

Efficacy of Fungicides against Pathogenic Mycoflora of Soybean Seeds

KEY WORDS

Pathogenic mycoflora,
Soybean seeds,
Alternaria,
Macrophomina,
Fusarium, fungicides

ABSTRACT

Soybean (*Glycine max* L. Merrill.) crop is affected by a number of pathogenic fungi, which are mostly seed borne, causing significant qualitative and quantitative losses. Therefore, present *in vitro* study was conducted to assess efficacy of fungicides against three major pathogenic seed borne fungi viz., *Fusarium verticillioides*, *Macrophomina phaseolina* and *Alternaria alternata*, by applying Poisoned Food 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 seven seed dressing fungicides significantly inhibited mycelial growth of *Fusarium verticillioides*, *Macrophomina phaseolina* and *Alternaria alternata*, over untreated control. However, the fungicides viz., tebuconazole 25 % WG and carbendazim 12 % + mancozeb 63 % 75 WP were found most effective with 100 per cent mycelial growth inhibition of *Fusarium verticillioides* and rest of the fungicides tested were comparatively less effective. However, mancozeb 75% WP, carboxin 37.5% + thiram 37.5 % 75 WP and carbendazim 12 % + mancozeb 63 % 75 WP were found most effective with 100 per cent mycelial growth inhibition of *Macrophomina phaseolina* and rest of the fungicides tested were comparatively less effective. However, tebuconazole 25% WG and carboxin 37.5 % + thiram 37.5 % 75 WP were found most effective with 38.52 and 85.93 %, respectively mycelial growth inhibition of *Alternaria alternata* and rest of the fungicides tested were comparatively less effective.

1. INTRODUCTION-

Soybean (*Glycine max* L. Merrill.) is a worldwide economic crop and the most important cultivated legume with hundreds of foods, feed and industrial uses. 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, glycerin and antibiotics (Endres *et al.*, 2013). Soybean seed is highly nutritious containing about 40 % protein, 30 % carbohydrates, 5 % fiber, 0.5 % lecithin, 4 % saponins and 18-20 % vegetable oil. 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 (Anonymous, 2017). 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*) (Nik, 1980), Downy mildew (*Perenospora manshurica*), *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, Anthracnose/pod blight (*Colletotrichum truncatum*), *Alternaria* leaf spot (*Alternaria alternata*), *Cercospora* leaf spot (*Cercospora sojina*), Purple seed stain (*Cercospora kikuchii*), Charcoal rot (*Macrophomina phaseolina*) etc. (Goulart, 1997). 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. Therefore, present study on *in vitro* efficacy of fungicides against pathogenic mycoflora of soybean seeds was planned and conducted at the Department of Plant Pathology,

College of Agriculture, Latur during 2017-18.

2. MATERIALS AND METHODS

2.1 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 color 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.

3. *IN VITRO* EVALUATION OF FUNGICIDES

A total of seven fungicides (Table. 1) were evaluated *in vitro* against *Fusarium verticillioides*, *Microphobia phaseolina* and *Alternaria alternata* separately, by applying Poisoned Food Technique (Nene and Thapliyal, 1993). Three separate experiments were planned and conducted with

Completely Randomized Design (CRD) and all the treatments replicated thrice. Observations on radial mycelial growth/colony diameter were recorded at 24 hrs interval and continued till the untreated control plates were fully covered with mycelial growth of the test fungus. Per cent mycelial growth inhibition of *F. verticillioides*, *M. phaseolina* and *A. alternata* over untreated control was calculated by applying following formula (Vincent, 1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where, C = growth of the test fungus in untreated control plate.

T = growth of the test fungus in treated plate.

The data obtained was statistically analyzed and the results were interpreted thereof.

4. RESULTS AND DISCUSSION

A total of seven seed dressing fungicides at their recommended field dosages were evaluated in vitro by Poisoned food technique, against three major seedborne fungi viz., *F. verticillioides*, *M. phaseolina* and *A. alternata* of soybean which was detected in seed health testing methods, and the results obtained on their colony diameter (mm) and per cent inhibition of mycelial growth are presented in Table 1 and depicted in PLATE-I A, B and C. The results obtained on per cent of mycelial growth inhibition of three test fungi viz., *F. verticillioides*, *M. phaseolina* and *A. alternata* with the test fungicides are presented in Table 1.

