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RESEARCH ARTICLE

METARHIZIUM ANISOPLIAE AS ENDOPHYTE HAS THE ABILITY OF PLANT GROWTH ENHANCEMENT

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ABSTRACT

Plants in natural ecosystem appear to be symbiotic with fungal endophytes. *Metarhizium anisopliae* is a fungal entomopathogen with the ability to colonize plants endophytically. In the present study we evaluated the effect of artificial inoculation of *M. anisopliae* on growth parameters of aerial and underground parts of tomato plants and also to determine its endophytic activities. *M. anisopliae* was applied as seed treatment, substrate treatments, foliar spray alone and in combination with other and comparison was made with the control. We observed significant increase in seed germination, shoot and root length and biomass production of 35 days old tomato seedlings. We could also recover the inoculated *M. anisopliae* from leaves, stem, roots and rhizosphere with highest per cent recovery of 55.55%, 77.77%, 33.33% and 106.66 cfu/g from leaves, stem, roots and rhizosphere respectively. The present experiment reveals some new information on the endophytic interaction between *M. anisopliae* and tomato plants but needs more research for understanding the actual mechanism of *M. anisopliae* by which the fungi cause growth promotion of plants.

INTRODUCTION

Indiscriminate use of chemical pesticides causes many problems like resistance, pest resurgence, environmental pollution and risks to human health. Considerable efforts have been directed toward the use of bio–control agents as an alternative to the use of chemical pesticides. Amongst the different bio-control agents entomopathogenic fungus like *Metarhizium anisopliae* and *Beauveria bassiana* have received considerable attentions as a viable alternative to chemical pesticides as both the fungi have potential to engage in plant fungus interaction. Endophytes are known to affect the interactions of plants with their environment, and to alter the course of their interactions with plant pathogens. Fungal endophytes mediate plant defense as a novel biological control mechanism. Moreover, endophytism offers several advantages to the host plants. They are (i) greater access to the nutrients, (ii) protection from desiccation, and (iii) protection from the surface feeding insects, parasitic fungi, etc. Pioneering work on entomopathogenic endophytes was conducted using maize (*Zea mays* L.), *B. bassiana*, and the European corn borer, *Ostrinia nubilalis* (Hu¨bner). Endophytes are gaining attention as a subject for research and applications in Plant Pathology because in some cases plants associated to endophytes have shown increased resistance to plant pathogens, particularly fungi and nematodes. The exploitation of *M. anisopliae* as a biological control agent against a wide range of insects and effect on plant growth has not been widely researched. The aim of the present study was thus to evaluate the effect of the inoculation with *M. anisopliae* on the growth of tomato plants and to determine its endophytic activity.

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MATERIALS AND METHODS

Source of the fungus

Pure and fresh culture of *M. anisopliae* (ITCC No. 8882.12) was taken from the culture collection of Mycology Research Section, Department of Plant Pathology, AAU, Jorhat, Assam and maintained by periodic transfer into petriplate containing PDA (potato dextrose agar) supplemented with antibiotic, streptomycin sulphate throughout the experimentation period. Monospores of *M. anisopliae* (@ $1x10^6$ spore /ml of water) was used in the study for its endophytic behavior and plant growth enhancement.

Tomato plants and treatment combination

The experiment was carried out during 2013-14 in a pot tray kept inside the net house of Mycology Research Section, AAU, Jorhat. Pot trays were filled with sterilized soil (100 g). Two healthy tomato seeds (var. Pusa Ruby) per pith of the pot tray

were sown with 10 replications for the following seven treatment combinations:

T_1: Untreated contol;
T_2: Seed treatment with *M. anisopliae* @ 5ml/kg of seeds;
T_3: Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate;
T_4: Foliar spray with *M. anisopliae* at 4 –leaf stage @ 5ml/lit of water;
T_5: Seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate;
T_6: Seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Foliar spray with *M. anisopliae* at 4 –leaf stage @ 5ml/lit of water;
T_7: Seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate + Foliar spray with *M. anisopliae* at 4 –leaf stage @ 5ml/lit of water.

