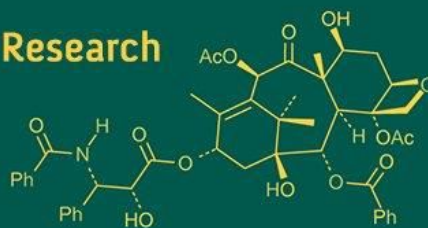
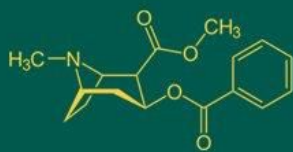


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In vitro efficacy of fungicides, bio-agents and phyto-extracts against *Colletotrichum truncatum*

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Abstract

Soybean [*Glycine max* (L.) Merrill] is one of the most important oilseed crop in India as well as in the World belonging to family leguminosae. *Colletotrichum truncatum*, the causal organism of anthracnose/ pod blight of soybean is one of the destructive pathogen. Seven systemic fungicides (@ 500, 1000 ppm each), seven bio-agents and seven botanicals @ 10% and 20% each were evaluated *in vitro* against *C. truncatum* using PDA as basal medium. The results revealed that all the fungicides, bio-agents and botanicals were found fungistatic and significantly inhibited the mycelial growth of the test pathogen over control. Among the fungicides, carbendazim and propiconazole recorded highest mean inhibition (96% @1000 ppm and 94.33% @ 500 ppm) of mycelial growth of the test pathogen. Among phyto-extracts tested, garlic extract recorded highest mean inhibition (79.66 @10% and 87.33 @20%) of mycelial growth of the test pathogen. Among bio-agents tested, *Trichoderma asperellum* recorded highest mean mycelial inhibition (74.88) of the test pathogen.

Keywords: *In vitro*, soybean, pod blight of soybean, *Colletotrichum truncatum*

Introduction

Soybean [*Glycine max* (L.) Merrill] is called as the miracle bean or golden bean crop of the planet. It is rich in oil (20%) and protein (40%). Soybean productivity in India is low at only 1.1 t/ ha, compared to the World's average of 2.2 t / ha. India shares only 4 per cent of World's soybean production, despite sharing 10 per cent of the World's soybean area under production (Ahamad *et al.*, 2018) [2]. Soybean is amongst the important crops of Marathwada region (constitutes Aurangabad, Jalna, Parbhani, Beed, Hingoli, Latur, Osmanabad and Nanded districts of Maharashtra) and is one of the reliable source of farmers economy in the region. Anthracnose caused by *Colletotrichum truncatum* (Schw.) Andrus and Moore which was first reported by (Verma and Upadhyay, 1973) [10] from India and the crop soybean is susceptible to *C. truncatum* at all stages of development particularly from flowering to pod filling stage. The disease causes damage by reducing plant stand, seed quality, seed germination, seedling vigour and yield (Vyas *et al.*, 1997) [12]. The 100 seed weight was significantly reduced from 29.5 per cent to upto 17.19 per cent because of attack of *Colletotrichum truncatum* (Chandrasekaran and Rajappan, 2002) [4]. The disease symptoms generally appeared at the early reproductive stages on stem, pods and petioles as irregularly shaped brown lesions and often as a result of latent infection (Cerkauskas, 1988) [3]. This disease thrives in warm and humid conditions, typically between 20-25 °C, and has been reported largely on soybean crop in tropical regions. Infected seeds may display brown discoloration, with up to 70 per cent seed infection observed in samples upto 30 per cent seed discoloration (Agam *et al.*, 2019) [1].

In view of this, *In vitro* studies were undertaken to evaluate the efficacy of fungicides, bio-agents and phyto-extracts against *Colletotrichum truncatum*.

Materials and Methods

The experiment was conducted during 2023-24 at Department of Plant Pathology, College of Agriculture, Badnapur. The culture of *C. truncatum* used in this study was isolated from pods of soybean plants collected from fields. To isolate pathogen, the sample were cut and were surface sterilized with 0.1% sodium hypochlorite solution for one minute and washing three

times by sterilized distilled water. The bits were placed in petriplates containing PDA medium. All the procedure was carried out in aseptic condition under laminar air flow. Then the plates were incubated at $27 \pm 2^\circ\text{C}$ for 10 days. The fungal growth that was developed around each bit was then transferred to PDA medium for sub culturing. The isolated fungi was identified as *C. truncatum* on the basis of morphological characters and available literature. The pathogenicity was proved using spore suspension spray method and was confirmed by Koch's postulates.