Plate. I In vitro efficacy of various seed dressing fungicides against *F. verticillioides*, *M. phaseolina* and *A. alternata* associated with soybean seeds.

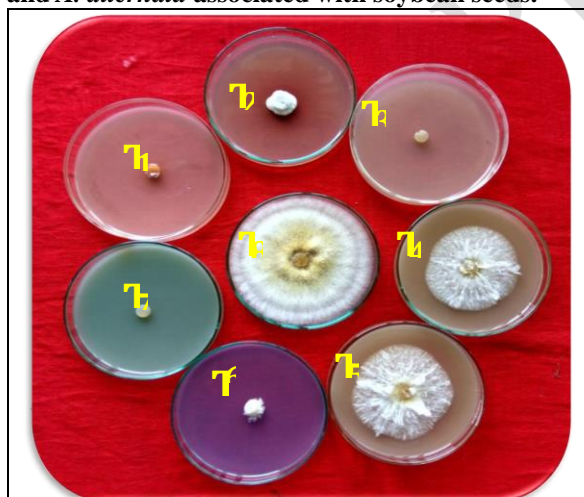


Plate I A Fungicides against *F. verticillioides*

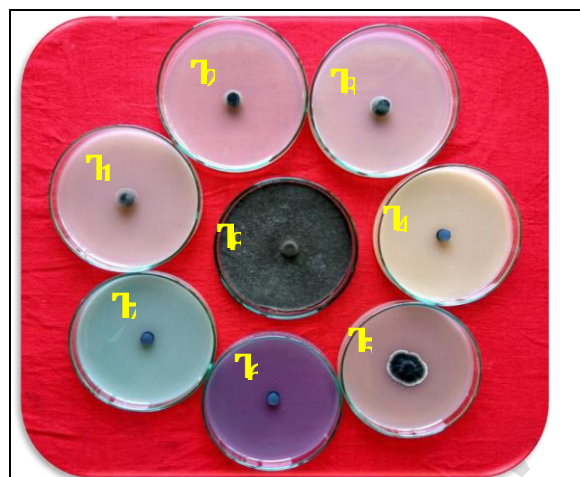


Plate I B Fungicides against *M. phaseolina*



Plate I C Fungicides against *A. alternata*

Table.1 In vitro efficacy of various seed dressing fungicides against *F. verticillioides*, associated with soybean seeds

	Treatments	Conc. (%)	Inhibition (%)
1	Carbendazim 50 % WP	0.1	88.88 (70.52)
2	Thiophanate methyl 70 % WP	0.1	83.61 (66.11)
3	Tebuconazole 25% WG	0.2	100.00 (90.00)
4	Mancozeb 75 % WP	0.25	33.43 (35.32)
5	Captan 75 % WP	0.3	39.54 (38.96)
6	Carboxin 37.5 % + thiram 37.5 % 75 WP	0.25	87.75 (69.51)
7	Carbendazim 12% + mancozeb 63% 75 WP	0.25	100.00 (90.00)
	Control (Untreated)	-	00.00 (00)
	SE±		0.64
	CD (P=0.01%)		1.88

Table.2 In vitro efficacy of various seed dressing fungicides against *M. phaseolina*, associated with soybean seeds

	Treatments	Conc. (%)	Inhibition (%)
1	Carbendazim 50 % WP	0.1	20.37 (26.82)
2	Thiophanate methyl 70 % WP	0.1	12.96 (21.10)
3	Tebuconazole 25 % WG	0.2	83.52 (66.04)
4	Mancozeb 75 % WP	0.25	44.91 (42.07)
5	Captan 75 % WP	0.3	66.38 (54.56)
6	Carboxin 37.5 % + thiram 37.5 % 75 WP	0.25	85.93 (67.96)
7	Carbendazim 12 % + mancozeb 63 % 75 WP	0.25	60.65 (51.14)
8	Control (Untreated)	-	00.00 (00)
	SE±		0.70
	CD (P=0.01%)		2.06

Table.3 In vitro efficacy of various seed dressing fungicides against *A. alternata* associated with soybean seeds

