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Efficacy of fungicides, botanicals and organic amendments to suppress the anthracnose of soybean caused by Colletotrichum truncatum (Schw.) Andrus and Moore

Ashok Kumar Gurjar, RN Bunker and Anil Kumar Sharma

Abstract
The present study was carried out with an aim to find out the effective fungicides, botanicals and organic amendments under *in vitro* and *in vivo* conditions. The *in vitro* evaluations revealed that Tebuconazole was found highly effective in controlling the mycelial growth of C. truncatum completely at 500 and 1000ppm concentration among the different fungicides tested by poison food technique. Among the botanicals and organic amendments Garlic bulb extract and Panchgavya found most effective, respectively. *In vivo* study revealed that among the eight treatments, combinations of Tebuconazole 0.1% (seed treatment + foliar spary) + Garlic extract 15% (foliar spray) + Panchgavya 15% (foliar spray) was found most effective in management of anthracnose of soybean with minimum percent disease index (PDI) 24.44% and maximum 62.06 per cent disease control (PEDC). The aim of present study was to find out an innovative and environmentally safe strategy for control of anthracnose in soybean by integrating of chemicals fungicides, botanicals and organic amendments.

Keywords: Colletotrichum truncatum, soybean anthracnose, fungicides, botanicals, organic amendments

1. Introduction
Soybean (*Glycine max* L. Merril) is one of the most important oil seed and pulse crop of the world, which contributes 25% of the total global edible oil (Anonymous, 2019) [1]. It is native of china and introduced to India from United States in 1968. It has highest protein content (40%) among pulse crops (Chandel, 2002) [4] so soybean is known as "Poor man's meat" and "complete protein". Soybean belongs to the family fabaceae and grows under warm and moist climate during kharif season. Soybean is known to be affected by more than 100 plant pathogens of which very few are of economic importance. Among them, anthracnose/pod blight in soybean caused by Colletotrichum truncatum (Schw.) Andrus and Moore, has assumed the major constraints in successful cultivation of soybean (Khan and Sinclair, 1992; Mittal 1993) [7, 12]. The disease was first time reported in Korea 1917 (Akem, 1996) [2] Pakistan, 1985 (Mirza 1985) [14] India 1969 (Lambert, 1969) [9]. In India anthracnose also considered the most serious soybean disease (Khare and Chacko, 1983) [8]. It is one of the most important limiting factor in successful cultivation of soybean and responsible for reduction in yield with the range of 16-26% (Backman *et al*., 1982) [3]. Colletotrichum truncatum is the most common species recorded on soybean (Lenne, 1992) [10] it is the primary pathogen, this is cause anthracnose disease. Soybean crop is susceptible to the pathogen at all growth stage particularly from stage of flowering and pod filling stage. It is overwinters in infected seeds and crop residues. Management strategies for soybean anthracnose include use of presumed disease free seeds, resistant cultivar and fungicidal sprays. Seed treatment is one of the best method for manage seed-borne disease. The continuous and indiscriminate use of chemical to manage the crop disease results in accumulation of harmful chemical residues in the soil. Development of fungicide resistant biotypes of the pathogen is a major problem to control the seed borne pathogen of soybean.
Hence present study has therefore been undertaken with objective to *in vitro* and *in vivo* management of C. truncatum causing anthracnose of soybean by using fungicides, botanicals and organic amendments.

