Harnessing the potential of efficient isolates of fungi as biocontrol agents against apple root rot-an emerging threat to apple industry in Kashmir

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ABSTRACT

Two hundred and twenty two VAM isolates were isolated from the rhizosphere of apple, pear and cherry crops from the four districts of Kashmir valley viz., Srinagar, Ganderbal,Baramulla and Shopian. During the study 29 species of VAM were identified, besides 16 could be identified only upto genus level. The identified VAM belonged to 7 genera. Glomus species was the most common and predominant VAM fungus in the soils of study sites with 57.72 per cent of frequency isolation, followed by Acaulospora (22.82%), Scutellospora (8.05%) and Septoglomus (4.69%). The VAM isolates, isolated from different host viz., apple, pear and cherry rhizosphere, and evaluated on maize revealed variable behaviour of isolates with respect to plant growth improvement and enzyme activities. Mycorrhizal root colonization in inoculated plants was in the range of 17.78 to 65.15 per cent.The root colonization by VAM isolates from cherry, apple and pear rhizosphere was 23.2 to 65.2, 19.8 to 53.1 and 17.8 to 61.5 per cent, respectively. The root phosphatase was in the range of 3.30-40.66 m mol PNP/g fresh root weight as compared to 3.66 m mol PNP/g fresh root weight in uninoculated control. The soil phosphatase activity varied from 3.23-30.73 m mole PNP /g soil in VAM inoculated plants as compared to uninoculated control. Amongst the isolates, Glomus geosporum (isolate SSC9), Acaulospora rehmii (isolate SHC17), Glomus diaphanum (isolate SBA21) and Gigaspora decipiens (isolate GBC34) proved significantly superior and selected to assess their efficacy in improving apple plant growth and suppressing root rot pathogen. The root rot pathogen, Dematophora necatrix R. Hartig, was isolated from infected samples of apple plants and pathogenicity proved as per Koch's postulates. Trichoderma viride Pers. was isolated from the rhizosphere of apple trees by dilution plate method. Five most efficient VAM species were assessed on apple cv. Red Delicious for their impact on plant height, girth, leaf area, leaf number, root rot index, root phosphatase activity, soil phosphatase activity and soil N and P under polyhouse conditions alongwith T. viride in presence and absence of root rot pathogen. The apple seedlings inoculated with G. geosporum showed maximum increase in growth parameters and phosphatase activity, followed by A. rehmii, G. diaphanum, G. decipiens and A. bireticulata. The soil N and P was also maximum in the plants inoculated with G. geosporum as compared to uninoculated control. The unicoculated control (without pathogen) showed the highest values of plant height, girth, leaf area and leaf number in apple plants. VAM inoculation reduced root rot incidence in apple by 8.36 to41.44 per cent. Inoculation of *T. viride* showed a significant reduction in controlling root rot of apple by35.23 per cent and also improved plant height, girth and leaf area but showed a non-significant effect on leaf number.

INTRODUCTION

Apple (*Malus domestica*Borkh.) is grown all over the world in the temperate areas and, to some extent, in cool highlands of sub-tropical areas such as East Africa, Northern parts of India, etc. In India, apple is grown on 55 % of the area falling under temperate fruits and account for 75 % of total temperate fruit production. Commercial cultivation of apple in India is confined to some Himalayan sates like Jammu & Kashmir, Himachal Pradesh, Uttaranchal, Arunachal Pradesh, Manipur and Sikkim. The successful cultivation of apple faces many production constraints including nutrition deficiency and disease, especially those incited by fungi. The major fungal diseases which inflict huge economic losses to apple crop allover India include scab, Alternaria leaf spot, powdery mildew, root rot and collar rot. Of these, root rot, caused by *Pythium, Phytophthora, Dematophora, Rhizoctonia, Armillaria, Fusarium,* etc.has posed a serious threat to apple plantations in recent years inflicting estimated annual loss worth approximately 1.3 million rupees (Gupta and Sharma, 2000).

Use of beneficial microbes as biological control deterrent and enhancers of plant health offers a great opportunity to restrict the activities of root rot pathogens. Several field and laboratory experiments have demonstrated that vesicular arbuscular mycorrhizal root colonization greatly helps in the sequestration of nutrients from the sources usually unavailable to the host plants (Sharma *et al.,* 2002) as well as suppress soil/root borne pathogens effectively through cross protection, competition and via release of phytohormones and pathogen inhibiting chemicals (Zargar *et al.,* 2000). These AM fungi not only reduce the damage caused by soil-borne plant pathogens but also impart resistance in plants. The effectiveness of AM fungi in biocontrol is dependent on the AM fungal species involved as well as the substrate and host plant. Among fungal antagonists, various species of *Trichoderma* are known to serve as effective biocontrol agents against soil borne diseases. *Trichoderma* species antagonize fungal pathogenic organisms by producing volatile and non- volatile antibiotics and/or by competing for nutrients and space as well as through mycoparasitism (Martinez *et al.,* 2003). .