	Treatments	Conc. (%)	Inhibition (%)
1	Carbendazim 50 % WP	0.1	85.37 (67.51)
2	Thiophanate methyl 70 % WP	0.1	85.74 (67.81)
3	Tebuconazole 25% WG	0.2	85.00 (67.21)
4	Mancozeb 75 % WP	0.25	100.00 (90.00)
5	Captan 75 % WP	0.3	73.15 (58.79)
6	Carboxin 37.5 % + thiram 37.5 % 75 WP	0.25	100.00 (90.00)
7	Carbendazim 12 % + mancozeb 63 % 75 WP	0.25	100.00 (90.00)
8	Control (Untreated)	-	00.00 (00)
	SE±		0.45
	CD (P=0.01%)		1.46

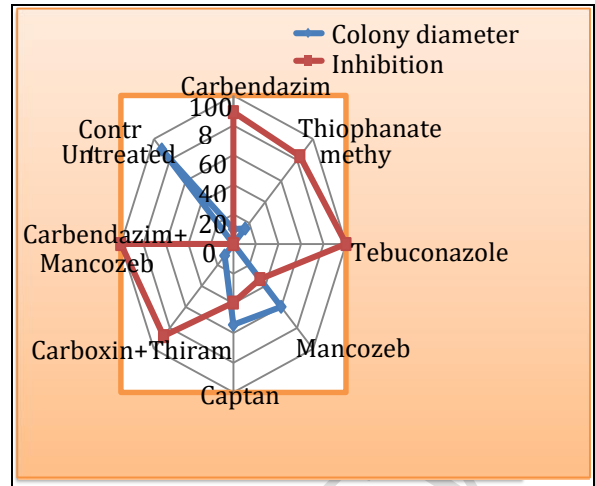


Fig. 1 A. In vitro efficacy of various fungicides against *F. verticillioides* associated with soybean seeds

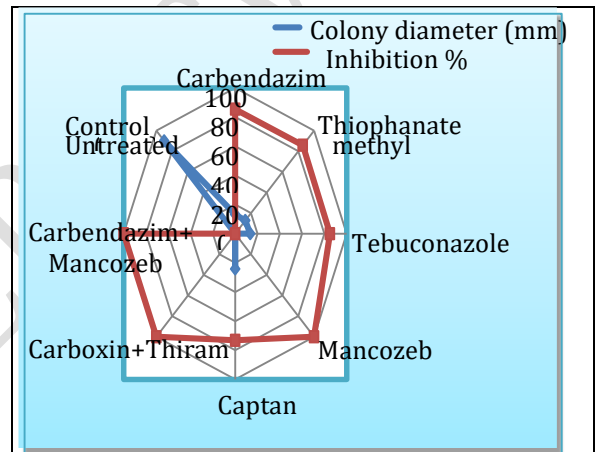


Fig. 1 B. In vitro efficacy of various fungicides against *M. phaseolina* associated with soybean seeds

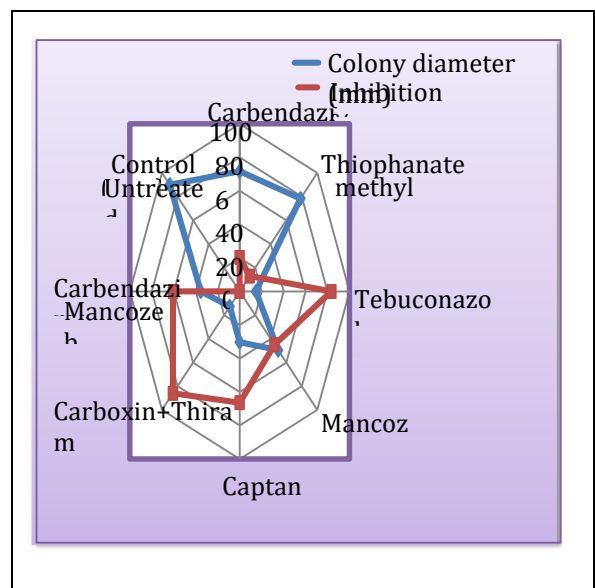


Fig. 1 C. *In vitro* efficacy of various fungicides against *Alternaria alternata* associated with soybean seeds.

