *S. rolfsii* and *A. niger* under *in vitro* conditions and the inhibitions were 66.6 per cent and 63.0 per cent respectively. Further, compatibility of effective fungal and bacterial bioagents *T. harzianum* (MBNRT-1) and *B. amyloliquifaciens* (MBNRB-3) with six fungicides and eight herbicides indicated that among the fungicides the azoxystrobin was highly compatible with both the bioagents *T. harzianum* (MBNRT-1) and *B. amyloliquifaciens* (MBNRB-3) whereas, among the herbicides imazethapyr + imazamox was found to be compatible with both the bioagents with all the concentrations. While, tebuconazole, thiram, mancozeb+carbendazim (fungicides) and quizolofop-p-ethyl and pendimethalin (herbicides) were highly inhibitory to the *T. harzianum* (MBNRT-1) and *B. amyloliquifaciens* (MBNRB-3) under *in vitro* conditions.

Key words: Biological control, groundnut, stem rot, collar rot, compatibility, fungicides, herbicides

Introduction:

Groundnut (*Arachis hypogaea* L.) is an important food legume grown in Asia and Sub Saharan Africa. Several soilborne diseases like, stem rot, collar rot and root rot causes severe yield losses in groundnut. In general management of soilborne diseases in crop plants is very difficult. Among various methods biological control is the most important method in managing Soilborne diseases. Several success stories have been reported regarding biological control of crop diseases using plant growth-promoting rhizobacteria (PGPR) and *Trichoderma* spp. in groundnut against the soilborne diseases (Chet, 1990; Cortes et al., 1998 and Sharma et al., 2011).

The use of biological control methods in soil-borne disease management either alone or in conjunction with other methods can be a sustainable option in groundnut. Weindling (1934) reported that the culture filtrate of *T. lignorum* was toxic to many soilborne fungi like *S. rolfsii*, *A. niger*, *R. solani*, *M. phaseolina* etc. Species of *Trichoderma* are widely distributed in soils and act through all the possible modes of antagonism such as antibiosis, competition and mycoparasitism (Papavizas, 1985., Chet, 1987; Howell, 1987). Among different PGPR, *Bacillus* spp. are the gram positive bacteria that are antagonistic to several soilborne plant pathogens (Kumar et al., 2009). Besides significant reduction in the soilborne pathogen population these bio agents also effective in enhancing crop yields in several crops including groundnut (Kubicek et al., 2001 and Podile & Kishore, 2002). Use of fungicide resistant strains of these bioagents is of extreme importance especially in the

ambit of integrated disease management, because when these bioagents are juxtaposed with chemical fungicides, their growth, multiplication and thus the efficacy can be reduced significantly. Several researchers have established the development of pesticide resistant bioagents (Gupta & Sharma, 2004 and Veena et al., 2006). In this context, the use of fungicide resistant bioagents in plant disease management assumes significance. The efficacy of bioagents can further be enhanced when used conjunctively with reduced dosages of fungicides (Korsten et al., 1992; Silimela & Korsten, 2001).

Material and methods

The rhizosphere microflora was isolated by following the serial dilution plate technique (Jhonson and Curl, 1972). Several fungi like were isolated from rhizosphere but the colonies resembling the *Trichoderma* sp and bacterial species were isolated, purified and were used to evaluate their antagonistic effect against *S. rolfsii* and *A. niger*. The isolates were designated to indicate the district from which they have isolated. One day old colonies of bacteria were picked up and purified by streak plate method. Different bacteria isolated from the rhizosphere were transferred to the culture plates containing appropriate media for further examination and also designated according to the district collected.

Screening of the native microflora against *Sclerotium rolfsii* and *Aspergillus niger*

The antagonistic activity of native bacterial and fungal isolates was determined by dual culture technique (Dennis & Webster, 1971).

Fungal isolates

Twenty ml of luke warm sterilized PDA was poured in 90 mm petriplates. Culture discs (5 mm) of rhizosphere fungal isolate and pathogen were taken from the margin of the actively growing cultures and transferred onto the solidified PDA on opposite sides approximately at one cm from the wall of the petriplate. A total of three replications were maintained for each fungal isolate and the petriplate without the fungal isolate served as control and all the inoculated petriplates were incubated at 28 ± 2^{o}C. The growth of the test pathogen and the ability of the antagonist to inhibit the pathogen were recorded by periodical observations.

Bacterial isolates

A dual culture plate technique was conducted for testing the efficacy of bacterial isolates against *S. rolfsii* and *A. niger*. Mycelium discs of 5 mm diameter were cut from the perifery of an actively growing fungal colony with a cork borer, and one disc was placed in the centre of each petriplate containing PDA. Two parallel streaks of bacteria 3.5 cm long were then made 2 cm apart on opposite sides of the mycelial disc. The uninoculated with the selective bacterial isolate served as control. The plates were incubated at 28 ± 2^{o} C. The experiment was conducted in Completely Randomized Block Design (CRBD) with four replications for each treatment.

The per cent growth reduction (I) of the test pathogen was calculated when the growth of the test pathogen was full in control plates by using the formula given below.

