Effect of *Trichoderma harzianum* Isolates Against Dry Root Rot Pathogen of Mungbean

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World Vegetable Center

THE WORLD FOOD PRIZE

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I would like to thank Priyakshi Buragohain, a PhD student at World Vegetable Center, for exposing me to true Indian culture. I'll remember our many talks and cultural stories you've shared with me for a lifetime. I appreciate the kindness and encouragement you extended to me since the first day I arrived.

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I will be forever appreciative of the encouragement from my friends and family. A special thanks to my siblings, Daniel and Katherine Swehla, for being there for me whenever I needed them. The many conversations and your continuous love and support are much appreciated. Most importantly, thank you to my parents, John and Dawn Swehla, for always believing in me. Thank you for your constant support and encouraging me to reach my goals.

Abstract

Mungbean [*Vigna radiata* (L.) Wilczek] is a legume crop grown for its production of protein-rich grain and sprouts. Mungbean is grown as a rotation crop in cereal farming system in Asia, particularly in rice fallows. Dry root rot caused by *Macrophomina phaseolina* is an emerging disease of mungbean in rice fallows. *M. phaseolina* causes substantial loss in production. A range of 27-44% loss of production has been reported due to this fungal pathogen (Bashir and Malik 1988). For the management of this disease, chemical and non-chemical methods have been considered. Non-chemical method control of this pathogen by bioagent (*Trichoderma* spp.) has been reported as an effective method.

In this study antagonistic activity of four *Trichoderma harzianum* isolates viz., Th-Dharwad, Th-Raichur, Th-Niphm and Th-Udaipur procured from different Indian Research Institutes was evaluated against *M. phaseolina* of mungbean by dual culture technique. The efficacy of these *T. harzianum* isolates were also compared with effective fungicides such as thiram and carbendazim. Results showed that among the *T. harzianum* isolates, isolate Th-Raichur was most effective exhibited 76.96% mycelial growth inhibition of the test fungus. However, in both fungicides carbendazim exhibited 100% mycelial growth inhibition that was more than isolate Th-Raichur. Biological spectrum of isolate Th-Raichur was studied against *M. phaseolina* of urdbean (*Vigna mungo* (L.) Hepper) and soybean (*Glycine max* (L.) Merr.) along with two other root pathogens viz., *Fusarium solani* of mungbean and *Sclerotium rolfsii* of urdbean. Isolate Th-Raichur showed maximum antagonistic activity against *M. phaseolina* and *F. solani* of mungbean. Thus, this isolate can be used as effective bioagent for management of dry root rot and *Fusarium* rot of mungbean after the field trials.

Review of Literature

Importance of Mungbean

Mungbean [*Vigna radiata* (L.) R. Wilczek] serves as an important pulse crop worldwide. The seed of the mungbean appears as a green, spherical bean which measures 4mm in diameter. Its ability to fix atmospheric nitrogen classifies it as a legume crop. The combination of leguminous characteristics and cheap source of protein-rich grain and sprouts makes the mungbean essential in developing countries such as in India. Kumari *et al.* (2012) stated that mungbean acts as the third most important pulse crop in India. Small-scale farmers rely on a healthy harvest for the sustainability of their families.

Tiwari and Shivhare (2016) reported an average of 30.41 lakh ha of mungbean production with 14.24 lakh tons harvested across India. Mungbean acts as the staple crop in many rural communities. Harvested mungbeans are used in a variety of preparations like halwa, dal, and snack. A healthy mungbean crop is essential for all production areas in India.

Dry root rot disease in mungbean and its economic impact

Dry root rot disease is also called as charcoal rot caused by *Macrophomina phaseolina* (asexual stage of *Rhizoctonia bataticola*), are widespread and serious diseases of mungbean in South Asia

and other Asian countries (Iqbal & Mukhtar, 2014). This pathogen has been detected as both a soil and seed borne. The pathogen can infect plants in both seedling and adult plant stages and show symptoms in both underground (root) and above ground plant parts (stem, branches, petioles, leaves and pods). The pathogen causes red to brown lesions on roots and stems with production of dark mycelia and black microsclerotia. When disease progresses, defoliation and wilting occur and plants die prematurely. The fungus produces resting structure, sclerotia, which can survive in the soil for several years and can infect plants when favorable environment exists. Dry conditions favor microsclerotia survival in soil, but moisture and temperature above 27 ˚C are suitable for mycelial growth and infection to the plants moisture (Hagedorn, 1991). In India, this pathogen causes 10.8% yield losses and 12.3% loss of protein content in mungbean seeds (Kaushik *et al*., 1987), while from Pakistan this yield loss was estimated up to 44% (Bashir and Malik, 1988). Although the pathogen affects all parts of the plant, it is generally most destructive and active in the fibro vascular systems of the roots (Khan, 2007). Infected roots develop a blackened color (Figure 2b). Kumari *et al.* (2012) reported *M. phaseolina* as the most catastrophic disease of mungbean. The sclerotial characteristics possessed by this pathogen make the management of this disease difficult (Kumari *et al.*, 2012).

Management options for dry root rot disease

For the management of dry root rot disease several methods such as seed treatment with fungicides and biocides, use of resistant varieties (Khan *et al*., 2007; Choudhary *et al*., 2011), soil application of bioagent and fungicides has been adopted. Limited resistant varieties exist for the management of dry root rot disease in mungbean. Chemical fungicides against *M. phaseolina* have yielded good results, but excess fungicide residue creates environmental and human health risks (Thilagavathi *et al*., 2007). The consideration of biological control measures has been reviewed in order to find a more environmentally safe approach. Thilagavathi *et al*. (2007) observed the antagonistic activity of biological agents produced defense enzymes which enabled plants to protect themselves against pathogenic invasion.