Materials and Methods

Isolation, purification and identification of pathogen The fresh anthracnose infected soybean leaves, twigs, stem and pods showing typical symptoms of anthracnose with profuse black acervuli were collected in early morning and brought to the laboratory. These infected plant parts were washed thoroughly in tap water to remove the surface adhering soil. Disease sample were cut into small pieces (4-5 mm) with adjoining healthy area with the help of sterilized blade. These pieces were washed in distilled water, surface sterilized by dipping in 0.1 per cent mercuric chloride solution (Hgcl2) for thirty seconds followed by rinsed three times with sterilized distilled water and then transferred in Petri plates filled with potato dextrose agar (PDA) under aseptic conditions in laminar flow cabinet. These were incubated at 27±20C in BOD for 7 to 10 days. Fungal growth appeared in two to three days from the infected tissues, it was sub-cultured aseptically on PDA slants by hyphal tip culture method. 10 days old pure culture was microscopically examined for identification and made confirmation based on the cultural characters *viz*; growth colour, growth pattern, and the morphological characters *viz*; Mycelia growth, acervuli, setae and conidia, its size and shape were studied under low (10X) and higher (40X) power magnification and were identified by key of Sinclair and Backman 1989.

In vitro evaluation of fungicides against C.

Efficacy of different fungicides were tested using poisoned food technique (Nene and Thapliyal, 1993) [15] against the C. truncatum *in vitro* with five fungicides *viz*., Propiconozole 25% EC (Folicure), Tebuconazole 25% EC (Tilt), Hexaconazole 5% EC (Contaf), Pyroclostrobin 13.3% + Epoxiconazole 5% SE (Opera), Chlorothalonil 75% WP (Kavach) were tested at four concentrations i.e. 100, 250, 500 and 1000 ppm. The technique includes cultivation of test organism on a medium containing the test chemical. In all experiments, PDA was used as basal medium. The required quantity of each chemical at different concentration was incorporated aseptically in 100 ml PDA in 250 ml flasks at the time of pouring the media in Petri plates. The medium was shaken well to give uniform distribution of the chemical. After that 20 ml of medium was poured in each Petri plates aseptically and allowed to solidify. The Petri plates were inoculated with 5 mm diameter mycelial disc cut from the periphery of 10 days old C. truncatum cultures. The mycelial disc was placed in the centre of the plates in an inverted portion to make a direct contact with the poisoned medium and incubated at 28+1 °C for 7-8 days. In each treatment three replications were maintained in Complete Randomize Design (CRD). At the same time a suitable control was also maintained by growing the fungus on chemical free PDA. Observations on linear growth were recorded when full growth of fungus observed in control Petri plate. The per cent inhibition of growth of the fungus in each treatment was calculated by using the following formula given by Vincent (1947) [18]

$$I = \frac{C - T}{C} \times 100$$

Where

I = Per cent inhibition

C = Growth of test fungus in control (mm)

T = Growth of test fungus in respective treatment (mm)

In vitro evaluation of plant extracts and organic amendments

An experiment was carried out to evaluate four plant extract *viz*., Neem leaf extract, Tulshi leaf extract, Garlic bulb extract and Parthenium leaf extract and two organic amendments *viz*., Vermiwash and Panchgavya at 5%, 10% and 15% concentration against the anthracnose of soybean pathogen C. truncatum by using poisoned food technique (Nene and Thapliyal, 1993) [15]. To prepare the phyto extract leaves of Neem, Tulshi, parthenium and bulb of garlic were first washed with tap water; surface sterilized (2% sodium hypochlorite), followed by three washings with sterile distilled water, and then were kept in the sterilized covered beaker and allowed to air dry. The plant materials were weighed and crushed with 80% ethanol in a Warring blender. The mixture was filtered through a double layered muslin cloth, the filtrate was evaporated, and the extract was diluted with sterile distilled water in a ratio of 1:1 (w/v). The concentration of this extract was considered as 100%; and it was used for stock solution. The stock solution of Vermiwash and Panchgavya were obtained from organic farming unit, Rajasthan College of agriculture, Udaipur. 5, 10 and 15 ml phyto extracts or organic amendments were taken separately from stock solution and mixed with 100 ml sterilized molten potato dextrose agar medium respectively, so as to get 5, 10 and 15 per cent concentrations. The medium was shaken thoroughly for uniform mixing of plant extract and organic amendment.