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

Wherein,

I = Per cent growth reduction of test pathogen

C = Radial growth of test pathogen in control (mm)

T = Radial growth of test pathogen in treatment (mm)

The potential fungal and bacterial antagonists against *S. rolfsii* and *A. niger* were selected and used for further studies

Identification of Rhizosphere Microflora

Pure cultures of the native rhizosperic fungal and bacterial isolates (the isolates which inhibit the test pathogens under dual culture assay) were grown on potato dextrose agar and nutrient agar slopes for four days. These cultures were sent to Macrogen Inc. Seoul, Korea for sequencing. The sequences obtained (through 18S rRNA (fungi) and 16S rRNA (bacteria) technique) were compared with those from the GenBank using the BLAST program (Alschul et al., 1990), aligned using the ClustalW software (Thompson et al., 1997), and phylogenetic trees inferred using the neighbor-joining method (Saitou and Nei, 1987). Bootstrap analysis was performed to estimate statistical stability of the branches in the cluster with 1000 replicates using MEGA version 6 programme (Tamura et al., 2013).

Compatibility of potential biocontrol agents with fungicides and herbicides

Based on dual culture studies, the potential fungal biocontrol agent against stem rot and collar rot was tested for its compatibility against commonly used fungicides and herbicides at recommended and half recommended doses by following poisoned food technique on PDA medium as described by Nene and Thapaliyal (1993).

The per cent inhibition was measured by using the formula:

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

Wherein,

I = Per cent inhibition of mycelia growth

C = Colony diameter in control (mm)

T = Colony diameter treatment (mm)

Similarly the compatibility of potential bacterial bioagent with fungicides and herbicides was tested by spectrophotometric method by measuring the optical density, using a UV Spectrophotometer. A loopful of antagonistic bacterial culture was inoculated in to the conical flask (250ml) containing Nutrient Broth and incubated overnight in an incubator shaker at 28±2°C at 180 rpm and then 50 µl of antagonistic bacteria culture was added to 250 mL conical flasks containing nutrient broth along with different fungicides and herbicides. Inoculated flasks were incubated at 28±2°C in incubator shaker rotation at 180 rpm. Bacterial growth was determined by measuring optical density (OD) at 610 nm after 24 hours of incubation. Each treatment consisted of three flasks per individual replication. The nutrient broth without fungicide/herbicide served as control and per cent inhibition was calculated. The experiment was conducted in Completely Randomized Block Design (CRBD) with four replications for each treatment.

Results and Discussion

All the rhizosphere isolates were screened against *S. rolfsii* and *A. niger* under *in vitro* condition to test their antagonistic potential by dual culture technique. The antagonistic effect of different native isolates was assessed based on their ability to inhibit the pathogen growth and development.

Evaluation of rhizosphere fungal isolates on growth of *S. rolfsii* and *A. niger* under *in vitro* conditions

Sclerotium rolfsii

In vitro evaluation of native fungal isolates indicated that all the tested isolates were inhibitory to the growth of *S. rolfsii* (Table 1). Highest per cent inhibition (70.58%) of *S. rolfsii* was noticed with the native bioagent *T. harzianum* (MBNRT-1) followed by MBNRT-2 (68.62%). These were found to be superior over other isolates with no significant difference. The next best inhibitions were found with the isolates ATPT-5, CHTT-2, MBNRT-4 and ATPT-1 and the inhibitions were in the range 58.43 to 61.17% with no significant difference among these isolates. The isolates ATPT-3, ATPT-2 and commercial (*T. viride*) were also effective in inhibiting the radial growth of *S. rolfsii* and the inhibition percentage was up to 57.25 and no significant difference among these isolates. Rest of the isolates (MBNRT-3, WGLT-1, WGLT-2 and ATPT-4) were shown inhibitions below 50 per cent and the inhibition varied from 28.62 to 45.48 per cent and the difference among these isolates were significant. Least inhibition of *S. rolfsii* was observed with isolates WGLT-2 and ATPT-4 and the inhibitions were 30.19 and 28.62 per cent respectively with no significant difference. Overall, the isolates T6 and T7 were highly effective in inhibiting the radial growth of *S. rolfsii* under *in vitro* conditions.

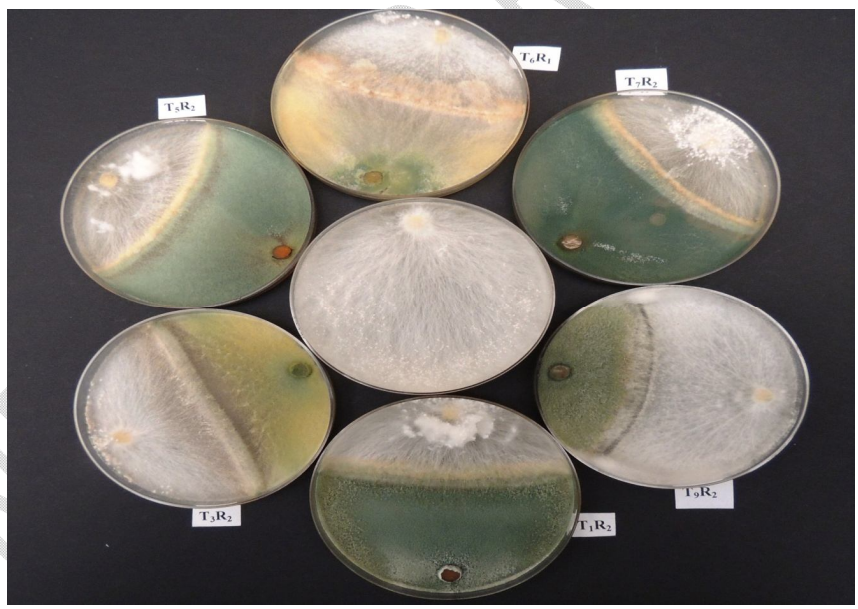

Figure 1: Effect of native *Trichoderma* isolates on radial growth of *Sclerotium rolfsii* under *in vitro* conditions

