Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 8 [7] June 2019 : 27-31

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Bioefficacy of Bioagents against Pathogenic Mycoflora of Soybean Seeds

S. S. Dhawan, S. J. Magar, M. D. Navale and H. N. Markad
Department of Plant pathology, College of Agriculture, Latur- 413512 (MS), India
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parabhani, Maharashtra, India
Email- shitaldhawanpatil1@gmail.com

ABSTRACT
Soybean (Glycine max L. Merrill.) crop is affected by number of pathogenic fungi, which are mostly seed borne, causing significant qualitative and quantitative losses. Though, majority of seed borne fungi are being managed with fungicidal seed treatments, but due to their harmful effects, their use needs to be reduced. Therefore, present in vitro study was conducted to asses bioefficacy of eight bioagents against three major pathogenic seed borne fungi viz., Fusarium verticillioides, Macrophomina phaseolina and Alternaria alternata, by applying Dual Culture Technique. Three separate experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. The results revealed that all of the eight test bioagents significantly inhibited mycelial growth of Fusarium verticillioides, Macrophomina phaseolina and Alternaria alternata, over untreated control. However, in case of F. verticillioides significantly highest mycelial growth inhibition was achieved with T.virens (85.93%), followed by, A. niger (84.63%), T. hamatum (66.85%), T. asperellum (63.52%), T. harzianum (62.77%), and T. koningii (52.96%). P. fluorescens (45.00%) was found least effective. Whereas, in M. phaseolina, significantly highest mycelial growth inhibition was resulted with T. hamatum (83.71%), followed by, T. virens (83.33%), T. harzianum (82.96%), A. niger (70.37%), T. asperellum (39.26%), P. fluorescens (30.55%) and T. koningii (24.07%). While for A. alternata, significantly highest mycelial growth inhibition was with T. hamatum (84.91%), followed by, T. virens (82.04%), A. niger (77.41%), T. asperellum (75.93%), T. harzianum (71.85%), T. koningii (55.37%) and P. fluorescens (43.15%).
Keywords: *Pathogenic mycoflora of soybean seeds, bioagent*

Received 02.01.2019 Revised 20.01.2019 Accepted 09.03. 2019

INTRODUCTION

Soybean (*Glycine max* L. Merrill.) is a worldwide economic crop and the most important cultivated legume with hundreds of food, feed and industrial uses [15]. Soybean is a protein rich oilseed, which is presently number one edible oil source globally. Soybean seed is the biggest source of vegetable oil in the World. Soybean oil is used as edible oil for manufacturing of chocolates, ghee, soaps, paints, rubbers, lubricants, explosives, glycerine and antibiotics. Soybean seed is highly nutritious containing about 40% protein, 30% carbohydrates, 5% fibre, 0.5% lecithins, 4% saponins and 18-20% vegetable oil [6]. In India during 2016-17, soybean was cultivated on an area of 10.60 M ha with a production of 7.13 million MT. The productivity was 0.61 MT/ha which is popularly grown in the state of Madhya Pradesh, Maharashtra and Rajasthan [2]. Several phytopathogenic and saprophytic fungal species have been reported on soybean seeds. The most important fungal seedborne diseases of soybean are *Fusarium* collar rot (*Fusarium semitectum*) [10], Downy mildew (*Perenospora manshurica*), *Aspergillus flavus*, *Aspergillus niger, Curvularia lunata, Penicillium digitatum, Rhizopus stolonifer*, Anthracnose/pod blight (*Colletotrichum truncatum*), *Alternaria* leaf spot (*Alternaria alternata*), *Cercospora* leaf spot (*Cercospora sojina*), Purple seed stain (*Cercospora kikuchii*) [3], Charcoal rot (*Macrophomina phaseolina*), Stem rot (*Sclerotinia sclerotiorum*) [6, 8]etc. These phytopathogenic fungi associated with soybean seeds cause qualitative losses by reducing oil quality due to increased free fatty acids as well as quantitative losses by seed deterioration and seedling mortality, leading to accountable seed yield losses [1, 14]. Therefore, present study on in vitro bioefficacy of bioagents against pathogenic mycoflora of soybean seeds was planned and conducted at the Department of Plant Pathology, College of Agriculture, Latur, during 2017-18.

