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Biocontrol activity of *Trichoderma viride, Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* (*in vitro*) against *Bipolaris oryzae*, causal agent of rice brown spot disease

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ABSTRACT: Rice is one of the crops that farmers cultivate on a regular basis, and it is a essential cereal grain for everyone. Diseases are one of the major causes which hampered the plant produce. Rice brown spot is a devastating disease of rice which is caused by *Bipolaris oryzae* and accounts for huge yield losses, despite the fact there are limited management practices available. Biocontrol agents offered environment friendly management of diseases and they are currently preferred over the usage of chemicals. In this view, dual culture technique was employed with biocontrol agents (*Trichoderma viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis*) against *Bipolaris oryzae*. *In vitro* studies indicated that of all the biocontrol agents utilised in this investigation, *Trichoderma viride* was shown to be the most antagonistic. *Trichoderma viride* (61.95%) was the most effective at inhibiting mycelial growth, followed by *Trichoderma harzianum* (59.78%). Among the bacterial biological control, *Bacillus subtilis* had the highest percent inhibition (45.52%), followed by *Pseudomonas fluorescence* (39.05%). This study may stimulate other researchers to evaluate different biocontrol agents against *Bipolaris oryzae* in order to better manage the disease.

Keywords: Brown spot, *Biopolaris oryzae*, Biocontrol agents, *in vitro.*

INTRODUCTION

Rice (*Oryza sativa* L.) is the most widely cultivated crop in the world, and it is a staple cereal food for millions of people. *Bipolaris oryzae* (teleomorph=*Cochliobolus miyabeanus*) causes Brown spot of rice and considered as one of the most important disease of rice throughout the world (Khalili *et al*., 2012; Reddy *et al*., 2010; Savary *et al*., 2000). It is responsible for a drop of 26 to 52 percent grain yield (Chakrabarti, 2001). In India, the catastrophic Bengal famine of 1942-43 was the result of this disease which accounts for 50-90 per cent yield loss and death of over 4 million people (Chakrabarti 2001; Padmanabhan, 1973). *Bipolaris oryzae* causes symptoms of dark brown spots, elongated to circular in shape which may reach upto 1 cm or more in length. Leaf sheaths and coleoptiles exhibit similar symptoms. The pathogen infects inflorescence which results in failure of grain development and establishes a major problem during the commercial production of rice. Chemicals are often applied for the management of brown spot disease but their use is known to have negative consequences in environment. They are creating vulnerable conditions for beneficial soil microorganisms. Besides this, they increased plant protection costs which directly affect farmer's livelihood. Treatments with fungicides are also inconsistent due to the complexity of soil ecology and year–to–year variations in climate conditions.

Biocontrol agents are now gaining popularity among phytopathologists as a new alternative for the control of various plant diseases. Biocontrol agents are safe, long lasting and ecofriendly. The fungal and bacterial antagonists have been shown to promote the growth of various crops (Singh *et al*., 2012). Biocontrol agents could be less harmful to the environment than synthetic fungicides. *Bacillus, Pseudomonas* and *Trichoderma* were the most commonly employed fungal and bacterial biocontrol agents against diverse plant diseases (Nakkeeran *et al*., 2005; Saravanakumar *et al*., 2007). Mycoparasitism and antagonism are the two main weapon used by *Trichoderma* spp. for the control of fungal disease (Singh *et al*., 2005) Several *Trichoderma* species have been demonstrated to have antagonistic activity against wide range of pathogens (Dutta and Das, 2002; Das *et al*., 2006; Dutta *et al*., 1999; Dutta *et al*., 2008) Moreover, *Trichoderma viride* (*T. viride*) and *Trichoderma harzianum* (*T. harzianum*)

are two well known widely used *Trichoderma* species. Biswas *et al*. (2010) found that application of *Trichoderma viride* or *T. harzianum* reduced the disease upto 70%. Bacterial biocontrol agents have been extensively utilized in plant disease management because it poses a range of biological control mechanism against different pathogens. They are associated with secondary metabolites production in agroecosystem and act as a potential antagonist against number of fungal pathogens. Biological control agents such as *Pseudomonas fluorescens* and *Bacillus subtillis* are commonly utilised. In previous studies mixture of *Pseudomonas fluorescens* and *Bacillus subtillis* are found effective against bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *Malvacearum* (Salaheddin *et al*., 2010). Talc based formulation of *Pseudomonas fluorescens* have been reported to suppress *Bipolaris oryzae* (Joshi *et al*., 2007). In future, there will be transition of disease management strategies from chemicals to biocontrol agents, necessitating the study of different antagonists as next-generation alternatives. Therefore, the efficiency of two fungal biocontrol agents, *Trichoderma viride* and *Trichoderma harzianum*, as well as two bacterial antagonists, *Pseudomonas fluorescens* and *Bacillus subtillis*, in suppressing rice brown spot disease was investigated under *in vitro* condition.