Observation on seed germination (%) was taken at 7 days of seeding. Similarly observation on growth parameters like shoot and root length, fresh and dry weight of shoot and root were taken after 35 days of seed germination. Pot trays were watered regularly twice in a day with double sterilized water.

Recovery of *M. anisopliae* as an endophyte

To study on the recovery of *M. anisopliae* as endophyte from the treatment combination mentioned elsewhere, tomato seedlings were carefully uprooted as per the treatments and washed thoroughly with double distilled water for twice. Roots, stems and leaf samples were cut into 6-8mm sections with sterilized sharp razor blade (Gillete) and kept carefully on sterilized petriplate under a laminar hood chamber (Labotech Laminar Air Flow Chamber). Samples were then surface sterilized by immersing them separately for two minutes each in 0.5 % sodium hypochlorite (NaOCl) and two minutes in 70 % ethanol followed by rinsing for three times in sterile distilled water and were allowed to dry in sterile paper towel. Outer edge of the samples was dissected with sterilized scissor/ blade and discarded. Six sections were cut from the trimmed sample, averaging 6x6 mm for leaves and 6 mm long for stems and roots and were being placed on a petriplate containing PDA medium supplemented with antibiotics tetracycline, streptomycin and penicillin at 2mg/litre. Three replications per treatment and samples were made. Plates were finally sealed with parafilm and inoculated petriplates were kept in BOD incubator at 28±1^{o}C under 12hr light alternating with 12hr dark period. Observations on fungal colonies were taken and those considered as positive results were randomly selected and transferred to PDA slants. All the colonies transferred were allowed to sporulate and based on their morphological characteristics (colour, conidial size, conidial shape, etc) identified as *M. anisopliae.*

Recovery of *M. anisopliae* from the rhizosphere

To study on the recovery of *M. anisopliae* from the rhizosphere of tomato, seedlings were uprooted carefully and were shaken gently to obtain the soil adhered to the root system. One gram of composite soil sample per treatment was suspended in 9 ml of sterile water, stirred in a vortex for 3 min and was used for analysis. One (1) ml of aliquot from the suspension was placed in petriplates containing *Metarhizium* specific media (Ghanbary *et al.,* 2009) and incubated at 28^{0}C for 5 days. Developing colonies with general characteristics of the genus *Metarhizum* (colour, conidial size, conidial shape, etc) was grouped. Quantification of *M. anisopliae* colonies were done to determine the number of colony forming unit (cfu) per gram of soil.

RESULTS

Effect of *M. anisopliae* on Plant Growth

Data presented in Table 1. showed 100% germination of tomato seeds when seeds and substrate were treated with *M. anisopliae* either alone or both i.e., seed treatment and substrate treatment with *M. anisopliae*. But when *M. anisopliae* was not used for seed treatment the germination percentage was found lower as compared to the previous two treatments and found at par with the control.

Highest shoot length (9.28 cm) and root length (3.22 cm) was observed in seed treatment with *M. anisopliae* @ 5ml/kg of seeds + substrate treatment with *M. anisopliae* @ 5ml/kg of substrate + foliar spray with *M. anisopliae* at 4-leaf stage @ 5ml/L of water. This was followed by seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate for shoot length (9.20 cm) and root length (2.60 cm). Seeds and substrate treatment alone though showed significant increased in shoot and root length but were statistically at par with each other. Lowest shoot and root length was observed in control where neither seeds and substrate were treated nor foliar spray of *M. anisopliae* was made; this was found at par with the treatment where foliar spray of *M. anisopliae* was made alone (Table 1).

Similarly, highest fresh and dry shoot and root weight were observed when *M. anisopliae* was used as seeds treatment in combination with substrate treatment and foliar spray at 4-leaf stage (Table 1). This was followed by seed treatment in combination with substrate treatment with *M. anisopliae* without any significant different between them. Lowest fresh and dry weight of shoots and roots was observed in control when *M. anisopliae* was neither applied as seed and substrate treatment nor as foliar spray.