MATERIALS AND METHODS

Isolation, identification and pathogenicity of seed borne fungi

Previous season stored seeds of soybean cultivars MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335 were collected from Oilseeds Research Station, Latur and Seed Processing Unit (National Seed Project) VNMKV, Parbhani. These seeds were plated aseptically onto autoclaved and cooled Potato Dextrose Agar medium, in separate sterile glass petri plates and incubated at room temperature. After a week of incubation, various fungal colonies developed on PDA plates were observed under stereomicroscope, distinguished on the basis of colony colour and growth habit, further re-isolated on fresh PDA plates and incubated at room temperature. Based on morpho-cultural characteristics and microscopic observations, the most predominant fungi identified were *Fusarium verticillioides, Macrophomina phaseolina* and *Alternaria alternata*. The pathogenicity of these three fungi was proved by seed inoculation and standard blotter paper techniques, by using the surface sterilized (2% Sodium hypochlorite solution) seeds of soybean cultivars MAUS-71, MAUS-158, MAUS-162, MAUS-612 and JS-335.

In vitro evaluation of bioagents

A total of eight biocontrol agents (Table. 1) were evaluated in vitro against *Fusarium verticillioides, Macrophomina phaseolina* and *Alternaria alternata* separately, by applying Dual Culture Technique [5]. Three separate experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. Observations on linear mycelial growth of the test fungi and test bio-agents were recorded separately at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungi. Per cent mycelial growth inhibition of the test fungi with the test bioagents, over untreated control was calculated by applying following formula [4].

$$\text{Per cent growth inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in Inter secting plate}}{\text{Colony growth in Control Plate}} \times 100$$

The data obtained was statistically analysed [11] and the results were interpreted thereof.

RESULT AND DISCUSSION

The results obtained on per cent of mycelial growth inhibition of two test fungi viz., *F. verticillioides, M. phaseolina* and *A. alternata* with the test bioagents are presented in Table 1 and Fig. 1.

F. verticillioides inhibition

The results (Table.1 and Fig.1A) revealed that all of the eight test bioagents significantly inhibited mycelial growth of *F. verticillioides*, over untreated control, which was ranged from 45.00– 85.93 per cent. However, significantly highest mycelial growth inhibition was achieved with *T.virens* (85.93%), followed by, *A. niger* (84.63%), *T. hamatum* (66.85%), *T. asperellum* (63.52%), *T. harzianum* (62.77%), and *T. koningii* (52.96%). *P. fluorescens* was found least effective (45.00%).

M. phaseolina inhibition

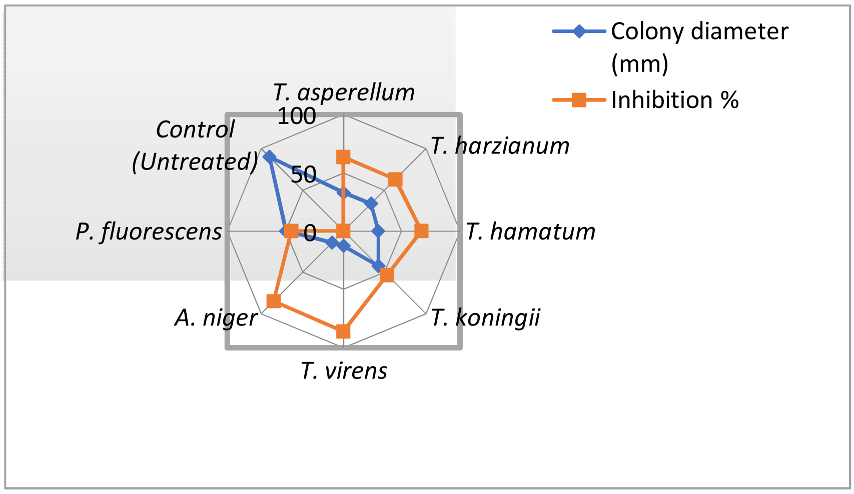
The results (Table.1 and Fig.1B) revealed that all of the eight test bioagents significantly inhibited mycelial growth of *M. phaseolina*, over untreated control and it was ranged from 24.07– 83.71 per cent. However, significantly highest mycelial growth inhibition was resulted with *T. hamatum* (83.71%), followed by, *T. virens* (83.33%), *T. harzianum* (82.96%), *A. niger* (70.37%), *T. asperellum* (39.26%), *P. fluorescens* (30.55%) and *T. koningii* (24.07%).