METHODS AND MATERIALS

Isolation and mass culture of *Dematophora necatrix*

Dematophora necatrix was isolated from the infected roots of apple trees cv. 'Red Delicious' collected from Batpora (Harwan) area of district Srinagar during August 2011 (Fig.1) The pure culture was maintained by subculturing of fungus at weekly intervals. Mass multiplication of root rot pathogen was done on sterilized maize:sand (1:2 ratio) medium as per Bhardwaj *et al.* (2000)(Fig.2 & Fig.3).

Evaluation of AM fungi against apple root rot pathogen

Two years old healthy apple cv. "Red Delicious" saplings of uniform height and girth were procured from a private nursery in Shopian (Kashmir). The saplings were transferred to 25 cm dia. pots containing 5 kg sterilized sandy loam soil. The five AM fungi *viz*., *G. geosporum, A. rehmii, G. diaphanum, G. decipiens* and *A. bireticulata* were evaluated for growth parameters and enzymatic activity of apple cv. Red Delicious under polyhouse conditions. These were evaluated individually and in combination with fungal bioagent (*Trichoderma viride*) for their effectiveness in combating apple root rot. The AM inoculum was applied @ 10 g/pot, pathogen @ 5 g/ pot and *T. viride* @ 5 g/pot around the rhizosphere of apple saplings in the month of February with the onset of spring.

Root rot estimation

The root rot index in rot-affected plants was estimated on the basis of per cent root area affected following 0-5 scale of Bharat and Bhardwaj (2001) wherein 0 % is apparently free from infection and 1, 2, 3, 4 and 5 represent 1-10, 11-20, 21-50, 4=51-75 and 5= 76-100 % root area affected.

Statistical analysis

The experiment was laid out in a factorial completely randomized design and three replications were maintained for each treatment.

RESULTS

The use of *T. viride* significantly improved the efficacy of AM fungi in sapling improving plant growth and height. *T. viride* caused 11.2% increase in plant height whereas AMF caused 9.1 to 40% increase with maximum increase by *G. geosporum* followed by *A. rehmii* (23.0%),*G. diaphanum*(16.1%) and *G. decipiens* (9.2%){Chart 1}. Minimum increase was due to *A. bireticulata*. *T. viride* caused 15.0% increase in plant girth whereas AMF caused 15.5 to 49.3% increase with maximum increase by *G. geosporum* followed by *A. rehmii* (33.8%), *G. diaphanum*(27.0%) and *G. decipiens* (21.6%). Minimum increase was recorded due to *A. bireticulata. T. viride* caused 2.5% increase in leaf area whereas AMF caused 5.8 to 23.2 % increase with maximum increase by *G. geosporum* followed by *A. rehmii* (16.6%),*G. diaphanum*(12.7%) and *G. decipiens* (9.2%). *A. bireticulata* showed the minimum increase in leaf area. *T. viride* caused 4.5% increase in leaf number whereas AMF caused 3.9 to 18.97 % increase with maximum increase by *G. geosporum* followed by *A. rehmii* (14.6%),*G. diaphanum*(8.6%) and *G. decipiens* (5.5%). Minimum increase was due to *A. bireticulata.* The interaction between AM fungi and *T. viride* was significantly high in case of plant height and girth while the interaction effect was non significant in case of leaf area and leaf number. In present investigation apple plant growth with respect to height, girth, leaf area and leaf number was inhibited by the presence of root rot pathogen (*Dematophora necatrix*). AMF inoculated apple saplings showed significant improvement in plant height, girth, leaf number and leaf area in comparison to uninoculated control (Table 1). The present study revealed that AMF inoculation reduced root rot incidence in apple from 8.36 to 41.44 per cent. Highest disease incidence of 24.67 per cent was observed in control and least root rot in the plants inoculated with *G. geosporum* (16.42%). *A. rehmii, G.diaphanum,G. decipiens* and *A. bireticulata* inoculated plants exhibited root rot incidence of 19.33, 19.42, 21.17 and 22.83 per cent, respectively(chart 2).