Aspergillus niger

It is evident from the results (Table 1) that all the tested isolates were more or less effective in inhibiting the radial growth of *A. niger* under *in vitro* conditions. The native bioagent *T. harzianum* (MBNRT-1) was found to be superior in inhibiting the radial growth of *A. niger* by 72.9 per cent. The isolates ATPT-2, ATPT-1, CHTT-2, ATPT-5 and ATPT-3 also inhibit the radial growth *A. niger* by 69, 66.7 (ATPT-1, CHTT-2, ATPT-5) and 66.3 per cent respectively but significant differences were not observed among these isolates. The

next best inhibitions were obtained with the isolates MBNRT-4, CHTT-1, ATPT-4, MBNRT-2, WGLT-1 and *T. viride* (Commercial) and the inhibitions varied from 56.6 to 63.9 per cent and there was no significant difference among these isolates. Among all the isolates tested least inhibition was recorded by the isolates WGLT-2 and MBNRT-3 with 54.9 per cent and 54 per cent respectively and were on par with each other. Overall, *T. harzianum* (MBNRT-1) was found to be superior in inhibiting the radial growth of *A. niger* under *in vitro* conditions.

Figure 2: Effect of native bioagent *T. harzianum* (MBNRT-3) on the growth of *A. niger* under *in vitro* conditions

Chemical control methods are extensively used in agriculture for the management of plant pathogens. However, due to the hazardous effects of these chemicals on human health and also on environment there is a need to go for alternate strategy. Biological control of soilborne diseases on the other hand is a sustainable, eco-friendly safe option and is widely reported on several (Deacon, 1988; Hornby, 1990). Among these biocontrol agents the fungal bioagent *Trichoderma* sp is the most promising one and it is effective against wide range of pathogens (*Sarhan et al., 1999*). In the present study also there was a profound effect with native fungal isolates on the test pathogens *S. rolfsii* and *A. niger* and all the native isolates have shown considerable inhibition against the test pathogens over the control. The isolate *T. harzianum* (MBNRT-1) recorded the highest inhibition of *S. rolfsii* (70.58 %) and *A. niger* (72.9%) and was also effective than the commercial bioagent *Trichoderma viride* where the inhibitions up to 56% against both the pathogens. The mode of inhibition of plant pathogens by *Trichoderma* is mainly due to mycoparasitism, competition or through production of antibiotics. Hyper parasitism is the main phenomenon where the bioagent *Trichoderma* coils the hyphae of the test pathogen (Elad et al., 1983) and causes lysis or breakdown of hyphae due production of extracellular lytic enzymes like β (1,3) glucanases, chitinases, lipases and proteases etc. which bring about lysis of host cell wall (Mukhopadhyay, 1987). Apart from these lytic enzymes *Trichoderma* also produces some volatile and non-volatile compounds which will reduce the growth of target pathogen (Dennis & Webster, 1971 and Mukhopadhyay, 1987). Present studies also showed the inhibition of pathogens *S. rolfsii* and *A. niger* which may be due to the production of these extracellular lytic enzymes and volatile compounds by the *Trichoderma* sp under *in vitro* conditions or may be due to the competition. Similar type of inhibition was observed with *Trichoderma* species against *S. rolfsii* (Iqbal et al., 1995; Rekha et al., 2012) and *A. niger* (Devi & Prasad, 2009; Gajera et al., 2011 and

Nandeesha et al., 2013) by several workers where they also confirmed the mechanisms by which *Trichoderma* inhibits the growth of these pathogens.

Antagonistic effect of rhizosphere bacterial isolates on *S. rolfsii* and *A. niger* under *in vitro* conditions

Sclerotium rolfsii

Evaluation of *Bacillus* sp and *Pseudomonas* sp isolates indicated that all the isolates are more or less inhibitory to the pathogen *S. rolfsii* (Table 2). Highest inhibition 66.6 per cent of *S. rolfsii* was obtained with *B. amyloliquifaciens* (MBNRB-3) was found to be superior over the other isolates. The next best isolates were WGLB-1, WGLB-2 and ATPB-2 with an inhibition of 63.7, 63.1 and 61.2% respectively and differences among these isolates were non significant. The isolates ATPB-1, MBNRB-4, MBNRB-2, commercial *Bacillus* sp and ATPB-3 were also effective in inhibiting the radial growth of *S. rolfsii* and the inhibition was 54.1 to 56.86% with no significant difference among these isolates. Isolate MBNRB-1 and ATPB-4 were also effective in inhibiting the radial growth of *S. rolfsii* and the inhibition was 45 and 36.8% respectively with significant differences between them. Overall, the *B. amyloliquifaciens* (MBNRB-3) was superior among all the isolates in inhibiting the radial growth of *S. rolfsii*.

Aspergillus niger

It is evident from the results (Table 2) that among the bacterial isolates the isolate *B. amyloliquifaciens* (MBNRB-3) was highly effective (63.08%) in inhibiting the radial growth of *A. niger* and this was followed by ATPB-2 (62.67%) and there was a significant difference between these two isolates and also differed significantly with the rest of the isolates. The next best inhibition was obtained with the isolates WGLB-1 (58.33%) and ATP-1 (58%) with no significant difference. The isolates ATPB-3 and MBNRB-2 were also effective in inhibiting the pathogen and the inhibition was up to 54 per cent. Rest of the isolates ATPB-4, MBNRB-1, MBNRB-4, commercial *Bacillus* sp and WGLB-2 were also relatively effective in inhibiting the radial growth of the *A. niger* and the inhibition was 38.21 per cent to 47.08 per cent. Overall, the isolate *B. amyloliquifaciens* (MBNRB-3) was superior among all the isolates in inhibiting the radial growth of *A. niger* under *in vitro* conditions.