Role of *Trichoderma* in disease management

Biological control is one of the sustainable and ecofriendly methods for suppressing plant diseases. Seed treatment and soil application of *Trichoderma* species has been reported as most effective for the management of dry root rot disease in mungbean. Most of these studies were carried out in the laboratories to evaluate the antagonistic effects of *Trichoderma* (biocontrol fungi) to inhibit growth of root rot pathogens, *Macrophomina*, few are in the glasshouse and field. In the field study, Kumari *et al.* (2012) examined that mixed application of vermicompost (10%) + bavistin (0.1%) + *T. harzianum* (4%) reduced seedling mortality caused by *M. phaseolina* where 5.74 and 5.04% mortality of seedlings were reported during pre and post emergence, respectively. Deshmukh *et al*. (2016) reported that in greenhouse, application of *T. harzianum* (4 g/kg seeds) with 25 g/kg of phosphate solubilizing bacteria (PSB) as seed dresser reduced 26% incidence of dry root rot. *T. viride* and *T. harzianum* reduced the mycelial growth (42.33 and 44.25 mm, respectively) of *Macrophomina* in dual culture method and combined application of *T. harzianum* at 4 g kg^{-1} + PSB at 25 g/kg seed as seed dresser with FYM in field trials had minimum dry root rot incidence in seedlings (26.0%) (Ebenezar & Yesuraja, 2000).

Materials and Methods

Source of *Trichoderma harzianum* isolates

Four isolates of *T. harzianum* were procured from four Indian Research Institutions namely, University of Agricultural Sciences Dharwad, University of Agricultural Sciences Raichur, (Karnataka State), National Institute of Plant Health Management, Rajendranagar Hyderabad and Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan state. These isolates were named as Th-Dharwad, Th-Raichur, Th-Niphm and Th-Udaipur.

These isolates were verified by studying their cultural characteristics on Potato dextrose agar medium PDA (Potato infusion 200 g, dextrose 20 g, agar 15 g, distilled water 1L) and morphological characteristics via a stereo-binocular microscope (Nikon-ci-digital, Olympus) by examine the conidia morphology; type of conidiophores as well as by using fungal key. Cultural characteristics including growth rate, color and colony appearance of each isolate of *T. harzianum* were examined following the procedure of Gams and Bisset (1998). The procured cultures were sub-cultured on PDA slants and were preserved at 4 °C for further experimentation.

Isolation and Identification of *M. phaseolina* from mungbean

Plants showing dry root rot disease symptoms were uprooted from the mungbean field of World Vegetable Center South Asia and brought to the laboratory in pre-sterilized polyethylene bags. Symptoms observed in the field were initially chlorosis of plants occurs followed by root and stem rots, which finally causes severe wilting and death of the plants (Figure 2a). Roots of the diseased plants were cut in small pieces and surface sterilized with 0.8% sodium hypochlorite for 2 min. Pieces of sterilized disease tissue were plated on pre-poured PDA medium in triplicates. Inoculated plates were incubated at 28± 2°C for 6-7 days and colonies appeared were purified on separate agar plates. Identification was conducted according to Dhingra and Sinclair (1978).

Cultural and morphological characteristics of *M. phaseolina* were carried out. Pathogen was identified by its mycelium structure, macroconidia morphology (size, shape of basal and apical cells), type of conidiophores (present or absent, arrangement), as well as by using fungal key. Culture was purified by single sclerotial isolation and maintained on PDA slants at 4°C for further study.

Sources of fungicides

Two fungicides viz., thiram and carbendazim used in this study were purchased. The fungicides (5 mg/ml) were directly mixed in the PDA medium before pouring in the Petri plates.

Screening of *T. harzianum* isolates and fungicides against *Macrophomina phaseolina*

In vitro antagonistic activity of *T. harzianum* isolates against test pathogen was assessed by dual culture technique (Dennis and Webster, 1971), while fungicides were screened by poison food method of Grover and Moore (1962). List of treatment is given in table 1.
In dual culture technique, 6 mm disc of 7 days old culture of test pathogen was placed on Petri plate (80 mm diam.) one cm away from the edge containing 10 ml pre-poured solidified PDA medium. Disc of *T. harzianum* (6 mm) of each isolate was placed at opposite side of the Petri plate, separately.

In poison food method, poisoned plates were prepared by pouring 10 ml of the PDA medium containing 5mg/ml of each fungicide in pre-sterilized Petri plates, separately. The plates were allowed to solidify. The poisoned plates were inoculated with test pathogen (6 mm diam. cut from the periphery of 7-day-old culture). Control plates were prepared without any treatment by inoculating the mycelia disc of the test pathogen on the agar surface.

Inoculated and control plates were kept in an incubator at 28 °C in randomize complete block design in three replicates and percent inhibition of test pathogens was recorded after 7 days of incubation. The percent growth inhibition by antagonistic fungi and fungicides was recorded by the following formula:

$\boldsymbol{PGI = \frac{C-T}{C} \times 100}$ (Vincent (Vincent 1927); where PI = percent inhibition, *C* = growth of test pathogen in absence of antagonist/fungicide (mm), *T* = Growth of test pathogen with antagonist/fungicide (mm).