Figure 1. Apple roots showing whitish mycelial growth of pathogen (*D. necatrix*)

Figure 2. Culture plate showing grayish mycelial growth of *D. necatrix*

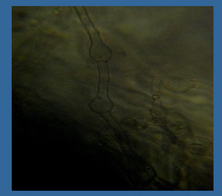

Figure 3. Mycelium of *D. necatrix*

Treatments	AM Isolates						Mean
	Control	*Acaulospora rehmii*	*Glomus diaphanum*	*Gigaspora decipiens*	*Glomus geosporum*	*Acaulospora rabiriculata*	
Plant height (cm), Pllant girth (mm),Leaf area(cm2) ana leaf number							
Control	10.73	12.85	12.24	11.51	14.90	11.01	12.20
T. viride	11.57	14.63	13.65	12.85	16.55	12.14	13.56
Mean	11.15	13.74	12.94	12.18	15.72	11.57	
Control	1.36	1.86	1.74	1.65	2.08	1.55	1.67
T. viride	1.59	2.09	2.01	1.94	2.33	1.86	1.92
Mean	1.48	1.98	1.88	1.80	2.21	1.71	
Control	17.88	20.92	20.36	19.81	22.16	19.25	20.06
T. viride	18.64	21.67	20.79	20.07	22.82	19.40	20.57
Mean	18.26	21.29	20.57	19.94	22.49	19.32	
Control	77.59	89.21	85.23	81.78	91.87	81.13	84.47
T. viride	81.43	93.02	87.44	86.07	97.33	84.17	88.24
Mean	79.51	91.11	86.33	83.92	94.60	82.65	

Table 1. Effect of coinoculation of AM fungi and *Trichoderma viride* on various growth parameters of apple cv. Red Delicious

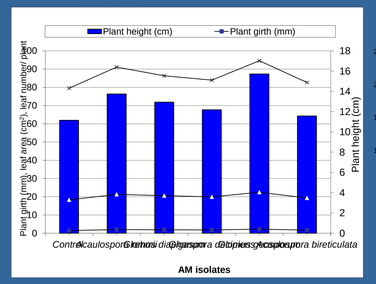

Chart 1 Effect of AM isolates on growth parameters of apple cv. Red. Delicious

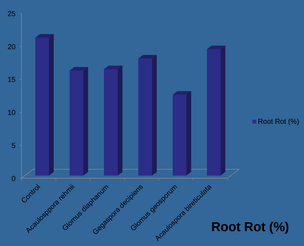

Chart 2. Effect of AM inoculation on root rot index of apple cv. Red Delicious

Discussion

The findings are in agreement with Chebet *et al.* (2008) who reported increase in plant height, leaf number, stem girth and biomass accumulation by AMF inoculation in passion fruit, rough lemon and papaya. Our results are also supported by Mathews *et al.* (2003) who reported significant increase in height, pseudostem girth, leaf area and shoot biomass of banana plantlets inoculated with *G. fasciculatum*. The inoculated plantlets exhibited higher leaf area of 459.19 cm^2 by 45 days after inoculation which was 2.2 times higher than uninoculated control. Sreenivasa and Bagyaraj (1989) attributed growth improvement in many AM inoculated plant species to the increase in nutrient uptake, production of growth promoting substances and induced tolerance to drought, salinity and transplantation shock as well as to the synergistic action of beneficial microbes such as nitrogen fixers and phosphorus solubilizers in soil. Our observations are in agreement with Granger *et al.* (1983), Gardener and Christensen (1991), Vidal *et al.* (1992) and Rapparani *et al.* (1994) who reported increased vegetative growth in mycorrhiza-inoculated fruit plants like apple, pear and avocado. Symbiotic association of plant roots with AM fungi often results in enhanced growth due to better acquisition of nutrients especially phosphorus (Kwapata and Hall, 1985). Meddad-Hamza *et al*. (2010) observed increased apical shoot growth in *G. mosseae*- and *G. intraradices*-inoculated apple, plum and olive plants. Meddad-Hamza *et al*. (2010) observed 376 and 226 per cent increase in vegetative matter in olive plantlets inoculated with *G. mosseae* and *G. intraradices,* respectively.

Conclusions

Five most efficient AM species were assessed on apple cv. Red Delicious for their impact on plant height, girth, leaf area, leaf number, root rot index, root phosphatase activity, soil phosphatase activity and soil N and P under polyhouse conditions alongwith *T. viride* in presence and absence of root rot pathogen. The apple seedlings inoculated with *G. geosporum* showed maximum increase in growth parameters and phosphatase activity, followed by *A. rehmii, G. diaphanum, G. decipiens* and *A. bireticulata*. The available soil nitrogen and phosphorus was also maximum in the plants inoculated with *G. geosporum* as compared to uninoculated control.

The inoculation of test VAM fungi on apple plants showed pronounced increase in growth during active phase i.e. during 90-180 days of growth with 2.7 to 28.3, 8.7 to 46.8, 6.9 to 23.0 and 3.4 to 18.2 per cent increase in plant height, girth, leaf area and leaf number respectively.

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