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Efficacy of Fungicides against Pathogenic Mycoflora of Soybean Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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. Therefore, present *In vitro* study was conducted to asses 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 the seven seed dressing fungicides significantly inhibited mycelial growth of *Fusarium verticillioides*, *Macrophomina phaseolina* and *Alternaria alternata*, over untreated control.

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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.

Keywords: Pathogenic mycoflora; soybean seeds; Alternaria; Macrophomina; Fusarium; fungicides.

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" [1]. "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" [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*)" [3], "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" [4,5]. "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" [6,7]. 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* [8,9,10]. 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 [11].

3. *In vitro* EVALUATION OF FUNGICIDES

A total of seven fungicides (Table 1) were evaluated in vitro against *Fusarium verticillioides, Macrophomina phaseolina* and *Alternaria alternata* separately, by applying Poisoned Food Technique [12]. "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 hours 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” [13].

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

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 1A, 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 1A. Fungicides against *F. verticillioides*

Plate 1B. Fungicides against *M. phaseolina*

Plate 1C. Fungicides against *A. alternata*

Plate 1. *In vitro* efficacy of various seed dressing fungicides against *F. verticillioides, M. phaseolina* and *A. alternata* associated with soybean seeds

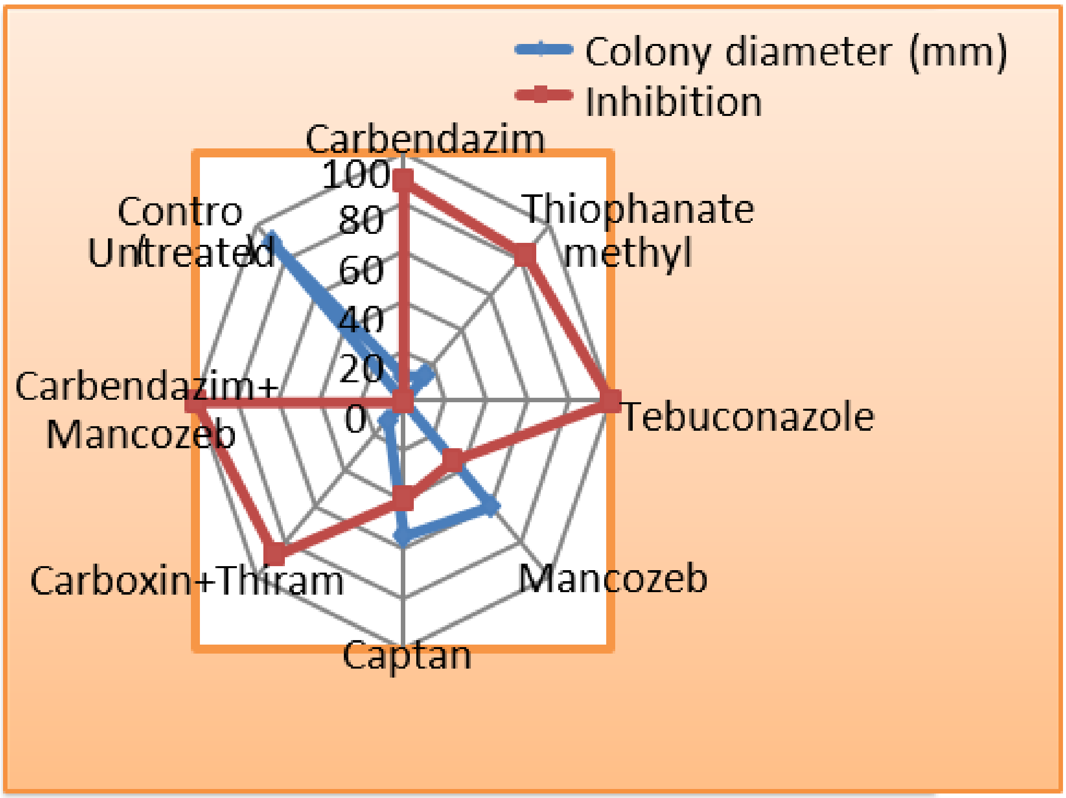

Fig. 1A. *In vitro* efficacy of various fungicides against *F. verticillioides* associated with soybean seeds