In the past bacterial bioagents were utilized because of their antifungal activity against several plant pathogens especially soilborne pathogens (Siala & Gray, 1974). These bacterial bioagents are broad spectrum in nature and controls several soilborne fungi. Among these *Bacillus* and fluorescent *Pseudomonads* are the important bioagents which inhibit the growth of several soilborne pathogens under *in vitro* as well as *in vivo* conditions (Utkhede, 1984). Bacterial bioagents inhibits the pathogens mainly due to the production of antimicrobial proteins namely, bacteriocins, chitinases, glucanases etc. and also through production of antibiotics through secondary metabolism pathway (Prabhakaran & Ravimycin, 2012). In present studies also the rhizosphere bacterial bioagents have shown considerable inhibition against both the soilborne pathogens *S. rolfsii* and *A. niger* of groundnut. Bacterial isolate *B. amyloliquifaciens* (MBNRB-3) showed highest inhibition against both the pathogens *S. rolfsii* the *A. niger* with an inhibitions of 66.66 and 63.08 per cent respectively. The mode of action of these bacterial bioagents is mostly due to rhizosphere colonization, production of extracellular antibiotics, lytic enzymes, and siderophores, and activation of host defence responses together might contribute to the reduction in the growth of pathogen (Podile & Prakash, 1996). However some bacterial

bioagents do not produce any antimicrobial compounds but they suppress the pathogens. For example *B. megaterium* (siderophore producers), was not antagonistic to *A. niger* but suppressed these pathogen by iron starvation in the rhizosphere by producing iron chelating compounds. Suppression of fusarium wilt from siderophore-mediated competition by *P. putida* WCS 358 has been shown (Duijff et al., 1993). In our present studies also the inhibition in growth of both the pathogens *S. rolfsii* and *A. niger* with bacterial bioagents may be due to the production these antimicrobial compounds and iron chelating compounds (siderophores). Similar type of inhibitions was obtained with bacterial bioagents *Bacillus* sp. and *Pseudomona*s sp. were obtained by several workers in *S. rolfsii* (Rengashwaran & Prasad, 2000; Lin et al., 2008) and *A. niger* (Kishore et al., 2005; Prabhakaran & Ravimycin, 2012).

Compatibility of *T. harzianum* (MBNRT-1) and *B. amyloliquefaciens* (MBNRB-3) with fungicides and herbicides

In order to include bioagent as a component in integrated disease management, the antagonist should be compatible with the commonly used agrochemicals. The effective biocontrol agents were tested for their compatibility with commonly used fungicides and herbicides under *in vitro* conditions using poisoned food technique.

Compatibility of *T. harzianum* (MBNRT-1) with fungicides and herbicides

Fungicides

A total of six fungicides were evaluated for their inhibition on growth of bioagent *T. harzianum* (MBNRT-1). Out of six fungicides three fungicides mancozeb+carbendazim, thiram and tebuconazole was showed cent per cent inhibition of bioagent *T. harzianum* (MBNRT-1) at all the three concentrations under study. But significant differences were not observed between different concentrations of above three fungicides. However, the strobilurin group fungicide, azoxystrobin did not inhibit (zero per cent inhibition) the *T. harzianum* (MBNRT-1) at all the concentrations under study. Though the fungicides Mancozeb (53.90 %) and metalaxyl (58.03%) inhibited bioagent at recommended concentrations significant difference was not observed between these fungicides (Table 3). But significant difference was observed at half the recommended concentrations by 20.39 per cent and 25.88 per cent, and were inferior to the inhibitions of bioagents at recommended rates. At double the recommended concentration metalaxyl inhibited the bioagent *T. harzianum* (MBNRT-1) by 83.91 per cent. While lowest inhibition (25.09%) was found with fungicide mancozeb. Present results indicate that the fungicide azoxystrobin was highly compatible with the bioagent *T. harzianum* (MBNRT-1) and can be integrated with bioagent and tested in field conditions.

Application of bioagents against soilborne diseases of groundnut is gaining momentum of late especially in the gamut of IDM. Earlier studies have indicated that the conjunctive usage of *Trichoderma* sp. with fungicides against stem rot (Csinos et al., 1983) and collar rot (Suresh, 2013). Basha et al., 2010 reported combined application of *Trichoderma* sp with fungicide mancozeb for effective management of stem rot. Similarly, earlier research indicated the effectiveness of *Trichoderma* sp. against collar rot in groundnut through their juxtapositioning with fungicides. For successful IDM it is essential to know the compatibility of biocontrol agents with commonly used agrochemicals. Earlier research on compatibility of fungicides with the bioagent *Trichoderma* has indicated that the bioagent is compatible with certain fungicides but not with other chemicals. In case of groundnut, studies indicated that tebuconazole is highly inhibitory to the growth of fungal bioagent