MATERIALS AND METHODS

A. Isolation of pathogen

Isolates of *Bipolaris oryzae* were collected from diseased rice crops at the Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. The leaves with typical brown spot symptoms induced by *Bipolaris oryzae* were cut with a sharp and sterilised blade, placed in paper bags, and brought to the laboratory. The necrotic patches of diseased leave along with some healthy tissue were cut into small pieces inside the laminar air flow and surfaces sterilized by dipping in 1 percent sodium hypochlorite for 20-30 seconds and were washed by sterilized water for three times. The cut pieces were plated in petri plate containing sterilize potato dextrose agar medium (PDA) (Riker and Riker, 1936) and kept in BOD incubator at 25±2°C for 3-4 days so that the fungi get ideal condition for growth. Purified culture were maintained in PDA slants and stored at 4°C.

B. In Vitro evaluation of biocontrol agents against Bipolaris oryzae

Efficacy of local biocontrol agent *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested on Potato Dextrose Agar against *B. oryzae* by dual culture technique. The local strain of biocontrol agents was collected from Department of Plant Pathology, B.C.K.V. Dual culture technique was carried out by picking a 5 mm disc from actively growing mycelium of *Bipolaris oryzae* with the help of sterilized cork borer and placed at one side of the Petri plate. After this, a 5 mm disc of fungal bioccontrol agents (*Trichoderma harzianum* or *T. viride*) was inoculated at opposite side of the previously inoculated test pathogen in the same plate (Dennis and Webster, 1971). Similarly, for bacterial biocontrol agent, *Bacillus subtilis* and *Pseudomonas fluorescens*, a 5 mm diameter disc was taken from 7 days old culture of *B. oryzae* and placed on one end of the Petri dish. Afterwards, bacterial antagonists were streaked with the help of inoculation needle on the opposite side of inoculated *B. oryzae*. Control plates were also maintained in PDA plates. These plates were incubated at 28 ±1°C temperature for 5 days. Completely Randomized Design (CRD) with three replications was taken to validate the result and the percentage inhibition of pathogen mycelial growth was calculated using the formula below (Vincent, 1927).

$$\text{Inhibition\%} = \frac{\text{Radial growth in control (mm)} - \text{Radial growth in treatment (mm)}}{\text{Radial growth in control (mm)}} \times 100$$

RESULT AND DISCUSSION

A. In vitro evaluation of fungal bio-control agents against Bipolaris oryzae causing brown spot of rice

Trichoderma are mostly found in rhizosphere of plant. They are excellent candidate of biocontrol agents and effectives against seed and soil borne fungus, root rot, stem rot, wilt, and blight and also to some extent control nematodes. The unique nature of *Trichoderma*, being non-pathogenic to plants is because of its ability to produce a number of substances that cause localized or systemic resistance responses in plants. They produce antibiotics and cell wall degrading enzyme like chitinases, β-1, 3-Glucanases etc. *Trichoderma* are known for their ability to parasitize other fungi, a process which is called as mycoparasitism and cause lysis of the pathogens (Majumdar *et al*., 1996).

In this study, both *Trichoderma* species significantly reduced the mycelial growth of the test pathogen, *Bipolaris oryzae*. *Trichoderma viride* was found to be the most antagonistic biocontrol agent of all the biocontrol agents used in this study (Table 1). It shows highest inhibition in radial growth of the pathogen (61.95%) followed by *T. harzianum* (59.78%) (Fig. 1 and 2). This result is in alignment with the earlier experiment conducted by Harish *et al*., (2008) in which *Trichoderma viride* (Tv2) was found to be significantly effective in inhibiting the pathogen's mycelial growth (62.92%) and spore germination (77.03%), followed by *Trichoderma harzianum* (Th5) and *Trichoderma reesei*