TREATMENT NUMBER	TREATMENT DETAIL
T1	Th-Niphm
T2	Th-Dharwad
T3	Th-Udaipur
T4	Th-Raichur
T5	Thiram
T6	Carbendazim
T7	Control

Table 1: List of treatments for efficacy of fungal antagonist and fungicide

Biological spectrum of effective *T. harzianum* isolate against other root pathogens

Biological spectrum of effective isolate of *T. harzianum* was studied against other root pathogens of vegetable legumes namely *M. phaseolina* from urdbean (MPU) and soyabean (MPS), *Fusarium solani* from mungbean (FSM), *Sclerotium rolfsii from* urdbean (SRU) including *M. phaseolina* from mungbean (MPM). All pathogens were isolated from World Vegetable Center South Asia legume field. *In vitro* antagonistic activity of effective *T. harzianum* isolate against these pathogens was assessed by dual culture technique as described earlier. The detail treatment

is given in table 2. Inoculated and controlled plates of each pathogen were kept separately in an incubator in three replicates in randomize complete block design at 28 ºC and percent inhibition of each test pathogen by fungal antagonist was recorded after 7 days of incubation. The percent growth inhibition of each test pathogen by antagonistic fungi was recorded by the formula as given above.

TREATMENT NUMBER	TREATMENT DETAIL
T1	MPM
T2	MPU
T3	MPS
T4	SRU
T5	FSM
T6	MPMC
T7	MPUC
T8	MPSC
T9	SRUC
T10	FSMC

Table 2: List of treatments for biological spectrum

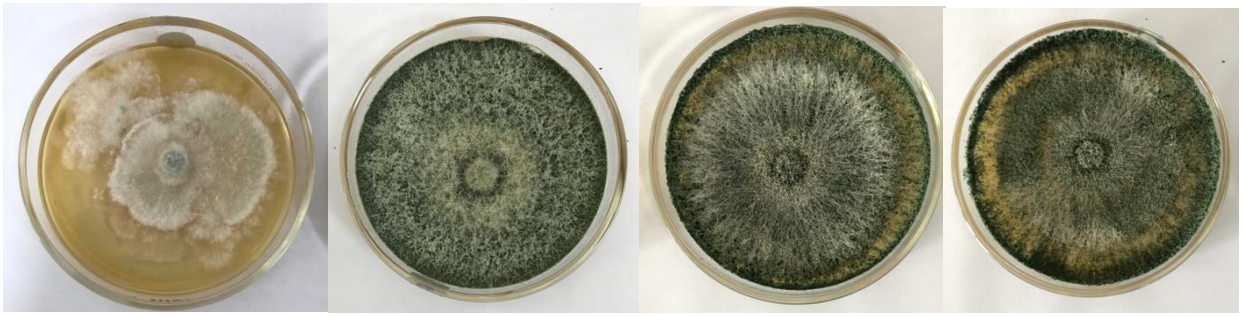
Statistical analysis

All the experiments were carried out in three replicates and data were statistically analyzed.

Results and Analysis

Cultural characteristics

Trichoderma harzianum: On PDA, all isolates of *T. harzianum* formed 1-2 concentric rings with green conidial production. The conidia production was denser in center then towards the margins. The isolate from NIPHM has slower growth rate (Figure 1). After 5 days, the mycelial growth measurements were 25 x 30, 35 x 35, 35 x 35, and 35 x 35 for Th-NIPHM, Th-Udaipur, Th-Raichur, and Th-Dharwad, respectively.



Th-Niphm Th-Udaipur Th-Raichur Th-Dharwad

Figure 1. Isolates of *Trichoderma harzianum*

Macrophomina phaseolina: Colonies were dark brown-greyish in color initially whitish on the PDA (Potato dextrose Agar) medium (Figure 2). Abundant aerial mycelium is found to be produced in the culture plate with sclerotia imbedded within the hyphae or engrossed in the agar.

a.

b.

c.

d.

Figure 2. Dry root rot symptoms of mungbean caused by *Macrophomina phaseolina* (a,b,c) and colonies on agar plate (d)

Morphological characteristics

Trichoderma harzianum: Conidia of *T. harzianum* (2.8 x 2.6 µm) were globose to sub globose in 7-days old culture. Conidia were light green color (Figure 3).

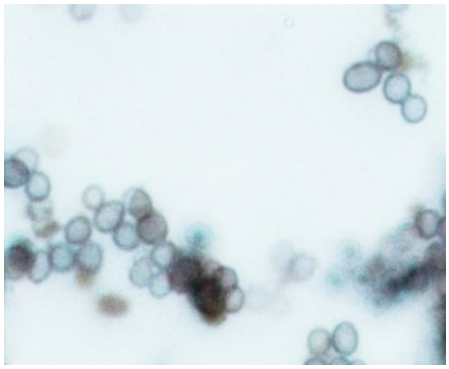
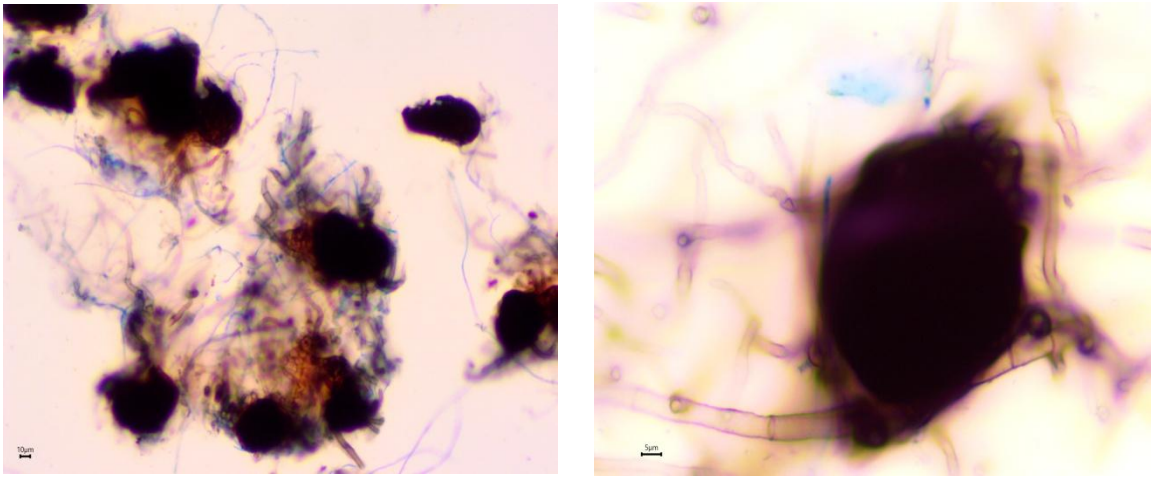