Trichoderma (Mc Lean et al., 2001). Though this fungicide is effective against stem rot at field level (Brenneman *et al.*, 1991), its application along with bioagents is limited and is confined to experimental conditions. Similarly, *in vitro* studies by Pandey et al. (2006) indicated 27.6 per cent inhibition of *Trichoderma* sp by azoxystrobin in contrast to the present study where zero per cent inhibition of *S. rolfsii* was observed with azoxystrobin. In the present study tebuconazole recorded 100 per cent inhibition in *T. harzianum* (MBNRT-1). Similar results were reported by Mc Lean *et al*. (2001) and Bagwan (2010). The results of the present study clearly indicate that the recommendation of application of *Trichoderma* and tebuconazole together should not be practiced in groundnut for the control of stem rot and collar rot. In our studies mancozeb was found effective against *A. niger* and *S. rolfsii* also it is compatible with *T. harzianum* (MBNRT-1) where can be used in IDM for the control of stem rot and collar rot pathogens. Although metalaxyl which was found compatible with *T. harzianum* (MBNRT-1) but was not effective against stem rot hence it cannot be recommended for field use. Though majority of earlier reports supported complete compatibility of *Trichoderma* sp with metalaxyl, contradictory reports on the inhibitory effect on *Trichoderma* by this acylalanine compound are also available (Tapwal et al., 2012). Based on our studies, it can be concluded that juxtapositioning of *Trichoderma* spp., particularly our strain, *T. harzianum* (MBNRT-1) should be at reduced rates only with either of these fungicides under the ambit of IDM against groundnut soilborne diseases. Ranganathswamy et al. (2012) reported that seed dressing fungicide, thiram, had significant inhibitory action against *T. harzianum* (MBNRT-1). Our results also indicated that thiram showed complete inhibition of *Trichoderma* sp.

The results of the present studies also revealed complete compatibility of fungal bioagent *T. harzianum* (MBNRT-1) with azoxystrobin. Earlier results indicated compatibility of fungal bioagents such as *Trichoderma* spp and *Gliocladium virens* with azoxystrobin (Ranganathswamy et al., 2012). Contradictory reports on the inhibitory effects of azoxystrobin to *Trichoderma* spp were also available (Sarkar et al., 2010). Reports on the conjunctive use of azoxystrobin with *Trichoderma* spp. however were also reported in groundnut under field conditions (Akgul et al., 2011).

Herbicides

Of all the herbicides screened, highest inhibition of bioagent *T. harzianum* (MBNRT-1) recorded with quizolofop-p-ethyl (90%) while zero per cent inhibition was obtained with imazethapyr+imazamox followed by pendimethalin (87.45%) and Cycloxydim (84.12%) at recommended dosages and significant differences were found among these herbicides. The herbicides Propaquizafop (77.65%) and Oxyfluorfen (76.67%) also inhibited the bioagent at recommended dosages and significant difference was not observed between these two chemicals. Similarly all these five herbicides were also inhibitory to *T. harzianum* (MBNRT-1) growth at half the recommended dosages significantly (Table 4). The inhibitory percentages at this concentration for these five chemicals ranged from 65.84 to 79.02%. Imazethapyr was also less inhibitory (5.88%) to the growth of MBNRT-1(*T. harzianum*) at its recommended dosage. These two chemicals, imazethapyr alone and in combination with imazamox as commercial formulations were also least inhibitory at half the recommended dosages (3.92% and zero percent respectively). Fenaxoprop recorded inhibited up to 43.06 and 46.31 per cent respectively at half the recommended and recommended rates respectively with no significant difference. Except for the chemicals imazethapyr; and its combined product comprising of imazamox, all other chemicals have shown increased inhibitions at double the recommended rates on bioagent *T. harzianum* (MBNRT-1) radial

growth. The herbicides imazithapyr; and imazethapyr + imazamox were least inhibitory to *T. harzianum* (MBNRT-1) growth when their concentrations were doubled. Overall, our results suggest that the herbicide formulation imazethapyr + imazamox was highly compatible and it can be conjunctively used with *T. harzianum* (MBNRT-1) with no signs of inhibition on the growth of bioagent at all the concentrations studied.

Herbicides though have significant role in boosting plant yields, they also have certain non-target effects especially on soil microflora that contribute to plant disease development (Glaze et al., 1984). This is especially true when these herbicides are used indiscriminately in field soils where weeds are problematic. IDM is successful when, these agrochemicals (herbicides) are compatible with the bioagents. Some herbicides have non target effects like control of plant pathogens along with weed. For the effective utilization *Trichoderma* sp as bioagents for control of soilborne diseases it is necessary to check the compatibility with commonly used herbicides in that particular crop. Previous studies indicated that some herbicides are compatible with *Trichoderma* sp., while some are non-compatible (Mondal et al., 1995; Sharma *et al*., 1999). In the present study herbicides propaquizafop and pendimethalin were highly inhibitory to both stem rot and collar rot pathogens but these herbicides are not compatible with the bioagent *T. harzianum* (MBNRT-1) which is also effective in inhibiting the stem rot and collar rot pathogens. Earlier reports also indicated that pendimethalin is highly inhibitory to the growth of bioagent *Trichoderma* (Madhavi et al., 2011). Similarly the herbicide quzalofop-p ethyl was also effective in inhibiting the collar rot pathogen *A. niger* under *in vitro* conditions, but it was highly inhibitory to *Trichoderma* sp which is in agreement with Madhavi et al. (2011). However, some herbicides like imazethapyr and 2, 4-D sodium salt are less inhibitory to the bioagent *Trichoderma* sp. (Gounder et al., 1999). In our studies the herbicides imazethapyr and imazethapyr+imazamox are highly compatible with the bioagent *T. harzianum* (MBNRT-1) but these herbicides were not effective against the collar rot and stem rot pathogens. Earlier studies also indicated the compatibility of *Trichoderma* sp with herbicide Imzethapyr (Gupta and Sharma 2004). Based on our results the herbicides imazethapyr and imazethapyr + imazamox are highly compatible with the bioagent *T. harzianum* (MBNRT-1) so these herbicides can be used in integrated disease management programme along with bioagents.

Compatibility of *B. amyloliquefaciens* (MBNRB-3) with fungicides and herbicides

Six fungicides and eight herbicides were evaluated for their compatibility against bacterial biocontrol agent under *in vitro* conditions and the results are presented in Table.