(Tr3). Similar result was obtained by Manimegalai *et al*. (2011) which showed an inhibition of 67.9 % with *T. viride*. *Bipolaris oryzae* is effectively suppressed (upto 61.72%) by *T. viride* (Kumar *et al*., 2016). Another species of *Trichoderma*, *T. harzianum* render significant inhibition (48%) against *Bipolaris oryzae* (Abdel-Fattah *et al.*, 2007). Singh *et al*. (2021) demonstrated that when *T. harzianum* was exposed to dual culture bioassays, it inhibited the growth of *F. oxysporum* (66%). *Trichoderma asperellum* showed potential in managing a range of fungal diseases and their antagonistic nature aid in enhancing medicinal plant productivity and quality. *Trichoderma* are opportunistic and avirulent plant symbionts capable of establishing strong colonization with root surfaces (Harman *et al*., 2004). The plant proteome and metabolism are significantly altered as a result of these root microbe interactions. Further, it was very well studied that *Trichoderma* developed over the pathogen, causing hyphal coiling, hyphal abnormalities and hyphae lysis (Malathi, 1996). The potential of *T. viride* on *B. oryzae* is primarily due to the production of lytic metabolites and through mechanism of mycoparasitism.

Table 1: Evaluation of fungal bio-control agents against *Bipolaris oryzae* causing brown spot of rice.

Bio-control agent	*Bipolaris oryzae*	
	Radial growth of myceliuum (mm)	Per cent inhibition (%)
Trichoderma viride	17.500	61.957
Trichoderma harzianum	18.500	59.783
Control	46.000	0.000
C.D.(0.05)	0.831	1.808
SE (m)	0.236	0.512

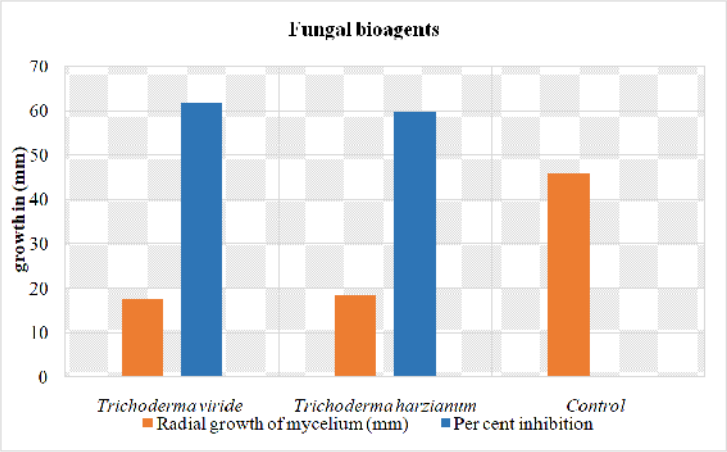

Fig. 1. *In vitro* effect of *Trichoderma viride* and *Trichoderma harzianum* on per cent growth inhibition of *Bipolaris oryzae*.

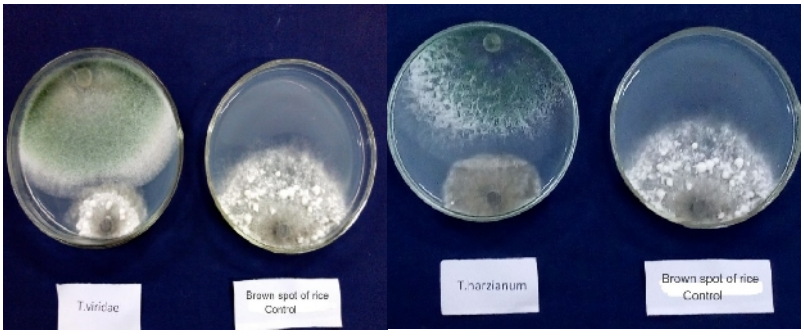

Fig. 2. In vitro effect of *Trichoderma viride* and *Trichoderma harzianum* on radial growth of of *Bipolaris oryzae*.

B. Evaluation of bacterial bio-control agents against brown spot of rice

Bacillus species posses a wide range of antimicrobial activities and they are used as anti-fungal agents (Milner *et al*., 1995). *Bacillus subtilis* has a profound antagonistic relationship with *B. oryzae* (Sarala *et al*., 2004). The data showed in the Table 2 revealed the antagonistic effect of selected bacterial biocontrol agents against *Bipolaris oryzae*. Both the pathogens cause reduction in radial growth of the pathogen. The percent inhibition over control ranges from 45.52% and 39.05%. Maximum percent inhibition