About 20 ml medium was poured into each of the 90 ml sterilized Petri plates. Four replications were maintained for each treatment. Suitable control plates were maintained without added of botanical extract and organic amendment in medium. Each plate was placed with 5 mm mycelial bit aseptically taken from the periphery of 7 days old culture and incubated at 27±2ºC

till the growth of the colony touched the periphery in control plate. Mean colony diameter in each case was recorded. The efficacy of plant extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula as given by Vincent (1947) [18].

Evaluation of promising fungicides, botanicals and organic amendments against incidence of anthracnose of soybean in the pot condition.

On the basis of *In vitro* study the one promising fungicides Tebuconazole 25% EC (0.1%), one botanical Garlic bulb extract (15%) and one organic amendment Vermiwash (15%) were evaluated as seed treatment and foliar spray for management of anthracnose of soybean in the pots. Plants of susceptible soybean cultivar “JS 335” were raised in 10x11 inch size pots contain five plants in each pot. The pots were arranged in eight treatments with three replications in completely randomized design (CRD) in the cage house. The different eight treatments were applied as seed treatment and foliar spray alone and their different combinations as follows:

Tr. No.	Treatments
T1	Tebuconazole 0.1% (ST+FS)
T2	Garlic extract 15% (ST + FS)
T3	Panchgavya 15% (ST + FS)
T4	Tebuconazole 0.01% (ST + FS) + Garlic extract 15% (FS)

T5	Tebuconazole 0.1% (ST + FS)+ Panchgavya 15% (FS)
T6	Garlic extract 15% (FS) + Panchgavya 15% (FS)
T7	Tebuconazole 0.1% (ST + FS) + Garlic extract 15% (FS) + Panchgavya 15% (FS)
T8	Control

Seed treatment was done before one day of sowing whereas the calculated quantities of different treatments (fungicides, botanical and organic amendments) were suspended in water and applied two times as foliar spray at 15 days intervals from the initiation of anthracnose symptoms. The observation of disease development and disease severity was recorded on a standard 0-9 disease rating scale (Mayee and Datar, 1986) [11] where, 0 = No infection, 1 = <1% infection, 3 = 1–10% infection, 5 = 11–25% infection, 7 = 26–50% infection and 9 = >51% infection. After 15 days of each spray per cent disease index was calculated by the formula given by Mc Kinney, 1923 [13].

$$\text{Per cent Disease Index} = \frac{\text{Sum of all disease rating}}{\text{No. of plants under observation x Maximum rating}} \times 100$$

The per cent efficacy of disease control (PEDC) was calculated by using following formula.

$$\text{PEDC} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Result and Discussion

In vitro evaluation of fungicides against C. truncatum All the tested fungicides significantly (P=0.05) inhibited the mycelial growth of C. truncatum at all concentration *in vitro* (Table-1). Tebuconazole was found most effective with completely inhibition of mycelial growth of C. truncatum at 500 and 1000 ppm concentrations followed by followed by Tebuconazole at 250 ppm (94.44%), Propiconazole 1000 ppm (93.33%), Hexaconazole 1000 ppm (91.48%).Chlorothalonil found least effective with inhibition 56.29, 61.85, 65.92 and 70.00% at 100, 250, 500 and 1000 ppm repectively.

Similarly result described by Rajashree *et al*. (2020) [16] repored that fungicides like Propiconazole 25% EC, Difenconazole 25% EC and Tebuconazole 25.9% EC recorded highest mycelial inhibition of C. truncatum at all the three concentrations (0.05, 0.1 and 0.15%) *in vitro*. Shashikumara *et al*. (2020) [17] also reported that Tebuconazole and propiconazole completely inhibited mycelial growth of C. truncatum *in vitro*.

***In vitro* evaluation of plant extracts and organic amendments**

These result revealed that none of the plant extracts or organic amendment could completely inhibited the growth of C. truncatum even at 15 per cent concentration (Table-2). Among the botanicals Garlic bulb extract inhibited maximum mycelial growth inhibition 78.33% and 70.28% at 15% and 10% concentration respectively followed by Neem leaf extract 69.44% at 15% concentration. In both organic amendments Panchgavya was more effective in inhibiting the pathogen as 50.00, 57.49 and 63.78 per cent compare to Vermiwash as 26.94, 35.00, 45.27 percent at 5, 10 and 15 percent concentrations, respectively.