Figure 3. Conidia of *T. harzianum*

Macrophomina phaseolina: Observations were made under the camera attached microscope. The mycelium of fungal was hyline, septate, pycnidia black, globose, homogenous in size, conidia hyaline, cylindrical, 1-celled (Figure 4).



a. **b.**

Figure 4. Pycnidia (a) and a single pycnidium with mycelium (b) of *M. phaseolina*

Efficacy of *T. harzianum* isolates and fungicides against *M. phaseolina*

All isolates of *T. harzianum* had significant antagonistic effect on the mycelial growth of *M. phaseolina*. Among four isolates of *T. harzianum*, isolate Th-Raichur showed highest antagonistic activity by exhibiting 76.96% mycelial growth inhibition followed by the isolates Th-Dharwad, Th-NIPHM, while least antagonistic activity was reported for isolate Th-Udaipur (66.67%) (Figure 5a). Among the fungicides, carbendazim was most effective with 100%

mycelial inhibition than that of thiram (69%). Carbendazim was found to be more effective than tested *T. harzianum* isolates (Figure 5a). The antagonistic activity of each isolate is shown in Figure 5b. The efficacy of fungicides by poison food method is shown in figure 5c.

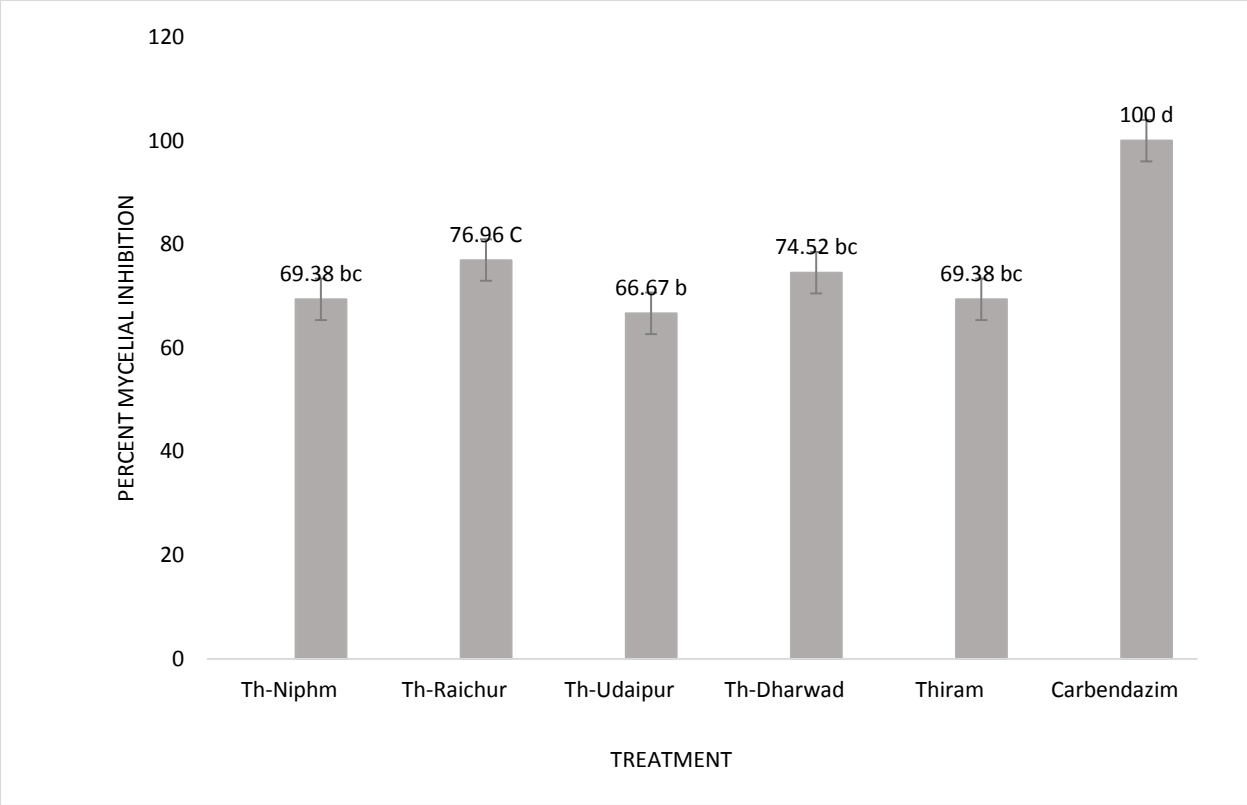
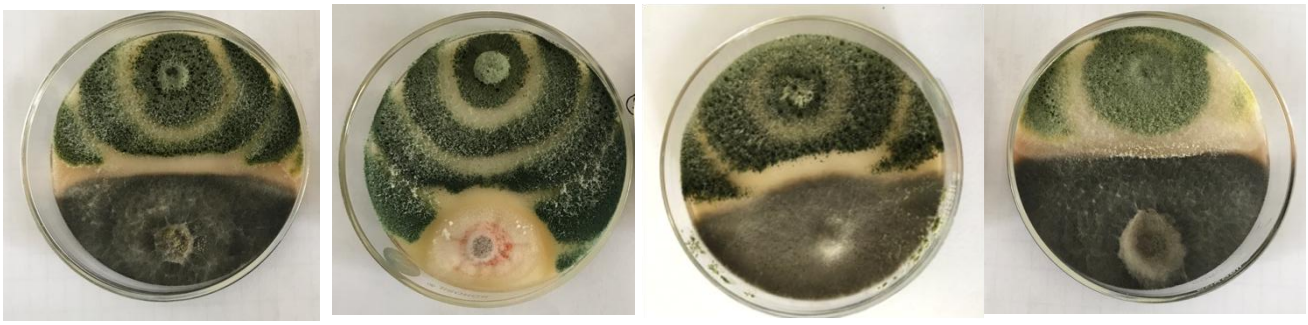
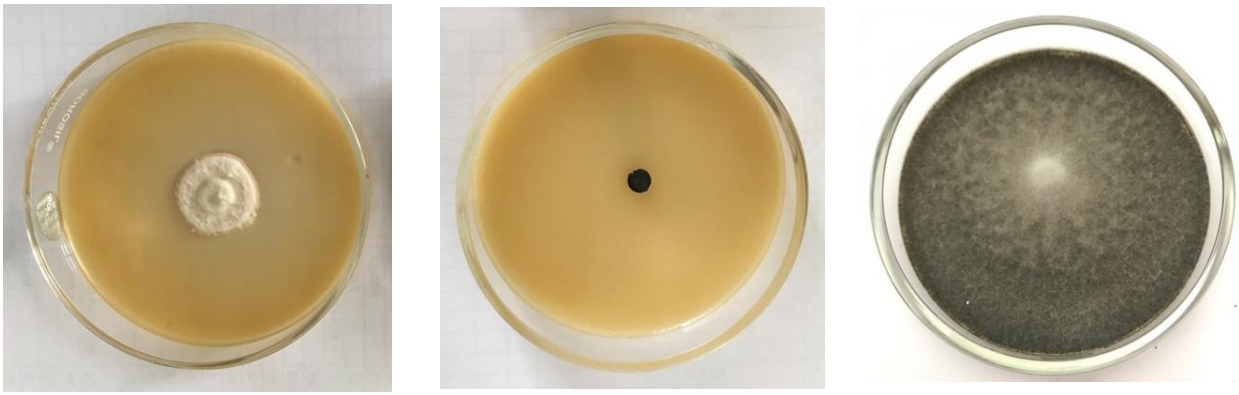