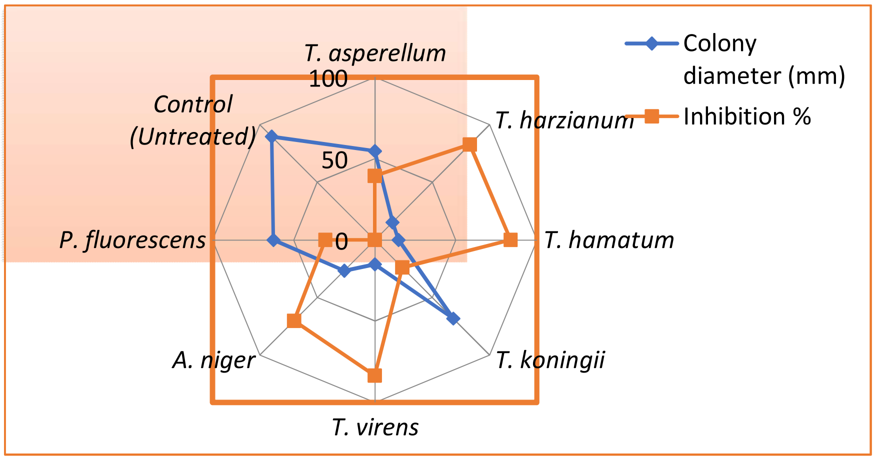
A. alternata inhibition

The results (Table.1 and Fig.1C) revealed that all of the eight test bioagents significantly inhibited mycelial growth of *A. alternata*, over untreated control and it was ranged from 43.15– 84.91 per cent. However, significantly highest mycelial growth inhibition was resulted with *T. hamatum* (84.91%), followed by, *T. virens* (82.04%), *A. niger* (77.41%), *T. asperellum* (75.93%), *T. harzianum* (71.85%), *T. koningii* (55.37%) and *P. fluorescens* (43.15%).

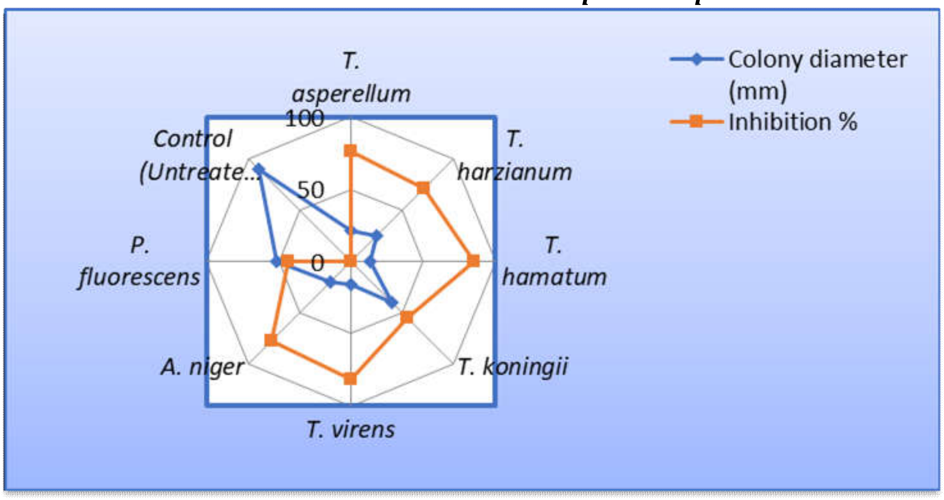
Various species of *Trichoderma, Aspergillus niger* and *P. fluorescens* are most commonly and commercially exploited bioagents/ antagonists to combat several seed borne and / or soil borne plant pathogens. Fungicidal / fungistatic effects of these bioagents have been attributed to various mechanisms exerted such as antibiosis, lysis, mycoparasitism, competition, production of volatile / nonvolatile compounds etc. In present study also, various species of *Trichoderma, Aspergillus niger* and *P. fluorescens* were found as efficient antagonists against three major seed borne pathogenic fungi *viz., F. verticillioides, M. phaseolina* and *A. alternata* of soybean.