***F. verticillioides* inhibition**

The results (Plate I A, Table 1 and Fig. 1 A) revealed that, all of the test fungicides exhibited significant mycelial growth inhibition of the *F. verticillioides*, over untreated control. However, the fungicides viz., tebuconazole 25 % WG at 0.2 % and carbendazim 12 % + mancozeb 63 % 75 WP at 0.25% resulted in 100 per cent inhibition of mycelial growth of *F. verticillioides*. Rest of the fungicides which also caused significant mycelial growth inhibition of *F. verticillioides* were carbendazim 50 % WP at 0.1 % (88.88 %), carboxin 37.5% + thiram 37.5 % 75 WP at 0.25 % (87.75 %), thiophanate methyl 70 % WP at 0.1 % (83.61 %), followed by captan 75% WP at 0.3 % (39.54 %) and mancozeb 75 % WP at 0.25 % (33.43 %). Thus, except the fungicides captan 75 % WP and mancozeb 75 % WP at their recommended dosage, rest of the five seed dressing fungicides tested were found highly effective against *F. verticillioides*.

***M. phaseolina* inhibition**

The results (Plate I B, Table 2 and Fig. 1 B) revealed that, all of the test fungicides exhibited significant mycelial growth inhibition of the *M. phaseolina*, over untreated control. However, the fungicides viz., mancozeb 75 % WP at 0.25 %, carboxin 37.5 % + thiram 37.5 % 75 WP at 0.25 % and carbendazim 12 % + mancozeb 63 % 75 WP at 0.25 % resulted in 100 per cent inhibition of mycelial growth of *M. phaseolina*. Rest of the fungicides which also caused significant mycelial growth inhibition of *M. phaseolina* were thiophanate methyl 70% WP @ 0.1% (85.74 %), carbendazim 50 % WP at 0.1 (85.37 %), tebuconazole 25 % WG at 0.2 % (85.00 %), followed by captan 75 % WP at 0.3 % (73.15 %). Thus, all the fungicides at their recommended dosage were found highly effective against *M. phaseolina*.

***A. alternata* inhibition**

The results (Plate I C, Table 3 and Fig. 1 C) revealed that, all of the test fungicides exhibited significant mycelial growth inhibition of the *A. alternata*, over untreated control. However, the effective fungicide was carboxin 37.5 % + thiram 37.5 % 75 WP at 0.25 % (85.93 %), followed by tebuconazole 25 % WG at 0.2 % (83.52 %), captan 75 % WP at 0.3 % (66.38 %) carbendazim 12 % + mancozeb 63 % 75 WP at 0.25 % (60.65 %), mancozeb 75 % WP at 0.25 (44.91 %), carbendazim 50 % WP at 0.1 % (20.37 %) and thiophanate methyl 70 % WP at 0.1 % (12.96 %), against *Alternaria alternata*. Thus, except the fungicides carbendazim 50% WP and thiophanate methyl 70 % WP at their recommended dosage, rest of the five seed dressing fungicides tested were found highly effective against *Alternaria alternata*.

The seed dressing fungicides viz., tebuconazole 25

% WG at 0.2 %, carbendazim 12 % + mancozeb 63 % 75 WP at 0.25 %, carbendazim 50 % WP at 0.1 %, carboxin 37.5 % + thiram 37.5 % 75 WP at 0.25 %, thiophanate methyl 70 % WP at 0.1 %, captan 75 % WP at 0.3 %, mancozeb 75 % WP at 0.25 %, etc at their recommend dosages as well as at various concentrations were reported earlier as most effective against many seed and / or soilborne pathogenic fungi, by several workers (Agarwal and Sushma 1989; Solanke *et al.* 1997; Goulart *et al.* 2000; Raj *et al.* 2002; Singh *et al.* 2003; Suryawanshi *et al.*,2008; Afzal *et al.* 2010 and Sonvane *et al.* 2011).

5. CONCLUSION

Among seven fungicides evaluated *in vitro*, the systemic fungicide carbendazim 50% WP, thiophanate methyl 70% WP and tebuconazole 25 % WG, followed by combi-fungicide (contact + systemic) carboxin 37.5 % + thiram 37.5 % 75 WP and carbendazim 12 % + mancozeb 63 % 75 WP and contact fungicides mancozeb 75 % WP and captan 75 % WP were found most effective against three major pathogenic fungi viz. *Fusarium verticillioides*, *Macrophomina phaseolina* and *Alternaria alternata* associated with the seeds of soybean.