Fungicides

Compatibility studies of bacterial bioagent *B. amyloliquefaciens* (MBNRB-3) with fungicides indicated that all the chemicals have shown more or less inhibition on the bioagent (Table 5). Among all the fungicides azoxystrobin and metalaxyl were least inhibitory to the *B. amyloliquefaciens* (MBNRB-3) and the inhibitions were 17.46 per cent and 17.69 per cent at their recommended concentration and the differences between these two fungicides were not significant. Whereas, at recommended concentration the highest inhibition in the growth of *B. amyloliquefaciens* (MBNRB-3) was found with the fungicides thiram and tebuconazole and the inhibitions were 47.4 per cent and 47.14 per cent respectively and the differences between these fungicides were non-significant. Further the fungicide mancozeb and combined product mancozeb+carbendazim were inhibited the bioagent *B. amyloliquefaciens* (MBNRB-3) in the range 32.42 per cent to 37.53 per cent and there was a reduction in the inhibition of bioagent with respect to the reduction in the concentration. Similarly, there was an

increased inhibition of bioagents with simultaneous increase in the concentrations of fungicides. Overall, the fungicide azoxystrobin was least inhibitory to the bioagent *B. amyloliquefaciens* (MBNRB-3) in the present studies and is considered as more compatible fungicide.

Herbicides

Of different herbicides evaluated, pendimethalin (88.7%) followed by quizalofop-P-ehtyl (80.1%) are highly inhibitory to *B. amyloliquefaciens* (MBNRB-3) at recommended dosages (Table 6). These two herbicides were also inhibitory at half the recommended rates showing inhibitions of 79.52 per cent and 70.97 per cent and are significantly different. These herbicides are followed by cycloxydim and oxyfluorfen showing inhibitions of 69.3 per cent and 68.5 per cent respectively at recommended dosages and the differences between these herbicides were not significant. However, these two herbicides differed at half recommended dosages showing inhibitions of 68.2% (cycloxydim) and 28 per cent (oxyfluorfen). The herbicides propaquizafop (53.75% inhibition), fenaxoprop (36.8% inhibition) and imazethapyr (20.8%) also have shown inhibitory effects at recommended dosages with significant differences among them and lowest inhibition (14.02%) was found with herbicide imazythapyr+imazamox. At half the recommended dosages, all the herbicides showed decreased inhibitions on *B. amyloliquefaciens* (MBNRB-3). At double the concentrations all the herbicides, showed increased inhibitory effects ranging from 21.3% (imazethapyr+imazamox) to pendemethalin (98%). Overall, our results suggested that imazethapyr+imazamox has least inhibitory effect on the growth of *B. amyloliquefaciens* (MBNRB-3) and is relatively a compatible herbicide with the bioagent.

For successful management of soilborne diseases with biocontrol agents in IDM it is essential to know the compatibility of biocontrol agents with commonly used agrochemicals. Earlier research on compatibility of fungicides with the bioagent *Bacillus* sp indicated that most of the commonly used fungicides were compatible with *Bacillus* species. Present studies also proved that bioagent *B. amyloliquefaciens* (MBNRB-3) was relatively compatible with all the fungicides used under study and recorded below 50 percent inhibitions and the inhibitions were in the range 17.46 to 47.4 per cent. Among six fungicides azoxystrobin was proved highly compatible at is recommended concentration and this was earlier reported by Devi and Prakasam (2013) however contradictory reports also reported by Prasad et al. (2014) with azoxystrobin where they found the fungicide azoxystrobin was highly inhibitory to the growth of Bacillus sp. under *in vitro* conditions. Kumar et al., 2011 also reported the compatibility of strain MBI 600 (*B. subtilis*) with commonly used fungicides and reported that strain MBI 600 (*B. subtilis*) was highly tolerant to hexaconazole, propiconazole and validamycin; moderately tolerant to tricyclazole; and poorly tolerant to benomyl and mancozeb at 1000 ppm. The MBI 600 strain also showed good compatibility with carbendazim and azoxystrobin at 400 ppm. Similarly Basha et al. (2010) studied the compatibility of *B. subtilis* PB18 with commonly used fungicides and reported that *B. subtilis* PB18 was more compatible with thiophanate methyl (96.07%) at 50 ppm followed by mancozeb, carbendazim, copper oxychloride and propioconazole. The compatibility was less (16.09%) with hexaconazole at 25 ppm compared to other fungicides. In the present studies also triazole group fungicide was relatively less compatible with *B. amyloliquefaciens* (MBNRB-3) and showed 47.1 per cent inhibition. Overall, the fungicide azoxystrobin was found to be highly compatible with *B. amyloliquefaciens* (MBNRB-3).

Compatibility of bioagents with herbicides also important because herbicides not only improve the crop yields by reducing the weed flora but also showing some non target effects against the soil borne pathogens

(Katan & Eshel, 1973) so it is important to study the compatibility of bioagents with herbicides. Present studies the biocontrol agent *B. amyloliquefaciens* (MBNRB-3) was highly compatible with herbicide imazethapyr + imazamox showing inhibition of 14.06 per cent. Whereas the herbicides pendimethalin and quizalofop-P-ehtyl were highly inhibitory to the bioagent MBNRB-3 even at half the recommended dosage and the inhibitions were 79.52 percent and 70.97 per cent respectively. In our present studies the herbicide propaquizafop also inhibited the *B. amyloliquefaciens* (MBNRB-3) at all the three concentrations tested similar results with propaquizafop was obtained by Prasad et al. (2014) who evaluated the compatibility of *B. subtilis* with different agrochemicals and reported that among all chemicals tested Azoxystrobin (fungicide), Flubendiamide (insecticide) and Propaquizafop (herbicide) were found to inhibit *Bacillus* at recommended / half recommended dosage.