Figure 5a. Efficacy of *T. harzianum* isolates and fungicides against *M. phaseolina*. Values with the same letter are not significantly different at p<0.0001 (CV: 14.57570, F value: 111.71).



a. Th-Niphm **b.** Th-Raichur **c.** Th-Dharwad **d.** Th-Udaipur

Figure 5b. Antagonistic activity of *T. harzianum* isolates against *M. phaseolina* in dual culture method



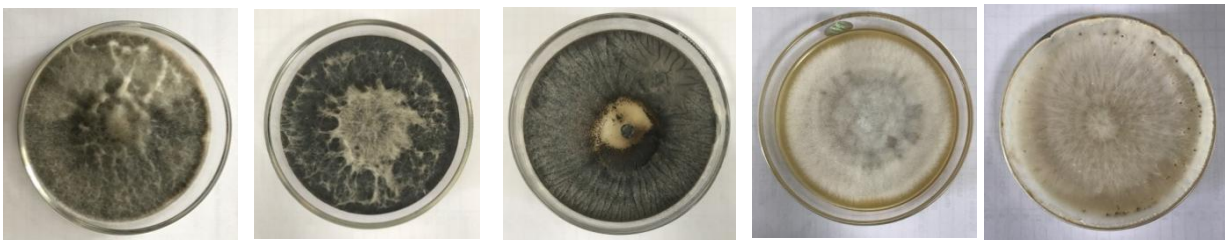
a. Thiram **b.** Carbendazim **c.** Control

Figure 5c. Efficacy of fungicides against *M. phaseolina* in poison food method.

Biological spectrum of isolate Th-Raichur against other root pathogens of vegetable legumes

Biological spectrum of isolate Th-Raichur was studied against *M. phaseolina* of mungbean (MPM), *M. phaseolina* of urdbean (MPU), *M. phaseolina* of soybean (MPS) causing dry root rot, *S. rolfsii* of urdbean (SRU) causing collar rot and *F. solani* of mungbean (FSM) causing root wilt. Culture of each pathogen is shown in figure 6a.

Isolate Th-Raichur showed a variable antagonistic activity when assessed by dual culture technique against root pathogens. Percent mycelial inhibition results are reported in figure 6b and antagonistic activity is shown in figure 6c. It is evident from the figure 6b that, Th-Raichur was most effective against *M. phaseolina* of mungbean exhibited 76.44% mycelial inhibition followed by *F. solani* (66.92%), while least effective against Macrophomina of soybean (50.12%). Additionally, 55-56% mycelial growth inhibition was reported against *M. phaseolina* and *S. rolfsii* of urdbean. Thus, it can be only used for the management of dry root rot and fusarium rot of mungbean.