Recovery of *M. anisopliae*

M. anisopliae was recovered from the treated plant as well as from the rhizosphere and no positive plate was obtained from the untreated control. The effectiveness of the disinfecting process in the recovery of endophytic association was confirmed because the rinse water did not yield any fungi. Highest recovery from leaves (55.55%), shoots (77.77%), roots (33.33%) and rhizosphere (106.66 cfu/g of soil) was recorded in seed treatment with *M. anisopliae* @ 5ml/kg of seeds + substrate treatment with *M. anisopliae* @ 5ml/kg of substrate + foliar spray with *M. anisopliae* at 4-leaf stage @ 5ml/L of water.

Table 1. Growth parameters of 35 day old tomato seedlings according to the different treatments used

Treatment	Germination (%)	Growth parameters											
		Shoot length (cm)	Increased shoot length (%) over control	Root length (cm)	Increased shoot length (%) over control	Fresh shoot weight (g)	Increased fresh shoot weight (%) over control	Fresh root weight (g)	Increased fresh root weight (%) over control	Dry shoot weight (g)	Increased dry shoot Weight (%) over control	Dry root weight (g)	Increased dry shoot Weight (%) over control
Untreated control	90 [c] (71.57)	6.14 [c]	-	1.68 [c]	-	0.097 [d]	-	0.005 [c]	-	0.004 [d]	-	0.0008[c]	-
Seed treatment with *M. anisopliae* @ 5ml/kg of seeds	95 [b] (77.07)	8.72 [b]	42.02	2.14 [b]	27.33	0.339 [b]	249.46	0.014 [b]	180.00	0.018 [b]	350	0.0014[b]	75.00
Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate	100 [a] (90)	8.74 [b]	42.35	2.22 [b]	32.14	0.349 [b]	259.79	0.016 [a]	220.00	0.018 [b]	350	0.0016[b]	100.00
Foliar spray with *M. anisopliae* at 4 leaf stage @ 5ml/lit of water	90 [c] (71.57)	6.74 [c]	9.77	1.74 [c]	3.57	0.304[c]	213.4	0.007 [c]	40.00	0.010[c]	150	0.0018[b]	125.00
Seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate	100 [a] (90)	9.2 [a]	49.84	2.6 [a]	54.76	0.429[a]	342.27	0.017[a]	240.00	0.019 [a]	375	0.0020[b]	150.00
Substrate treatment with *M. anisopliae* @ 5ml/kg of seeds + Foliar spray with *M. anisopliae* at 4 leaf stage @ 5ml/lit of water	90 [c] (71.57)	8.5 [b]	38.44	2.1 [b]	25.0	0.308[c]	217.53	0.014 [b]	180.00	0.016 [b]	300	0.0010[c]	250.00
Seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate + Foliar spray with *M. anisopliae* at 4 leaf stage @ 5ml/lit of water	100 [a] (90)	9.28 [a]	51.14	3.22 [a]	91.66	0.443[a]	78.10	0.020 [a]	300.00	0.022 [a]	450	0.0030[a]	275.00

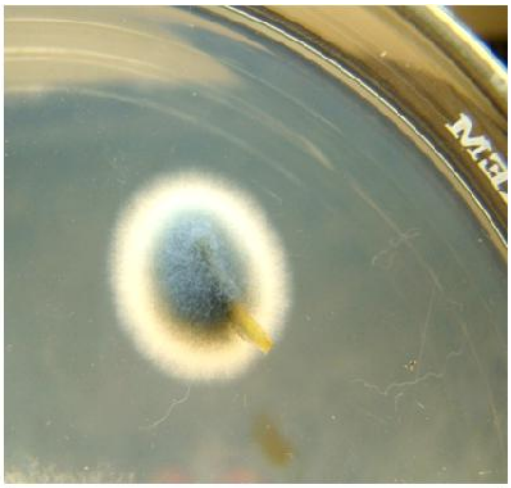
*Data in the parentheses are arc sine transformed value, Data are mean of 10 replications

Table 2. Percentage of positive plates with *M. anisopliae* recovered from 35 day old tomato plants