Similarly Jagtap *et al*. (2012) [5] tested the *in vitro* efficacy of nine different aqueous leaf extract against C. truncatum and reported Garlic recorded significantly highest growth inhibition (81.82%) of the test pathogen. Shashikumara *et al*., (2020) [17] also reported that Garlic extract recorded highest mean mycelial growth inhibition (53.22%) of the C. truncatum followed by the onion extract (47.11%), neem (46.44%).

Evaluation of promising fungicides, botanical and organic amendments against incidence of anthracnose of soybean in the pot condition.

Data presented in table-3 showed that all the treatments are significantly effective over control. Anthracnose severity (PDI) 15 days after second spray minimum per cent disease severity (PDI) in Tebuconazole 0.1% (ST+FS) + Garlic extract 15% (FS)+ Vermiwash 15%(FS) (24.44) with maximum disease control (62.06%) has been recorded followed by Tebuconazole 0.1% + Garlic extract 15% (27.40) and disease control (57.47%). The individual application of fungicide Tebuconazole 0.1% exhibited (30.36) disease severity with 50.57% disease control while, combination of Tebucanazole 0.1% + Garlic 15% showed (27.40) disease severity with 57.47% disease control and combination of Tebuconazole 0.1% + Vermiwash 15% showed (28.88) disease severity with 55.18% disease control. The solely application of Garlic 15% and Vermiwash 15% showed (36.29) disease severity with 43.67% disease control and (39.25) disease severity with 39.08% disease control, respectively while combination of Garlic 15% + Vermiwash 15% showed 34.07 disease severity with 55.18% disease control. Maximum disease severity (51.11 PDI) was observed in control.

Although, there are limited work conducted on integrated management of soybean anthracnose while similar work conduct on other pulse crops like mungbean, greengram, where the integration of fungicides + botanicals and biorationals/biocontrol found promising in suppress of anthracnose. Similarly work done by Hingole *et al*. (2017) [6] to managed pod blight of soybean by using fungicides, botanicals and bioagents and resulted that Carbendazim 0.1% found most effective in reduction in disease intensity and pod infection with 47.14 and 70.98 percent respectively. Other treatment like fungicide Mancozeb, Carbendazim + Mancozeb, Propiconazole; bioagent T. viride and botanical A. sativum also found effective.

These observations suggest combinations of fungicides, botanicals and organic amendments need to widely adopt for sustainable soybean cultivations.

Table 1: Comparative efficacy of different fungicides on the growth of Colletotrichum truncatum in *In vitro*.

S. No	Treatments/Fungicides	Colony diameter (mm) at different conc. (PPM)*				Per cent growth inhibition*			
		100	250	500	1000	100	250	500	1000
1	Propiconozole 25 EC	17.33 (24.58)	14.33 (22.20)	9.33 (17.75)	6.00 (14.14)	80.74 (63.95)	84.07 (66.48)	89.62 (71.20)	93.33 (75.03)
2	Tebuconazole 25 EC	10.33	5.00	0.00	0.00	88.51	94.44	100.00	100.00