a. MPM **b.** MPU **c.** MPS **d.** FSM **e.** SRU

Figure 6a. Root pathogens of vegetable legumes

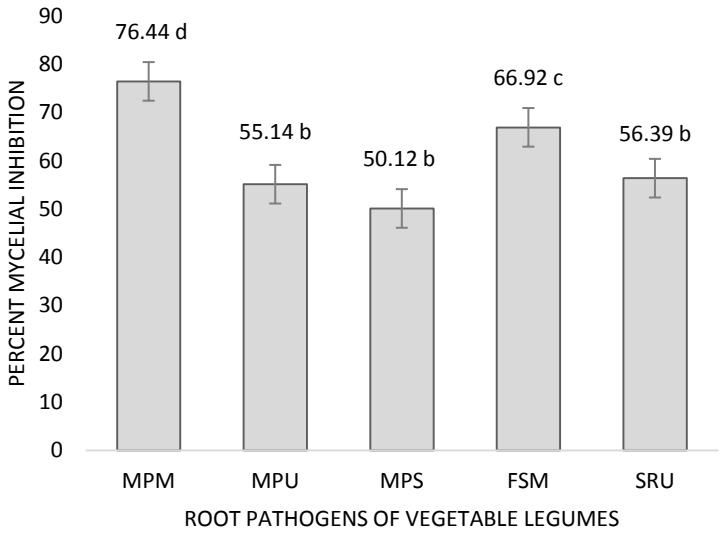
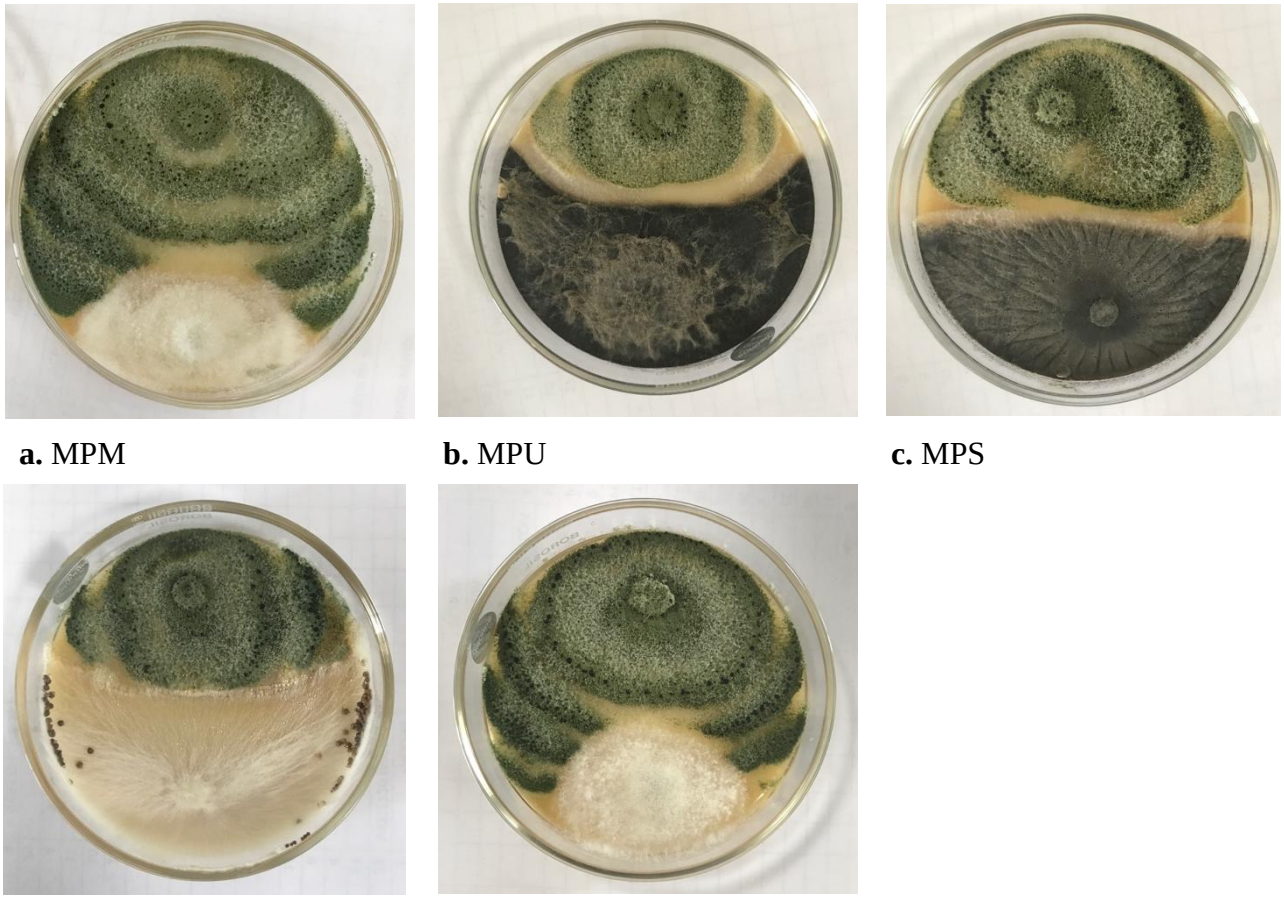


Figure 6b. Antagonistic activity of isolate Th-Raichur against different root pathogens. Values with the same letter are not significantly different at p<0.0001 (CV: 5.693919, F value: 207.55).



a. MPM **b.** MPU **c.** MPS

d. SRU **e.** FSM

Figure 6c. Dual culture study of isolate Th-Raichur against different root pathogens

Conclusion

In this study, efficacy of *Trichoderma harzianum* isolates was investigated against dry root rot pathogen of mungbean by dual culture method. Cultural study of the four isolates of *T. harzianum* revealed that isolate procured from NIPHM had slow growth rate than that of other three isolates. Screening results showed that among the *T. harzianum* isolates, isolate Th-Raichur was most effective: exhibiting 76.96% mycelial growth inhibition of the *M. phaseolina*. This indicates Th-Raichur is effective in the management of dry root rot in mungbean. Although this is great discovery, it's important to consider its efficacy against a variety of root pathogens associated with mungbean and other vegetable legumes. Biological spectrum of Th-Raichur against other root pathogens namely, *M. phaseolina* of urdbean (MPU) and soybean (MPS) causing dry root rot, *S. rolfsii* of urdbean (SRU) causing collar rot and *F. solani* of mungbean (FSM) causing root rot by dual culture technique showed that isolate was effective against *F. solani* of mungbean. The remaining root pathogens were not significantly inhibited by Th-Raichur isolate. These results indicate the Th-Raichur isolate was effective against root rot in mungbean, but it lacked the diverse resistance to various root pathogens. Further studies are required in search of *T. harzianum* isolates having a broad range of antagonistic activity against root pathogens.