7. 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. Agarwal, S.C. and Sushma, N. (1989). Effect of carbendazim on *Macrophomina* leaf blight of blackgram and greengram. *Ind. J. Pl. Prot.*, 17: 147.
3. Anonymous (2017). India Agristat revealing agriculture in India. Statistical Information, www.indiastat.com.
4. Anuja, G. and Aneja, K.R. (2000). Field efficacy of seed dressing chemicals on seedling emergence, seed yield and seed weight in soybean. *Seed Res.*, 28: 54-58.
5. Ellis, M.A., Ilyas, M.B. and Sinclair, J.B. (1975). Effect of three fungicides on internally seedborne fungi and germination of soybean seeds. *Dept. of Pl. Pathol., University of Illinois, Urbana*, 65: 553-556.
6. Endres, J., Barter, S., Theodora, P. and Welch, P. (2013). Soybean enhanced lunch acceptance by preschoolers. *J. American Dietetic Asso.*, 103: 346351.
7. Gawade, D.B., Suryawanshi, A.P., Pawar, A.K., Apet, K.T. and Devgire, S.S. (2009). Field evaluation of fungicides, botanicals and bioagents against anthracnose of soybean. *Agri. Sci. Digest.*, 29(3): 174-177.
8. Goulart, A.C.P. (1997). Fungi in soybean seed detection and importance. *Documents EMBRAPA centro de pesquisa agropecua ria dooestei.*, 11: 58.
9. Goulart, A.C.P., Andrade, P.J.M. and Borges, E.P. (2000). Control of soybean seedborne pathogens by fungicide treatment and its effect on emergence and yield. *Summa Phytopath.*, 26(3): 341-356.
10. Meena Kumari, K.V.S., Rajeswari, B. and Reddy,

- B.M. (2002). Impact of seedborne diseases on seed quality and seed dressing fungicides on storability of soybean. *Ind. J. Pl. Prot.*, **30**(2): 139-143.
11. Nene, Y.L. and Thapliyal, R.N. (1993). Fungicides in Plant Disease Control. 3rd edition. IBH Pub. Co. New Delhi, India:225.
12. Nik, W.Z. (1980). Seedborne fungi of soybean (*Glycine max* (L.) Merrill) and their control. *Pertanika.*, **3**(2): 125-132.
13. Pradhan, A., Lakpale, N. and Khare, N. (2014). Effect of fungicidal seed treatment on seedborne mycoflora and seedling vigour of pigeon pea (*Cajanus cajan* L.) millsp. *J. Mycol. Pl. Path.*, **44**(4): 447-449.
14. Rajeswari, B. and Meena Kumari, K.V.S. (2009). Bioagents and fungicides for the management of seed and seedling diseases of soybean. *Ind. J. Pl. Prot.*, **37**(2): 121-131.
15. Raju and Krishnamurthy (2002). Efficacy of fungicides in the management of collar rot of groundnut caused by *A. niger* Van Tieghem. *Ind. J. Pl. Prot.*, **28**:197-199.
16. Solanke, R.B., Kore, S.S and Sudewad, S.M. (1997). Detection of soybean seedborne pathogens and effect of fungicides. *J. Agril.*, **22**(2):168170.
17. Sonavane, A. A., Barhate, B. G. and Bade, S. J. (2011). Efficacy of bioagents, botanicals and fungicides on seed mycoflora of soybean. *J. Pl. Dis. Sci.*, **6**(1): 74-76.
18. Srinivasan, R. and Kannan, G.S. (2013). Studies on the use of new fungicides as seed dressers for management of collar rot disease of groundnut. *J. Pl. Dis. Sci.*, **8**(2): 179-181.
19. Suryawanshi A. P., Gore, D. D., Gawade, D. B., Pawar, A. K. and Wadje, A.G. (2008). Efficacy of fungicides against *Macrophomina* blight of mungbean. *J. Pl. Dis. Sci.*, **3**(1): 40-42.
20. Tripathi, D.P. and Singh, B.R. (1991). Mycoflora of soybean seed and their control. *Madras Agril. J.*, **78** (1-4): 130-132.
21. Vincent, J.M. (1927). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*. **59**: 850.