		(18.71)	(12.91)	(0.00)	(0.00)	(70.19)	(76.33)	(90.00)	(90.00)
3	Hexaconazole 5 EC	21.67 (27.72)	17.67 (24.82)	11.67 (19.87)	7.33 (15.65)	75.92 (60.59)	80.37 (63.69)	87.03 (68.95)	91.48 (73.09)
4	Pyroclostrobin 13.3 +	32.00	27.33	24.00	20.33	64.44	69.62	73.33	77.40
	Epoxiconazole 5 SE	(34.42)	(31.49)	(29.30)	(26.78)	(53.37)	(56.54)	(58.89)	(61.60)
5	Chlorothalonil 75 WP	39.33 (38.82)	34.33 (35.85)	30.67 (33.61)	27.00 (31.29)	56.29 (48.59)	61.85 (51.83)	65.92 (54.26)	70.00 (56.76)
6	Control	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 0.00)
S.Em ±		0.556	0.638	0.729	0.568	0.595	0.681	0.776	0.703
CD (P=0.05)		1.733	1.987	2.272	1.771	1.855	2.123	2.417	2.19

*Mean of three replications; Figures given in parentheses are arcsine√ Per cent angular transformed values.

Table 2: Comparative efficacy of different botanicals and organic amendments on the growth of Colletotrichum truncatum) in *In vitro*.

S.NO	Treatments/Botanicals	Colony diameter (mm) at different conc. (per cent)*			Per cent growth inhibition*		
		5	10	15	5	10	15
1	Neem leaf extract	41.50 (40.08)	35.50 (36.55)	27.50 (31.61)	53.89 (47.21)	60.55 (51.07)	69.44 (56.42)
2	Tulshi leaf extract	55.75 (48.28)	51.00 (45.55)	47.25 (43.40)	38.05 (38.06)	43.33 (41.15)	47.49 (43.54)
3	Garlic Bulb extract	34.75 (36.10)	26.75 (31.13)	19.50 (26.19)	61.39 (51.56)	70.28 (56.94)	78.33 (62.23)
4	Parthenium leaf extract	47.50 (43.54)	42.50 (40.67)	35.50 (36.55)	47.22 (43.38)	52.78 (46.57)	60.55 (51.07)
5	Vermiwash	65.75 (54.15)	58.50 (49.87)	49.25 (44.55)	26.94 (32.25)	35.00 (36.25)	45.27 (42.27)
6	Panchgavya	45.00 (42.11)	38.25 (38.18)	33.50 (35.34)	50.00 (44.98)	57.49 (49.28)	62.78 (52.38)
7	Control	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em ±		0.182	0.189	0.267	0.203	0.210	0.290
CD (P=0.05)		0.540	0.559	0.790	0.601	0.621	0.857

*Mean of three replications; Figures given in parentheses are arcsine√ Per cent angular transformed values.

Table 3: Evaluation of promising fungicides, botanicals and organic, amendments in integration for suppression of soybean anthracnose in cage house.

S.NO	Treatments/Botanicals	Colony diameter (mm) at different conc. (per cent)*			Per cent growth inhibition*		
		5	10	15	5	10	15
1	Neem leaf extract	41.50 (40.08)	35.50 (36.55)	27.50 (31.61)	53.89 (47.21)	60.55 (51.07)	69.44 (56.42)
2	Tulshi leaf extract	55.75 (48.28)	51.00 (45.55)	47.25 (43.40)	38.05 (38.06)	43.33 (41.15)	47.49 (43.54)
3	Garlic Bulb extract	34.75 (36.10)	26.75 (31.13)	19.50 (26.19)	61.39 (51.56)	70.28 (56.94)	78.33 (62.23)
4	Parthenium leaf extract	47.50 (43.54)	42.50 (40.67)	35.50 (36.55)	47.22 (43.38)	52.78 (46.57)	60.55 (51.07)
5	Vermiwash	65.75 (54.15)	58.50 (49.87)	49.25 (44.55)	26.94 (32.25)	35.00 (36.25)	45.27 (42.27)
6	Panchgavya	45.00 (42.11)	38.25 (38.18)	33.50 (35.34)	50.00 (44.98)	57.49 (49.28)	62.78 (52.38)
7	Control	90.00 (71.53)	90.00 (71.53)	90.00 (71.53)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em ±		0.182	0.189	0.267	0.203	0.210	0.290
CD (P=0.05)		0.540	0.559	0.790	0.601	0.621	0.857

*Per cent disease index; ** Per cent efficacy of disease control; Mean of three replications; Figures given in parentheses are arcsine √ Per cent angular transformed values.