Fig.1 *In vitro* efficacy of various bioagents against *F. verticillioides, M. phaseolina* and *A. alternata* associated with soybean seeds





A. *Fusarium verticillioides* **B. *Macrophomina phaseolina***



C. *Alternaria alternata*

Table.1 In vitro efficacy of various bioagents against *F. verticillioides, M. phaseolina* and *A. alternata* associated with soybean seeds

Tr. No.	Treatments	Inhibition* (%)		
		F. verticillioides	***M. phaseolina***	***A. alternata***
T_1	*Trichoderma asperellum*	63.52 (52.84)	39.26 (38.79)	75.93 (60.61)
T_2	*Trichoderma harzianum*	62.77 (52.39)	82.96 (65.61)	71.85 (57.95)
T_3	*Trichoderma hamatum*	66.85 (54.84)	83.71 (66.19)	84.91 (67.14)
T_4	*Trichoderma koningii*	52.96 (46.69)	24.07 (29.38)	55.37 (48.08)
T_5	*Trichoderma virens*	85.93 (67.96)	83.33 (65.90)	82.04 (64.92)
T_6	*Aspergillus niger*	84.63 (66.91)	70.37 (57.02)	77.41 (61.62)
T_7	*Pseudomonas fluorescens*	45.00 (42.13)	30.55 (33.55)	43.15 (41.06)
T_8	Control (Untreated)	00.00 (00)	00.00 (00)	00.00 (00)
SE±		**0.91**	**0.70**	**0.76**
CD (P=0.01%)		**2.66**	**2.06**	**2.23**

*: Mean of three replications. Figures in parentheses are Arcsine values

These results of the present study are in agreement with previous findings of several workers [8, 12, 14, 15]. Gupta and Ansari [8] revealed that seedling mortality was reduced maximum with *T. viride* and *P. fluorescens* individually or in combination with fungicide and bioagent seed treatments, rather it enhanced seed germination over untreated control. Rahman *et al*. [12] reported that all the three species of *Trichoderma viz., T. harizanum, T. hamatum* and *T. viride* showed excellent control of seedborne *M. phaseolina* and also increased germination significantly and thereby production of healthy seedlings. Rajeswari *et al.* [13] reported that seed treatments with *T. viride* (6g/kg), mancozeb (2.5g/kg) and neem oil (10 ml/kg) not only enhanced the seed quality but also were effective in reducing total seed mycoflora and seedling mortality. Seeds of safflower cultivars Nira and Manjeera treated with *T. viride* @ 6g kg-1 also 32 recorded higher seed germination (90% and 87%) and seedling vigour (1529,1383) with less number of total fungal colonies (7% and 12%).
Hence, from ongoing results and discussion, it is concluded that soybean pathogenic three major seedborne fungi *viz., F. verticillioides*, *M. phaseolina* and *A. alternata* could be managed with the bioagents viz., *A. niger*, various species of *Trichoderma* and *P.fluorescens*.