Treatment	Leaves (%)	Shoots (%)	Roots (%)	Rhizosphere (cfu/g soil)
Untreated control	0.00 [e]	0.00 [f]	0.00 [d]	0.00 [g]
Seed treatment with *M. anisopliae* @ 5ml/kg of seeds	33.33 [c] (35.06)	33.33 [c] (35.06)	11.11 [c] (19.36)	84.66 [d]
Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate	33.33 [c] (35.06)	33.33 [c] (35.06)	22.22 [b] (27.97)	90.33 [c]
Foliar spray with *M. anisopliae* at 4 leaf stage @ 5ml/lit of water	11.11 [d] (19.36)	11.11 [e] (19.36)	11.11 [c] (19.36)	60.33 [f]
Seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate	44.44 [b] (41.55)	55.55 [b] (47.87)	22.22 [b] (27.97)	98.33 [b]
Substrate treatment with *M. anisopliae* @ 5ml/kg of seeds + Foliar spray with *M. anisopliae* at 4 leaf stage @ 5ml/lit of water	11.11 [d] (19.36)	22.22 [d] (27.97)	11.11 [c] (19.36)	75.33 [e]
Seed treatment with *M. anisopliae* @ 5ml/kg of seeds + Substrate treatment with *M. anisopliae* @ 5ml/kg of substrate + Foliar spray with *M. anisopliae* at 4 leaf stage @ 5ml/lit of water	55.55 [a] (47.87)	77.77 [a] (61.34)	33.33 [a] (35.06)	106.66 [a]

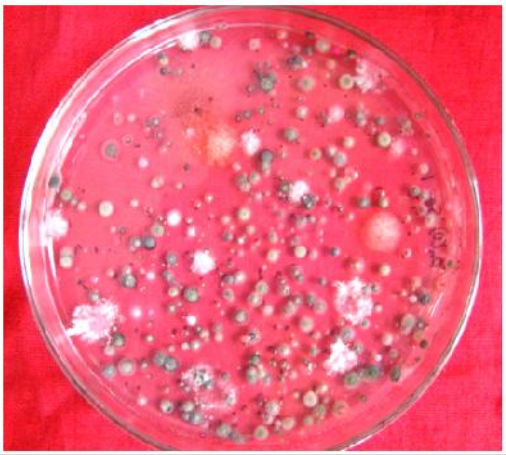
Percentage of positive plates on the total number of sample (n=10). Considered positive when atleast one colony of *M. anisopliae* grew in any of the samples

a. Recovery of *M. anisopliae* from leaf



b. Recovery of *M. anisopliae* from shoot

c. Recovery of *M. anisopliae* from root



d. Recovery of *M. anisopliae* from soil

This was followed by combination of seed and substrate treatment with *M. anisopliae* where recovery of 44.44%, 55.55%, 22.22% and 98.33 cfu/g of soil was recovered from leaves, shoots, roots and rhizosphere respectively. When substrate was treated alone recovery of *M. anisopliae* was recorded as 33.33%, 33.33%, 22.22% and 90.33 cfu/g of soil for leaves, shoots, roots and rhizosphere respectively (Table 2.). Lowest recovery of *M. anisopliae* with 11.11% and 60.33 cfu/g of soil was recovered from all the plant parts and rhizosphere respectively.

DISCUSSION

Increased germination percent observed in the present study is not in agreement with the earlier work of Kabulak and Ericsson (2007) who did not observe increased germination of corn seeds. But Elena *et al.* (2011) reported growth promotion activity of *M. anisopliae* on tomato plants. The increased seed germination observed in the study may be due to the release of growth hormone by the fungus when used as either seed treatment or substrate treatment or both before seeding of the tomato seeds. In the present study we observed the increased growth parameter of aerial parts as well as below ground parts of tomato plants in the treatment where *M. anisopliae* was used as seed treatment and substrate treatment alone and in combination with each other. When we applied *M. anisopliae* as seed treatment in combination with substrate treatment and foliar spray at 4-leaf stage, it showed highest enhancement of plant growth parameters of both aerial pats and below ground parts of the tomato plants (shoot length 51.14%, root length 91.66%, shoot fresh weight 78.10%, root fresh weight 300%, shoot dry weight 450%, and root dry weight 275%). The growth enhancement of tomato plants observed in this study might be due to the endophytes stimulative behavior for different growth parameters of the plants as observed by Sasan and Bidochka (2012) for *M. robertsii*. Elena *et al.* (2011) reported that *M. anisopliae* has endophytic activity and promotes plant growth. Moreover, *M. anisopliae* being a clavicipitaceous fungus has the ability to remain as endophyte and colonize, transmit and enhance plant growth (Rodriguez, 2009). In an another experiment Wille *et al.* (1999) reported that clavicipitaceous endophytes can frequently increase plant biomass. The mechanisms by which *Metarhizium* boosts plant growth are likely to be multifactorial, as reported for species of *Trichoderma* where the plant-growth-promoting effects include antibiosis, parasitism, induction of host plant resistance, and competition (Mukherjee *et al.*, 2013). Endophyte promotes plant growth by secreting different hormonal substances.