The Experience

Personal Growth and Experience

My time at World Vegetable Center South Asia has proved to be my most life-changing experience. In addition to the research I conducted, the people I've met and the cultural experiences I've gained also played important roles in the overall impact of my internship.

During the duration of my internship, the main sites I visited include Chilkur Balaji Temple, Golconda Fort, Charminar, Chowmahalla Palace, and Kasu Brahmananda Reddy National Park. Although these are mostly tourist destinations, I was still able to look beyond the scenic beauty and find true Indian culture. At Golconda Fort, women were painting the stone stairs as part of a ritual they practice before their religious holiday Eid al-Fitr. At Chilkur Balaji Temple, individuals and families pray to their god for a specific wish and walk eleven laps around the temple. Once their wish is fulfilled, they must return and walk one-hundred and eight laps. Although these destinations had many beautiful sights, it's important to understand these sights are not common throughout India.

Though I enjoyed visiting various tourist destinations, I found that I liked having conversations with local community members more. I was surprised by their openness and willingness to discuss multiple topics. They always answered any questions I had ranging from farming techniques to the concept of marriage in India. Every day I was amazed by the genuine attitude and politeness possessed by everyone I met. I have never encountered a more courteous and gracious group of people. I felt welcomed whenever and wherever I was. Their demeanor inspires me to become a better person and increases my aspiration to continue my education so one day I'm able to return with helpful, innovative ideas.

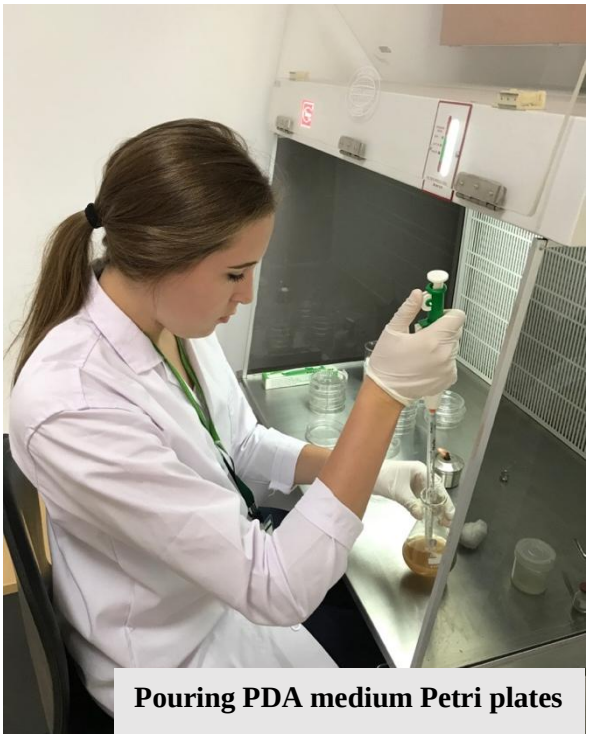
Through my experiences, I have gained so much respect for the values and beliefs held by local people. I believe this understanding is essential when trying to advance and improve current practices. It's illogical to believe locals will immediately change the practices they've been using for decades. It will take time and gradual acceptance for continued development.

The experience of working in a plant pathology lab and studying various techniques used to culture microorganisms has made me consider this type of work as a potential future career. Plant pathology is so complex, but the amount of information I learned in that short amount of time has made me want to learn more. The aspect of lab research and searching for the best isolate of fungi was very intriguing to me. During my time at World Vegetable Center South Asia, my supervisor Dr. Ram, legume breeder, was kind enough to give me weekly lessons on the basics of plant breeding. These weekly teachings allowed my interest in plant breeding and genetics to grow. I am very thankful this internship was able to help confirm my interests in lab research and international agriculture. It has influenced by decision to continue my education at Iowa State University with a double major in agronomy and global resource systems. In the future, I plan to attend graduate school to receive a PhD in plant breeding.

Photographs

Preparing microscopic slide of *M. phaseolina*

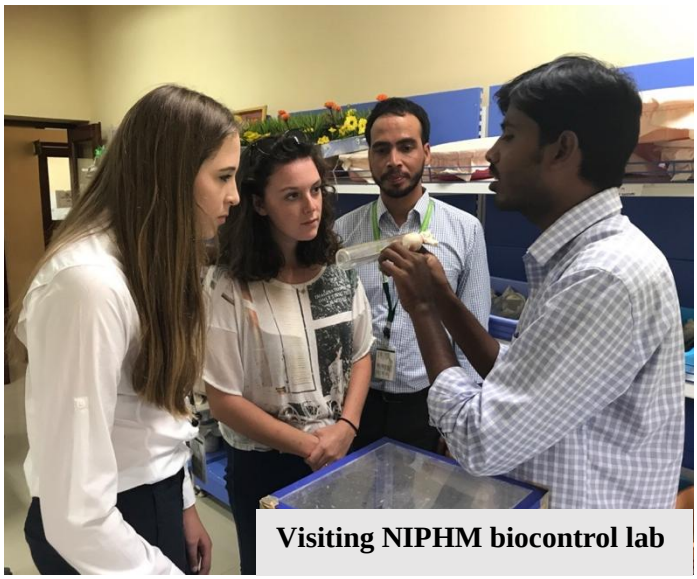
Transplanting seedlings from glasshouse to field