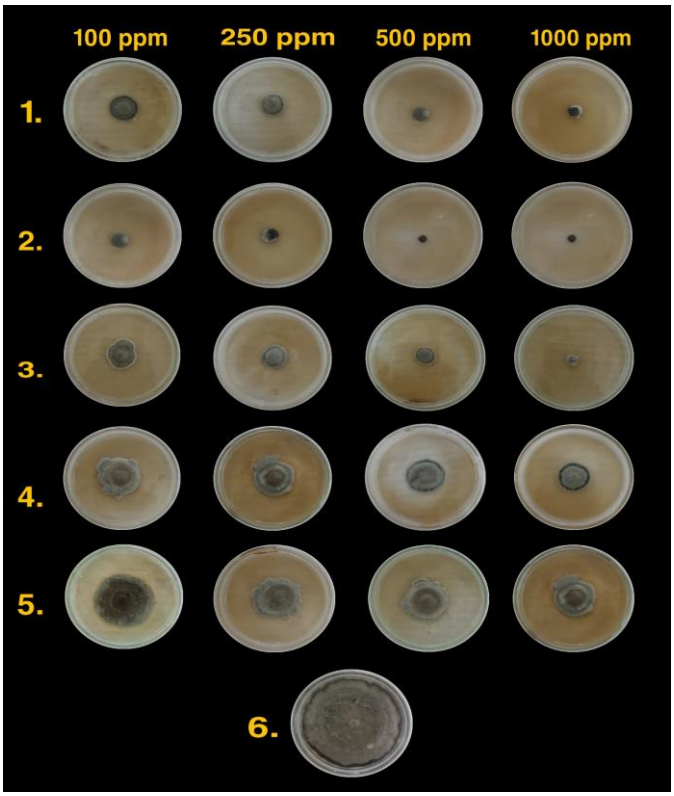


Fig 1-7: Inhibition of mycelia growth of Colletotrichum truncatum at different concentration of various fungicides *in vitro*. (1). Propiconazole 25 EC (2). Tebuconazole 25 EC (3). Hexaconazole 5 EC (4). Pyroclostrobin 13.3 + Epoxiconazole 5 EC (5). Chlorothalonil 75 WP (6). Control

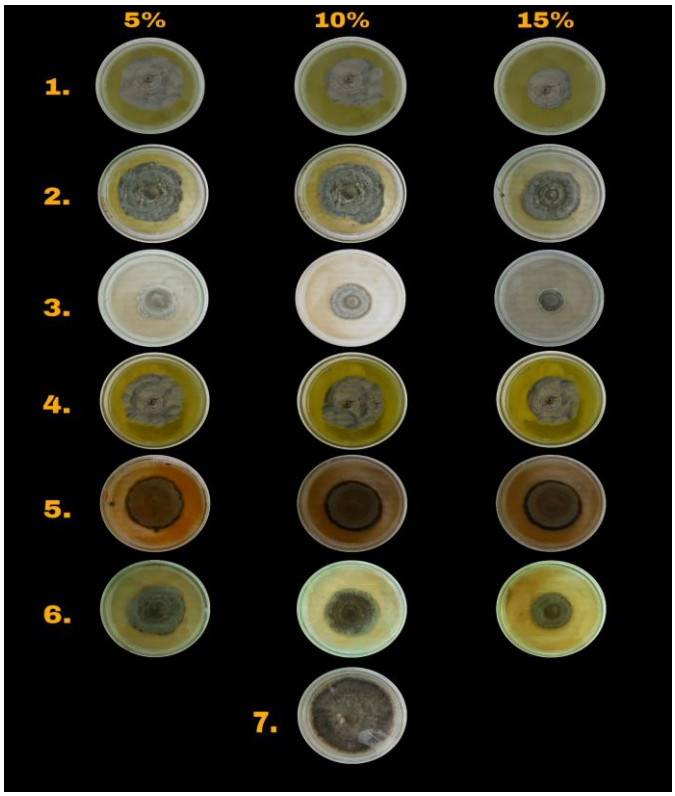
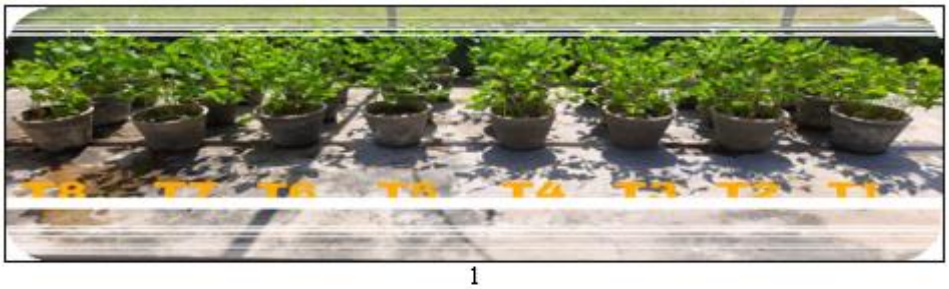