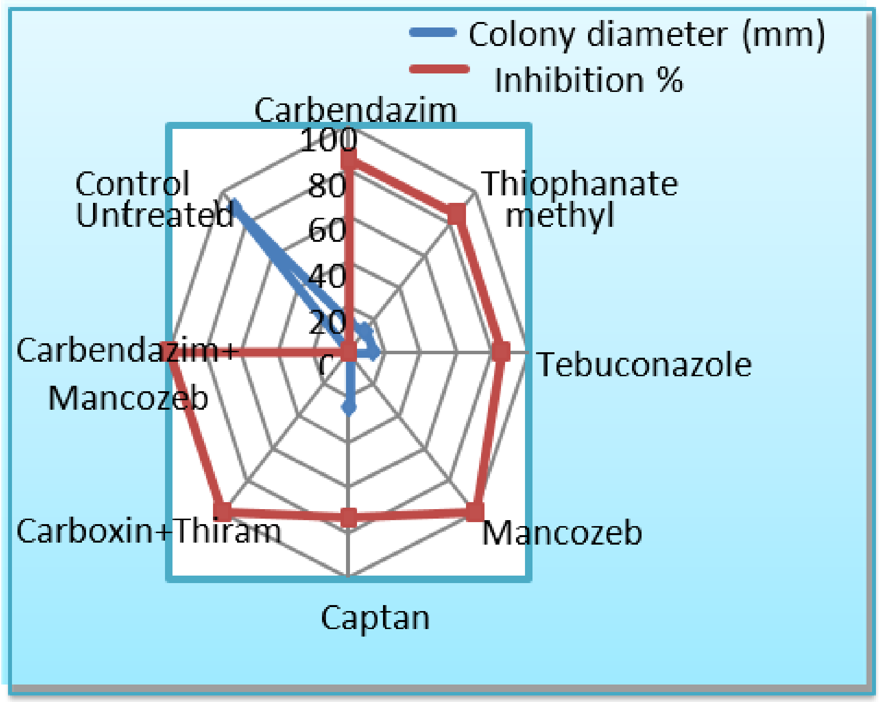

Fig. 1B. ***In vitro*** **efficacy of various fungicides against** ***M. phaseolina*** **associated with soybean seeds**

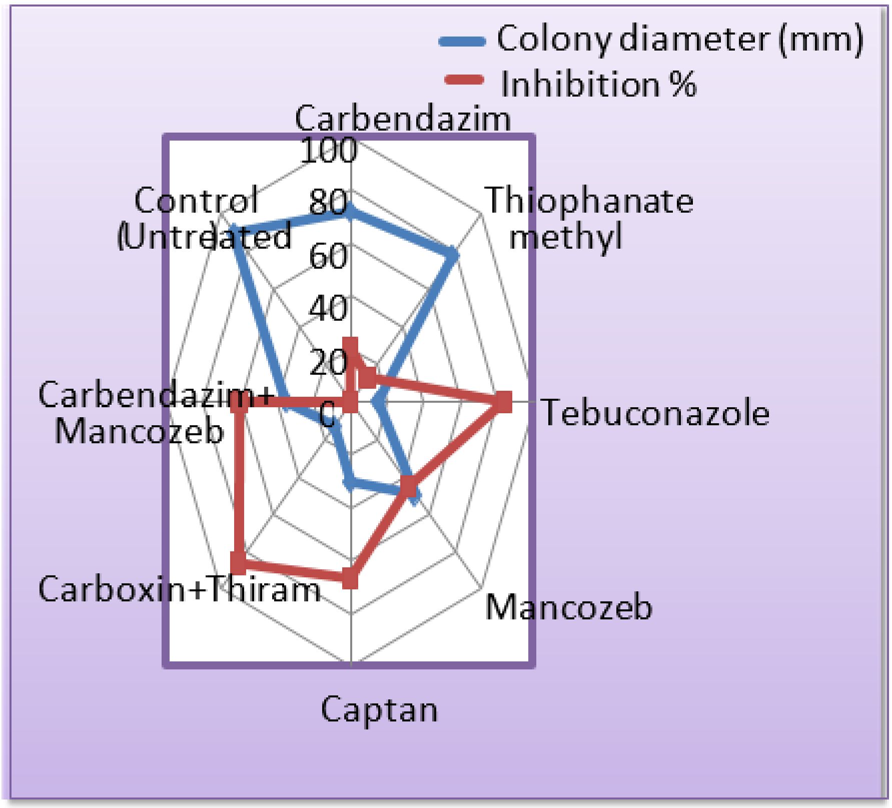

Fig. 1C. ***In vitro*** **efficacy of various fungicides against** ***Alternaria alternata*** **associated with soybean seeds**