Conclusions

In vitro evaluation of native fungal isolates under *in vitro* conditions indicated that all the tested isolates were inhibitory to the growth of *S. rolfsii* and *A. niger*. The bioagent *T. harzianum* (MBNRT-1) was superior among all the isolates in inhibiting the pathogens *S. rolfsii* and *A. niger* and the per cent inhibitions were 70.58% in case *S. rolfsii* whereas; in *A. niger* the inhibition was 72.9 per cent. Among native bacterial isolates the isolate *B. amyloliquifaciens* (MBNRB-3) and is significantly superior over the other isolates in inhibiting the pathogens *S. rolfsii* and *A. niger* under *in vitro* conditions and the inhibitions were 66.6 per cent and 63.07 per cent.

Compatibility of superior bioagents *T. harzianum* (MBNRT-1) and *B. amyloliquifaciens* (MBNRB-3) with six fungicides and eight herbicides indicated that among the fungicides the azoxystrobin was highly compatible with both the bioagents *T. harzianum* (MBNRT-1) and *B. amyloliquifaciens* (MBNRB-3) whereas, among the herbicides imazethapyr + imazamox was found to be compatible with both the bioagents with at all the concentrations. While, tebuconazole, thiram, mancozeb+carbendazim (fungicides) and quizolofop-p-ethyl and pendimethalin (herbicides) were highly inhibitory to the *T. harzianum* (MBNRT-1) and *B. amyloliquifaciens* (MBNRB-3) under *in vitro* conditions.

Tables

Table 1. Evaluation of native fungal isolates against *Sclerotium rolfsii* and *Aspergillus niger* under *in vitro* conditions

Fungal isolate (Identity Number)	Identification by using 18S *r*RNA technique	Inhibition of test pathogen over control (%)	
		Sclerotium rolfsii	***Aspergillus niger***
ATPT-1	*Trichoderma viride* strain SBTTv-001(2)	58.43 (49.83)	66.7 (54.73)*
ATPT-2	*Trichoderma harzianum* strain K Air-15	56.07 (48.47)	69.0 (56.15)
ATPT-3	*Trichoderma asperellum* strain T42	57.25 (49.15)	66.3 (54.48)
ATPT-4	*Trichoderma harzianum* strain BpT10a	28.62 (32.32)	62.0 (52.27)
ATPT-5	*Trichoderma virens* isolate Tc13	61.17 (51.44)	66.7 (54.72)
MBNRT-1	*Trichoderma harzianum* strain CEN830	70.58 (57.19)	72.9 (58.63)
MBNRT-2	*Penicillium marneffei* isolate M22	68.62 (55.92)	60.0 (50.89)
MBNRT-3	*Trichoderma* sp	45.48 (42.39)	54.0 (47.28)
MBNRT-4	*Trichoderma harzianum* strain CEN830	58.82 (50.08)	63.9 (53.06)
CHTT-1	*Trichoderma strigosum* strain T83	69.01 (56.15)	62.7 (52.37)
CHTT-2	*Trichoderma harzianum* isolate T-HV1	60.00 (50.74)	66.7 (54.71)
WGLT-1	*Penicillium* sp. 4 TMS-2011	37.64 (37.82)	56.9 (48.93)
WGLT-2	*Trichoderma asperellum* strain SBTT-076	30.19 (33.30)	54.9 (47.82)
T. viride (Commercial)		55.68 (48.24)	56.6 (48.78)
Control		0.00 (0.00)	0.00 (0.00)
CD at 5% SE(d) SE(m) CV %		2.938 1.427 1.009 3.690	7.370 3.602 2.547 9.008

*Values in the parenthesis are angular transformed values and are mean of three replications

Table 2. Evaluation of bacterial isolates against *Sclerotium rolfsii* and *Aspergillus niger* under *in vitro* conditions

Bacterial isolate (Identity number)	Identified species by using 16S *r*RNA technique	Inhibition of test pathogen (%)	
		Sclerotium rolfsii	***Aspergillus niger***
ATPB-1	*Bacillus sp.*B12	56.3 (*48.59)	58.00 (*49.59)
ATPB-2	*Bacillus cereus strain* BS1	61.2 (51.46)	62.67 (52.31)
ATPB-3	*Leucobater aridicollis*	54.1 (47.34)	54.11 (47.34)
ATPB-4	*Bacillus amyloliquefaciens*	36.8 (37.30)	42.83 (40.86)
MBNRB-1	*Bacillus subtilis*	45.0 (42.11)	41.95 (40.35)
MBNRB-2	*Pseudomonas sp.* 22	54.1 (47.34)	54.11 (47.34)
MBNRB-3	*Bacillus amyloliquefaciens strain* BHR3P1B2	66.66 (54.72)	63.08 (52.56)
MBNRB-4	*Bacillus cereus strain* NXUGDS005	55.1 (47.92)	39.60 (38.97)
WGLB-1	*Bacillus sp.* BCH532	63.7 (52.96)	58.33 (49.80)
WGLB-2	*Bacillus subtilis strain* PVR-YHB-1-1	63.1 (52.56)	38.21 (38.15)
Commercial (*Bacillus* sp)		56.86 (48.23)	47.08 (45.06)
Control		0.00 (0.00)	0.00 (0.00)
CD at 5% SE(d) SE(m) CV %		2.565 1.221 0.863 3.100	3.186 1.517 1.072 4.062

Values in the parenthesis are angular transformed values and are mean of three replications