Fig 1-7: Inhibition of mycelia growth of Colletotrichum truncatum at different concentration of various botanicals and organic amendments *in vitro* (1). Neem leaf extract (2). Tulshi leaf extract (3). Garlic leaf extract (4). Parthenium leaf extract (5). Vermiwash (6). Panchgavya (7). Control



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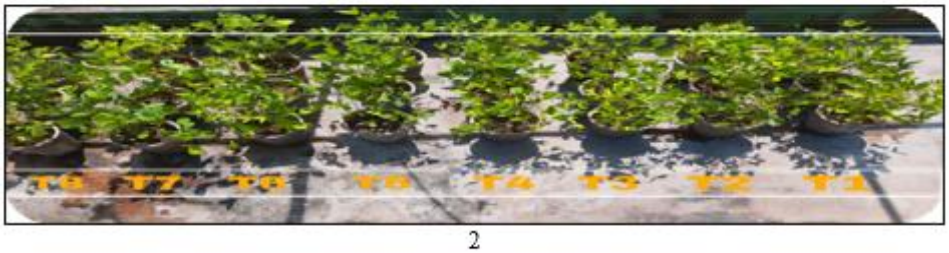

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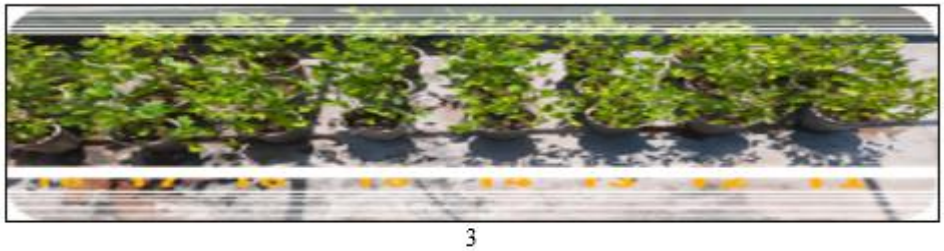

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Fig 1-3: Evaluation of promising fungicides, botanicals and organic amendments for suppression of anthracnose of soybean under pot condition. (1). At 1st appearence (2). After 1st spray (3). After 2nd spray T1- Tebuconazole 0.1% (ST+FS), T2- Garlic extract 15% (ST + FS), T3- Panchgavya 15% (ST + FS), T4- Tebuconazole 0.01% (ST + FS) + Garlic extract 15% (FS), T5- Tebuconazole 0.1% (ST+FS) + Panchgavya 15% (FS), T6- Garlic extract 15% (FS) + Panchgavya 15% (FS), T7- Tebuconazole 0.1% (ST + FS) + Garlic extract 15% (FS) + Panchgavya 15% (FS), T8- Control

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