(Porter *et al*., 1979) reported that endosymbionts produced auxin which enhanced the vegetative growth of the endophyte infected plants.

In our experiment we could able to recover *M. anisopliae* from all the plant parts and rhizospheric substrate in all the treatment combination, this suggests some degree of vertical movement of the fungus either through colonization or via percolating water or through passive movement through xylem. Presence of *M. anisopliae* in plant parts at locations distant from the point of inoculation may be due to the movement within the plants and its colonization, as we observed fungal hyphae within the vascular elements of treated tomato plants. Earlier, studies showed that when *B. bassiana* when injected into the corn stem, it colonize and moves within the plants (Bing and Lewis, 1991 & 1992).

Conclusion

The present study showed that for better endophytic activity and enhancement of plant growth parameters, *M. anisopliae* should be used as seed treatment, substrate treatment as well as foliar spray. But further research should be done to understand the mechanism through which *M. anisopliae* enhances plant growth and effect on host physiology, tolerance to biotic factor, and/or triggering host resistance. The use of endophytic microorganism for the plant disease control is relatively new and unexplored area of research. Little is known about the interaction between the endophyte, host plant and pathogen. Understanding of this interaction is essential for the development of proper biocontrol strategy. Although scientific approaches on the diversity of endophytes have just recently got momentum, information on endophytes behavior, their mutualistic interaction with crops remain scanty and more studies are needed to explore their full potentiality in plant protection sector.

REFERENCES

Bing, L.A. and Lewis, L.C. 1991. Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. *Environ. Entomol.*, 20: 1207-1211.

Bing, L.A. and Lewis, L.C. 1992. Endophytic *Beauveria bassiana* (Balsamo) Vuillemin in corn: the influence of the plant growth stage and *Ostrinia nubilalis* (Hübner). *Biocontrol Sci. Technol.*, 2: 39-47.

Elena, G.J., Beatriz, P.J., Alejandro, P. and Roberto, L. 2011. *Metarhizium anisopliae* (Metschnikoff) Sorokin promotes growth and has endophytic activity in tomato plants. *Advances in Biological Research*. 5 (1): 22-27.

Ghanbary, T.A., Asgharzadeh, A., Hadizadeh, A.R. and Sharif, M.M. 2009. A quick method for *Metarhizium anisopliae* isolation from cultural soils. *American Journal of Agril. and Biological Sciences.*4(2): 152-155.

Kabaluk, J.T. and Ericsson, J.D. 2007. *Metarhizium anisopliae* seed treatment increases yield of field corn when applied for wireworm control. *Agron. J*., 99: 1377-1381.

Mukherjee, P.K., Horwitz, B.A., Herrera-Estrella, A., Schmoll, M. and Kenerley, C.M. 2013. *Trichoderma* research in the genome era. *Annu Rev Phytopathol*. 51(1):105–129

Porter, J.K., Bacon, C.W. and Robins, J.D. 1979. Ergosine, ergosinine and chanoclaveine I from *Epichloe typhina*. *J Agricult Food Chem.* 27: 595-598.

Rodriguez, R.J., White, J.F., Arnold, A.E. and Redman, R.S. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist.* www.newphytologist.org .1-17.

Sasan, R.K. and Bidochka, M.J. 2012. The insect pathogenic fungus, *Metarhizium robertsii* (Clavicipitaceae) is an endophyte that stimulate plant root development. *American J. of Botany*.99: 101-107.

Wille, P.A., Aeschbacher, R.A. and Boller, T. 1999. Distribution of fungal endophyte genotypes in doubly infected host grasses. *Plant Journal.* 18: 349–358

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