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

Tr. No.	Treatments	Conc. (%)	Inhibition of *F. verticillioides*
T_1	Carbendazim 50%WP	0.1	88.88 (70.52)
T_2	Thiophanate methyl 70%WP	0.1	83.61 (66.11)
T_3	Tebuconazole 25%WG	0.2	100.00 (90.00)
T_4	Mancozeb 75% WP	0.25	33.43 (35.32)
T_5	Captan 75%WP	0.3	39.54 (38.96)
T_6	Carboxin 37.5% + thiram 37.5% 75 WP	0.25	87.75 (69.51)
T_7	Carbendazim 12% + mancozeb 63% 75 WP	0.25	100.00 (90.00)
T_8	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

Tr. No.	Treatments	Conc. (%)	Inhibition of *M. phaseolina*
T_1	Carbendazim 50%WP	0.1	85.37 (67.51)
T_2	Thiophanate methyl 70%WP	0.1	85.74 (67.81)
T_3	Tebuconazole 25%WG	0.2	85.00 (67.21)
T_4	Mancozeb 75% WP	0.25	100.00 (90.00)
T_5	Captan 75%WP	0.3	73.15 (58.79)
T_6	Carboxin 37.5% + thiram 37.5% 75 WP	0.25	100.00 (90.00)
T_7	Carbendazim 12% + mancozeb 63% 75 WP	0.25	100.00 (90.00)
T_8	Control (Untreated)	-	00.00 (00)
SE±			**0.45**
CD (P=0.01%)			**1.46**

***F. verticillioides* inhibition:** The results (Plate IA, Table 1 and Fig. 1 A) revealed that, all the test fungicides exhibited significant mycelial growth inhibition of the *F. verticillioides*, over untreated control. However, the fungicides viz., tebuconazol25 % WG @ 0.2 % and carbendazim 12% + mancozeb 63% 75WP @ 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 @ 0.1% (88.88%), carboxin 37.5% + thiram 37.5% 75WP @ 0.25% (87.75%), thiophanate methyl 70% WP @ 0.1% (83.61%), followed by captan 75% WP @ 0.3% (39.54%) and mancozeb 75% WP @ 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*.

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

Tr. No.	Treatments	Conc. (%)	Inhibition (%) of *A. alternata*
T_1	Carbendazim 50%WP	0.1	20.37 (26.82)
T_2	Thiophanate methyl 70%WP	0.1	12.96 (21.10)
T_3	Tebuconazole 25%WG	0.2	83.52 (66.04)
T_4	Mancozeb 75% WP	0.25	44.91 (42.07)
T_5	Captan 75%WP	0.3	66.38 (54.56)
T_6	Carboxin 37.5% + thiram 37.5% 75 WP	0.25	85.93 (67.96)
T_7	Carbendazim 12% + mancozeb 63% 75 WP	0.25	60.65 (51.14)
T_8	Control (Untreated)	-	00.00 (00)
SE±			**0.70**
CD (P=0.01%)			**2.06**

***M. phaseolina* inhibition:** The results (Plate IB, Table 2 and Fig. 1 B) revealed that, all the test fungicides exhibited significant mycelial growth inhibition of the *M. phaseolina*, over untreated control. However, the fungicides viz., mancozeb 75% WP @ 0.25 %, carboxin 37.5% + thiram 37.5% 75WP @ 0.25% and carbendazim 12% + mancozeb 63% 75WP @ 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 @ 0.1 (85.37%), tebuconazole 25 % WG @ 0.2 % (85.00%), followed by captan 75% WP @ 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 IC, Table 3 and Fig. 1 C) revealed that, all 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% 75WP @ 0.25% (85.93%), followed by tebuconazole 25 % WG @ 0.2 % (83.52), captan 75% WP @ 0.3% (66.38%) carbendazim 12% + mancozeb 63% 75WP @ 0.25% (60.65%), mancozeb 75% WP @ 0.25 (44.91%), carbendazim 50% WP @ 0.1% (20.37%) and thiophanate methyl 70% WP @ 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 @ 0.2 %, carbendazim 12% + mancozeb 63% 75WP @ 0.25%, carbendazim 50% WP @ 0.1 %, carboxin 37.5% + thiram 37.5% 75WP @ 0.25%, thiophanate methyl 70% WP @ 0.1%, captan 75% WP @ 0.3%, mancozeb 75% WP @ 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 [14,15,16,17,18,19,20,21,22,23]

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% 75WP and carbendazim12% + mancozeb 63% 75WP 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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