was observed by *Bacillus subtilis* (45.522%) followed by *Pseudomonas fluorescens* (39.05%) (Fig. 2 and 3). In a study it was found that *Bacillus amyloliquefaciens* (BS5) exerted mycelial growth inhibition (76.66%) against *Bipolaris oryzae* (Prabhukarthikeyan *et al*., 2019). *Bacillus species* (*B. subtilis, B. polymyxa, B. brevis, B. licheniformis, B. circulans, B. cereus* etc) are active producer of antibiotics (Yilmaza *et al.*, 2006). In previous studies, *P. fluorescens* was found to be antagonistic to *B. oryzae*, with the highest percent inhibition (75.22%), followed by *S. marcescens* (72.78%) and *B. subtilis* (70.56 %) (Sanjeevkumar *et al*., 2016). Alagesaboopathi and Selvankumar (2011) showed antagonistic effect of *P. fluorescens* against a wide range of fungal pathogen including *Bipolaris oryzae, Rhizoctonia solani, Fusarium oxysporum, Alternaria brassica, Cochliobolus lunatus, Aspergillus niger* and *Trichothecium reseum*. It was found that seed treatment with *Pseudomonas* spp. has lessen the disease severity upto 70% or more in brown spot (Joshi *et al*., 2007; Ludwig *et al*., 2009). *Pseudomonas* are potent producer of antibiotics such as 2, 4-DAPG, pyrrolnitrin and phenazine which enables it to work as a strong antagonistic agent.

Table 2: Evaluation of bacterial bio-control agents against *Bipolaris oryzae* causing brown spot of rice.

Bio-control agent	*Bipolaris oryzae*	
	Radial growth of mycelium (mm)	Percent inhibition (%)
Bacillus subtilis	30.500	45.522
Pseudomonas fluorescence	26.167	39.055
Control	67.000	0.000
C.D.(0.05)	0.679	1.013
SE(m)	0.192	0.287

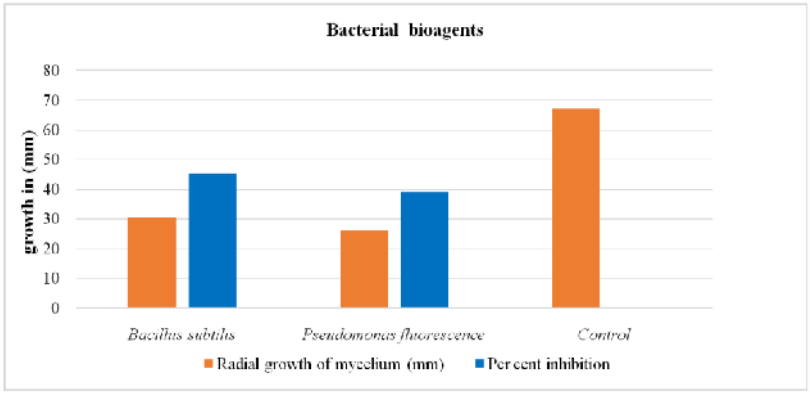

Fig. 3. *In vitro* effect of *Bacillus subtillis* and *Pseudomonas fluoruescens* and on per cent growth inhibition of *Bipolaris oryzae.*

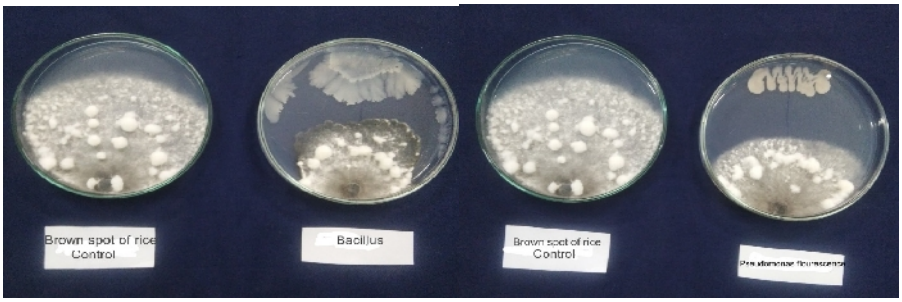

Fig. 4. *In vitro* effect of *Bacillus subtillis* and *Pseudomonas fluoruescens* on radial growth of *Bipolaris oryzae.*

CONCLUSION

In the present study, among fungal biocontrol agent, *Trichoderma viride* perform exceptionally well in controlling the brown spot of rice under in vitro condition. Microorganisms are naturally active in suppressing pathogens by micoparasitism, antibiosis, or the formation of pathogen-inhibiting volatile chemicals. According to the findings of this study, *Bacillus subtilis* emerged as a promising candidate for suppressing rice brown spot in vitro. The result concludes that indigenous species of *Trichoderma* and *Bacillus* strain has a great possibility of becoming a viable antagonist against *Bipoaris oryzae*. Biocontrol agents can be our new pillar in disease management strategies and their

mechanism of action, compatibility with other components of integrated disease management and their efficiency in field are all key topics that need to be thoroughly researched. These findings could pave the way for the agriculture industry to adopt more ecologically friendly farming practices.