Table 3 Compatibility of effective bio agent *T. harzianum* (MBNRT-1) with fungicides under *in vitro* conditions

Fungicides	Inhibition of *T. harzianum* (MBNRT-1) (%)		
	Recommended**	Half the recommended	Double the recommended
Mancozeb	53.90 (47.59)	20.39 (26.14)	25.09 (30.05)
Mancozeb+Carbendazim	100 (90.00)	100 (90.00)	100 (90.00)
Azoxystrobin	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Metalaxyl	58.03 (49.64)	25.88 (30.47)	83.91 (66.33)
Thiram	100 (90.00)	88.23 (69.90)	100 (90.00)
Tebuconazole	100 (90.00)	100 (90.00)	100 (90.00)
Factors Fungicides (A) Concentration (B) A X B	CD (5%) 6.48 4.2 11.22	SE (d) 3.21 2.10 5.56	SE (m) 2.27 1.48 3.93

Values in the parentheses are angular transformed and are means of three replications

**The recommended doses are 1000 ppm (azoxystrobin and tebuconazole); 2000 ppm (mancozeb, metalaxyl, thiram) and 2500 ppm (mancozeb+carbendazim)

Table 4. Compatibility of bioagent *T. harzianum* (MBNRT-1) with commonly used herbicides under *in vitro* conditions

Herbicide	Inhibition of *T. harzianum* (MBNRT-1) (%)		
	Recommended **	Half Recommended	Double Recommended
Propaquizafop	77.65 (61.76)	72.43 (58.30)	81.57 (64.56)
Cycloxydim	84.12 (66.50)	70.27 (56.94)	87.45 (69.23)
Oxyfluorfen	76.67 (61.09)	65.84 (54.22)	82.94 (65.58)
Imazythapyr	5.88 (14.02)	3.92 (9.35)	5.88 (14.02)
Imazythapyr + Imazamox	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Pendimethalin	87.45 (69.24)	79.02 (62.71)	82.35 (65.16)
Quizalopop- P- Ethyl	90.00 (71.54)	67.22 (55.04)	90.98 (72.51)
Fenaxoprop	46.31 (42.86)	43.06 (40.99)	49.88 (44.91)
Factors Herbicides (A) Concentration (B) (A X B)	C.D at 5 % 1.31 0.75 2.27	SE(d) 0.65 0.37 1.13	SE(m) 0.46 0.26 0.80

Values in the parentheses are the angular transformed values and are means of three replications
**The recommended doses are 430 ppm (Imazythapyr + Imazamox); 1500 (propaquizapop), 1700 ppm (oxyfluorfen), 2000 ppm (Imazythpyr, Fenaxoprop,Quizalopop- P- Ethyl); 3000, ppm (cyclodixim) and 5000 ppm (pendimethalin)

Table.5 Compatibility of *B. amyloliquefaciens* (MBNRB-3) with fungicides under *in vitro* conditions

Fungicide	Optical density at 610 nm			Inhibition over control (%)		
	(R)*	(0.5R)	(2R)	(R)*	(0.5R)	(2R)
Mancozeb	1.47	2.08	1.50	32.4	4.02	31.00
Mancozeb+ Carbendazim	1.36	2.08	1.55	37.5	4.06	28.4
Azoxystrobin	1.79	2.07	1.77	17.4	4.9	18.6
Metalaxyl	1.79	2.08	1.62	17.6	4.02	25.4
Thiram	1.14	1.19	1.10	47.4	44.8	49.3
Tebuconazole	1.15	1.62	1.05	47.14	25.4	51.7
Control	2.17	2.17	2.17	0.00	0.00	0.00
Factors Fungicides (A) Concentration (B) A X B				CD at 5 % 2.92 3.2 5.06	SE(m) 1.02 0.4 2.51	SE(d) 1.44 0.67 1.77

*The recommended doses are 1000 ppm (azoxystrobin and tebuconazole); 2000 ppm (mancozeb, metalaxyl, thiram) and 2500 ppm (mancozeb+carbendazim)

Table.6 Compatibility of *B. amyloliquefaciens* (MBNRB-3) with herbicides under *in vitro* conditions

Herbicide	Optical density at 610nm			Inhibition over control (%)		
	Concentration of Fungicide			Concentration of Fungicide		
	(R)**	(0.5R)	(2R)	(R)	(0.5R)	(2R)
Propaquizafop	0.21	1.12	2.01	*53.75	44.38	91.32
Cyclodixin	0.11	0.33	0.74	69.32	68.20	95.51
Oxyfluorfen	0.41	1.74	0.76	68.54	27.98	83.09
Imazythapyr	1.70	2.14	1.91	20.81	11.09	29.53

Imazythapyr + Imazamox	1.90	2.14	2.07	14.02	11.19	21.32
Pendimethalin	0.05	0.49	0.27	88.70	79.52	98.01
Quizalopop- P- Ethyl	0.15	0.70	0.48	80.10	70.97	93.70
Fenaxoprop	0.07	1.99	1.55	35.83	17.61	97.18
Control	2.41	2.41	2.41	0.00	0.00	0.00
Factors				CD at 5 %	SE (m)	SE(d)
Herbicides (A)				3.90	1.37	1.93
Concentration (B)				2.38	0.83	1.18
(A X B)				6.75	2.37	3.35

*Means of three replications

**The recommended doses are 430 ppm (Imazythapyr + Imazamox); 1500 (propaquizapop), 1700 ppm (oxyfluorfen), 2000 ppm (Imazythpyr, Fenaxoprop,Quizalopop- P- Ethyl); 3000 ppm (cyclodixim) and 5000 ppm (pendimethalin)

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