Pouring PDA medium Petri plates

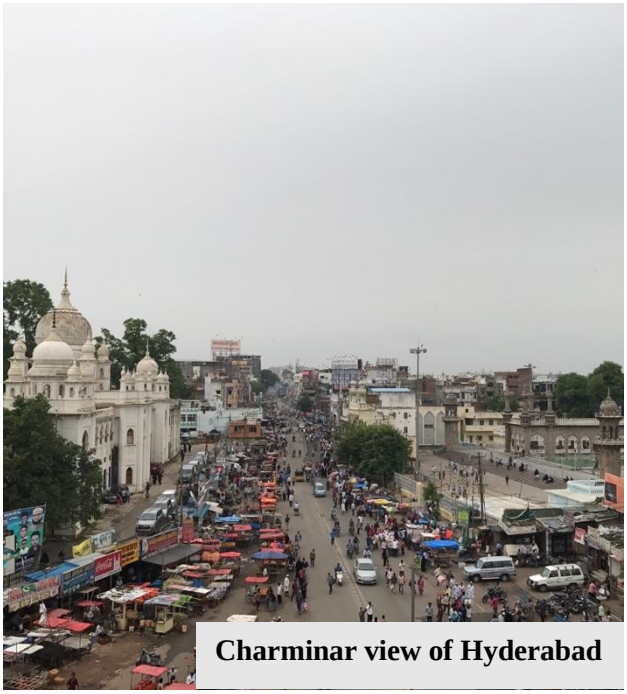
Emasculating mungbean bud

Visiting ICRISAT Genebank



Visiting NIPHM biocontrol lab

Chowmahalla Palace



Charminar view of Hyderabad

References

Choudhary S, Choudhary AK., Sharma OP. 2011. Screening of mungbean (*Vigna radiata*) genotypes to identify source of Resistant to dry root rot. Journal of Food Legumes 24: 117-119.

Dennis C, Webster J (1971) Antagonistic properties of species-groups of *Trichoderma* II: Production of volatile antibiotics. Trans British Mycol Soc 84: 41-48.

Deshmukh MA, Gade RM, Belkar Y.K. and Koche M.D. 2016. Efficacy of bioagents, biofertilizers and soil amendments to manage root rot in greengram. Legume Research 39: 140-144.

Dhingra OD, Sinclair JB (1978). Biology and pathology of *Macrophomina phaseolina*. Imprensa Universitaria, Universidade Federal De Vicosa, Vicosa- 36.570 Minas Gerais-Brazil, p.166.

Ebenezar, E. G., & Yesuraja, I. (2000). Effect of fungal and bacterial antagonists on *Macrophomina* phaseolina (Tassi. Goid) causing root rot in green gram. Journal of Tropical Agriculture 38(1/2): 73-76.

Gams, W., and Bisset, J. (1998). Morphology and identification of *Trichoderma*. Pages 3-34 in: *Trichoderma* & Gliocladium. Vol. 1. G. E. Harman and C. P. Kubicek, eds. Taylor and Francis, London.

Grover RK, Moore JD (1962) Toximetric studies of fungicides against brown rot organism *Sclerotinia fructicola* and *S. laxa*. Phytopathology 52:876–880.

Hagedorn DJ. (1991). Ashy stem blight. In: Hall R. (ed.) Compendium of Bean Diseases. St. Paul, MN, USA, APS Press, pp 70.

Iqbal U. and Mukhtar T. (2014). Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiata* L.) Wilczek from Pakistan. The Scientific World Journal. Article ID 950175, 1-9.

Kaushik CD, Chand JN, Saryavir (1987). Seedborne nature of *Rhizoctonia bataticola* causing leaf blight of mungbean. Indian J Mycol Plant Pathol 17: 154-157.

Khan KSH, Shuaib M., Shuaib M. (2007). Identification of sources of Resistant in mungbean (*Vigna radiata* L.) against charcoal rot *Macrophomina phaseolina* (Tassi) Goid. African Crop Science Conference Proceedings 8: 2101-2102.

Khan, Salik Nawaz. (2007). *Macrophomina phaseolina* as causal agent for charcoal rot of sunflower. Mycopath 5(2): 111-118.

Kumari R., Shekhawati KS, Gupta R and Khokhar MK (2012). Integrated management against root rot of mungbean (*Vigna radiata* L. Wilczek) incited by *Macrophomina phaseolina.* Journal of Plant Pathology and Microbiology 3: 1-5.

Kumari Rekha, Shekhawat KS, Gupta Renu, and Khokhar MK. (2012). Integrated Management against Root-rot of Mungbean [*Vigna radiata* (L.) Wilczek] incited by *Macrophomina phaseolina*. J Plant Pathol. Microb. 3: 136.

M. Bashir and B. A. Malik. (1988). Diseases of major pulse crops in Pakistan—a review. Tropical Pest Management 34(3): 309–314.

Thilagavathi Rasu, Saravanakumar Duraisamy, Ragupathi Nachimuthu, and Samiyappan Ramasamy. (2007). A Combination of Biocontrol Agents Improves the Management of Dry Root Rot (*Macrophomina phaseolina)* in Greengram. Phytopathol. Mediterr. 46: 157-167.

Tiwari, A.K. and Shivhare, A.K. (2016). Pulses in India: Retrospect and Prospects. Directorate of Pulses Development 2: 60-68.