FUTURE SCOPE

Biological control has long been an important part of plant disease management because of the numerous benefits it provides to both the plant and the environment. There are millions of evidences which showed the potential of biocontrol agents to be used in disease management strategies and therefore different strain of biocontrol can be tested against a range of pathogen for effective management of plant diseases. Biocontrol is mostly used to combat plant diseases since it has antifungal and antimicrobial capabilities. Gene expression analysis should be monitor in biocontrol host-pathogens system to know the behavior of biocontrol agents to limit pathogenic growth at the molecular level. The biocontrol agents should be monitor under different field conditions. Furthermore, future research should targetted on developing various bioformulations and integrating them with carriers in order to improve their stability and efficiency in the field. In the end, more information about biocontrol agents would be revealed, allowing for more effective disease management while avoiding harm to other biosystems. This research will aid in the performance of practical tests and the commercialization of biocontrol as a protective agent.

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Conflict of Interest. None

REFERENCES

Abdel-Fattah, G. M., Shabana, Y. M., Ismail, A. E. and Rashad, Y. M. (2007). *Trichoderma harzianum*: a biocontrol agent against *Bipolaris oryzae*. *Mycopathologia*, *164*, 81–89.

Alagesaboopathi, C. and Selvankumar, T. (2011). Antagonistic activities of *Pseudomonas fluorescens* and strain improvement of *Rhizobium* species. *International Journal of Biosciences*, *1*(6): 54-63.

Biswas, C., Srivastava, S. S. L. and Biswas, S. K. (2010). Effect of biotic, abiotic and botanical inducers on crop growth and severity of brown spot in rice. *Indian Phytopathology*, *63*, 187–191.

Chakrabarti, N. K. (2001). Epidemiology and disease management of brown spot of rice in India. In: *Major Fungal Disease of Rice: Recent Advances* (pp. 293-306). Kluwer Academic Publishers.

Das, B. K., Das, B. C., Dutta, P., and Sarmah, D. K. (2006). Bioformulation of *Trichoderma harzianum* Rifai for management of stem rot of soybean caused by *Rhizoctonia solani* Kuhn. *Journal of Biological Control*, *20*(1): 57-64.

Dennis, C. and Webster, J. (1971). Antagonism properties of species groups of *Trichoderma* III, Hyphal interaction. *Transactions British Mycological Society*, *57*(3): 363-369.

Dutta, P. and Das, B. C. (1999). Effect of seed pelleting and soil application of *Trichoderma harzianum* in the management of stem rot of soybean. *Journal of Mycology and Plant Pathology*, *29*(3): 317-322.

Dutta, P. and Das, B. C. (2002). Management of collar rot of tomato by *Trichoderma* sp. and chemicals. *Indian Phytopathology*, *55*(2): 235-237.

Dutta, P., Das, B. C. and Islam, M. (2008). Eco- friendly strategies of management of *Sclerotinia* rot of French bean. *Journal of Biological Control*, *22*(2): 405-410.

Harish, S., Duraiswamy, S., Ramalingam, R., Ebenezar, E.G. and Seetharaman, K. (2008). Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. *Biocontrol*, *53*, 555-567.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. (2004). *Trichoderma* species: opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, *2*, 43–56.

Joshi, N., Brar, K.S., Pannu, P.P.S. and Singh, P. (2007). Field efficacy of fungal and bacterial antagonists against brown spot of rice. *Journal of Biological Control*, *21*, 159-162.

Khalili, E., Sadravi, M., Naeimi, S. and Khosravi, V. (2012). Biological control of rice brown spot with native isolates of three *Trichoderma* species. *Brazilian Journal of Microbiology*, *43*, 297-305.

Kumar, H., Ahmad, S. and Zacharia, S. (2016). Efficacy of fungal, bacterial bioagents and botanicals against brown spot (*Helminthosporium oryzae*) of rice (*Oryza sativa* L.) *New Agriculturist*, *27*(2): 343–348.

Ludwig, J., Moura, A. B., dos Santos, A. S. and Ribeiro, A.S. (2009). Seed microbiolization for the control of rice brown spot and leaf scald. *Tropical Plant Pathology*, *34*(5): 322-328.