CONCLUSION

Agroforestry can support food production, increase the total efficiency and stability of the system in North Eastern region. It holds promise to satisfy all human needs (food, fuel, fodder, timber *etc*.) and it also can act as an insurance against drought, flood and natural calamities those are familiar to north-east region of India. With inclusion of agroforestry with different crop components and livestocks one can be benefitted in terms of monetary as well as ecological sustainability. Thus, area based effective research strategies in North Eastern region is required for meeting the diversified needs of the people as well as for increasing the food production

ACKWOLEDGEMENT

Author very thankful to Department of Plant pathology, College of Agriculture, Latur-413512 (MS), India Vasantrao Naik Marathwada Krishi Vidyapeeth, Parabhani, Maharashtra, India provide all the necessary facility for research works.

REFERENCES

1. Agarwal, V.K., Singh, O.V. and Nene, Y.L. (1974). Influence of fungicidal seed treatment on the mycoflora of stored seed and seedling emergence. *Ind. J. Agric. Sci*., 43(8): 820-824.
2. Anonymous (2017). India Agristat revealing agriculture in India. Statistical Information, www.indiastat.com.

3. Anwar, S.A., Abbas, S.F., Gill, M.M., Rouf, C.A., Mahamood, S. and Butta, A.R. (1995). Seedborne fungi of soybean and their effect on seed germination. *Pak. J. Phytopath*., 7: 184-190.
4. Arora, D. K. and Upadhyay, R. K. (1978). Effect of fungal staling substances on colony interaction. *Pl. Soil*., 49: 685-690.
5. Dennis, C. and Webster, J. (1971). Antagonistic properties of species groups of Trichoderma. *Trans. Br. Mycol. Soc*., 57:25-39.
6. Endres, J., Barter, S., Theodora, P. and Welch, P. (2013). Soybean enhanced lunch acceptance by preschoolers. *J. American Dietetic Asso*., 103: 346- 351.
7. Goulart, A.C.P. (1997). Fungi in soybean seed detection and importance. Documents EMBRAPA centro de pesquisa agropecua ria dooestei., 11: 58.
8. Gupta, G.K. (2001). Management strategies for soybean diseases in India. Souvenir, Harnessing the soy potential for health and wealth. *India Soy Forum*,: 38-45.
9. Gupta, G.K. and Ansari, M.M. (1998). Studies on survey, surveillance, epidemiology and other biological aspects of major root and seed diseases of soybean. *Annual progress report*, *National Res. Centre for Soybean*, Indore, M.P.:24-25.
10. Nik, W.Z. (1980). Seedborne fungi of soybean (Glycine max (L.) Merril) and their control. *Pertanika*., 3(2): 125-132.
11. Panse, V.G. and Sukhatme, P.V. (1963). Statistical Methods for Agricultural Worker. *IARI,* New Delhi Pub: 157-165.
12. Rahman, S., Verasilp, S. and Srichuwong, S. (2002). Control of seedborne Macrophomina phaseolina in mungbean through biological seed treatment. *J. of Agriculture*., 18(1): 33-39.
13. Rajeswari, B., Keshavulu, K. and Krishnarao, V. (2012). Management of seed mycoflora on safflower (Carthamus tinctorius L.). *J. Oilseed Res*., 29: 332-335.
14. Sarbhoy, A. K. and Vishwadhar, P. (1992). Soybean rust in India and its management: farming system and integrated management (Eds.J.P.Verma and A. Verma). *Malhotra Publishing House,* New Delhi-64, India,:117-120.
15. Shovan, L.R., Bhuiyan, M.K.A., Sultana, N., Begum, J.A. and Pervezi, Z. (2008). Prevalence of fungi associated with soybean seeds and pathogenicity tests of the major seedborne pathogens. *Inter. Pl. Prot*.,:72-75.

CITATION OF THIS ARTICLE

S. S. Dhawan, S. J. Magar, M. D. Navale and H. N. Markad. Bioefficacy of Bioagents against Pathogenic Mycoflora of Soybean Seeds Bull. Env. Pharmacol. Life Sci., Vol 8 [7] June 2019: 27-31