Majumdar, V. L., Jat, J. R. and Gour, H. N. (1996). Effects of biocontrol agents on the growth of *Macrophomina phaseolina* the incitant of blight of moth bean. *Indian journal of Mycology and plant Pathology*, *26*(2): 202–203.

Malathi, P. (1996). Biocontrol of groundnut (*Arachis hypogaea* L.) dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid. Ph.D Thesis Tamil Nadu Agricultural University, Coimbatore.

Manimegalai, V., Ambikapathy, V. and Panneerselvam, A. (2011). Biological control of paddy brown spot caused by *Bipolaris oryzae* (Breda de Haan). *European Journal of Experimental Biology*, *1*(4): 24-28.

Milner, J. L., Raffel, S.J., Lethbridge, B. J. and Handelsman, J. (1995). Culture conditions that influence accumulation of zwittermicin A by *Bacillus cereus* UW85. *Applied Microbiology and Biotechnology*, *43*,685–691

Nakkeeran, S., Fernando, W. G. D. and Siddiqui, Z.A. (2005). Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. In: Siddiqui Z.A. (eds), *PGPR: Biocontrol and Biofertilization* (pp. 257-296). Springer, Dordrecht.

Padmanabhan, S. Y. (1973). The great Bengal Famine. *Annual Review of Phytopathology*, *11*, 11-24.

Prabhukarthikeyana, S. R., Yadav, M. K., Anandan, A., Aravindan, S., Keerthana, U., Raghua, S., Baitea, M. S., Parameswaran, C., Panneerselvam, P. & Ratha, P. C. (2019). Bio-protection of brown spot disease of rice and insight into the molecular basis of interaction between *Oryza sativa*, *Bipolaris oryzae* and *Bacillus amyloliquefaciens. Biolgical control*, *137*, 104018.

Reddy, C. S., Laha, G. S., Prasad, M. S., Krishnaveni, D., Castilla, N. P., Nelson, A. and Savary S. (2010). Characterizing multiple linkages between individual diseases, crop health syndromes, germplasm deployment and rice production situations in India. *Field Crops Research*, *120*(2): 241–253.

Riker, A. J. and Riker, Eegina S. (1936). *Introduction to research on plant diseases. A guide to the principles and practice for studying various plant-disease problems*, pp.117.

Salaheddin, K., Valluvaparidasan, V., Ladhalakshmi, D. and Velazhahan, R. (2010). Management of bacterial blight of cotton using a mixture of *Pseudomonas fluorescens* and *Bacillus subtilis. Plant Protection Science*, *46*, 41-50.

Sanjeevkumar, K., Sivakumar, T. and Balabaskar, P. (2016). Efficacy of combination of delivery systems with *Bacillus subtilis* on the Incidence of Damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. and growth and yield of tomato. *Bulletin of Environment, Pharmacology and Life Sciences*, (Spl. Issue 2): 100-105.

Saravanakumar, D., Harish, S., Loganathan, M., Vivekananthan, R., Rajendran, L., Raguchander, T. and Samiyappan, R. (2007). Rhizobacterial bioformulation for the effective management of *Macrophomina* root rot in mungbean. *Archives of Phytopathology and Plant Protection*, *40*(5): 323-337.

Savary, S., Willocquet, L., Elazegui, F. A., Teng, P. S., Du, P. V. and Zhu, D. (2000). Rice pest constraints in tropical Asia: characterization of injury profiles in relation to production situations. *Plant Disease*, *84*, 341–356.

Singh, D., Yadav, D. K., Sinha, S. and Upadhyay, B. K. (2012). Utilization of plant growth promoting *Bacillus subtilis* isolates for the management of bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* race 1 biovar 3. *Indian Phytopathology*, *65*, 18-24.

Singh, R. K., Singh, C. V. and Shukla, V. D. (2005). Phosphorus nutrition reduces brown spot incidence in rainfed upland rice. *International Rice Research Notes*, *30*(2): 31–32.

Singh, S., Balodi, R., Meena, P.N. and Singhal, S. (2021). Biocontrol activity of *Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas fuorescens* against *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani. Indian Phytopathology*, *74*: 703–714.

Vincent, J. M. (1927). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, *59*, 850.

Yilmaza, M., Sorana, H. and Beyatli, Y. (2006). Antimicrobial activities of some *Bacillus* spp. strains isolated from the soil. *Microbiological Research*, *161*(2): 127-131.

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