In vitro evaluation of fungicides by poisoned food technique

Seven systemic fungicides viz., Azoxystrobin, Metalaxyl, Carbendazim, Difenconazole, Hexaconazole, Propiconazole, Tebuconazole were tested against *C. truncatum* at concentrations of 500 ppm and 1000 ppm on PDA medium using poisoned food technique (Nene and Thapliyal, 1993) [8] under *in vitro* conditions. The PDA medium was prepared and liquefied. The fungicides were added in melted media to obtain the required concentrations. About 20 ml of media was poured in each sterilized petriplate. Proper control treatment was also maintained. 5 mm mycelial disc was taken from the 8 days old colony and was placed in the centre of petriplates and was incubated at $27 \pm 2^\circ\text{C}$ for 15 days. Three replications were maintained for each treatment. The diameter of the colony was measured when maximum growth of the pathogen was observed in control plates. The percent inhibition was calculated by using following formula (Vincent, 1927) [11].

$$\text{PI} = C - T / C \times 100$$

Where,

PI = percent inhibition

C = growth of fungi in control

T = growth of fungi in treatment

In vitro evaluation of bio-agents by dual culture method

Bio-agents viz., *Trichoderma asperellum*, *T. harzianum*, *T. hamatum*, *Verticillium lecani*, *Metarhizium anisopliae*, *Aspergillus niger*, *A. flavus* were evaluated for their effectiveness against *C. truncatum* by using dual culture technique (Dennis and Webster, 1971) [5] under laboratory conditions. Three replications were maintained along with one control. The plates were incubated at $27 \pm 2^\circ\text{C}$ and the diameter of the colony of both bio-agents and the pathogen was measured when maximum growth of the pathogen was observed in control plates and then per cent mycelial growth inhibition was calculated using above formula.

In vitro evaluation of phyto-extracts

Aqueous extracts of seven different plant species reported to show antifungal qualities were used against *C. truncatum*. The plants used were neem, garlic, onion, ghaneri, parthenium, ginger, tulsi. 100gm of healthy plant parts (leaves, rhizomes, bulbs) collected from field were washed with distilled water and were crushed in 100 ml of sterile water. The crushed product was filtered through muslin cloth and collected the filtrate. The prepared solution was further diluted to obtain required concentrations of 10% and 20%. The extracts were tested using poisoned food technique (Nene and Thapliyal, 1993) [8] against *C. truncatum* using PDA medium under *in vitro* conditions. The percent

inhibition was calculated by using above formula (Vincent, 1927) [11].

Results and Discussion

Effect of fungicides

Among the fungicides tested at 500 ppm concentration, per cent mycelial growth inhibition was ranged from 73.00 (Azoxystrobin) to 94.33 (Propiconazole and Carbendazim). However, significantly highest mycelial growth inhibition was recorded with the fungicides Propiconazole and Carbendazim (each 94.33%). This was followed by the fungicides, Hexaconazole (91.66%), Tebuconazole (91.00%), Difenconazole (86.00.00%), Metalaxyl (79.00%) and Azoxystrobin (73.00%), respectively.

At 1000 ppm concentration, similar trend of mycelial growth inhibition was observed and it was ranged from 81.66 (Azoxystrobin) to 96.00 (Propiconazole and Carbendazim). However, significantly highest mycelial inhibition was recorded with the fungicides Propiconazole, Carbendazim (each 96.00%), Hexaconazole (94.33%) and Tebuconazole (93.00%). This was followed by the fungicides, Difenconazole (90.33%), Metalaxyl (85.33%) and Azoxystrobin (81.66%). Azoxystrobin was found least effective with least inhibition of 81.66%.

The results obtained were similar to that of earlier reported by Gawade (2009) [6], in which he observed that, all the tested fungicides notably inhibited the mycelial growth of the pathogen over untreated control. But amongst all the fungicides tested, Carbendazim, Propiconazole, Hexaconazole, Difenconazole and Chlorothalonil showed inhibition in the mycelial growth of the pathogen but Carbendazim showed maximum (90.59%) per cent inhibition and was found highly effective.

Effect of bio-agents

Results indicated that, all the bio-agents evaluated, exhibited fungistatic activity against *C. truncatum* and significantly inhibited mycelia growth of the test pathogen over untreated control.

Out of the seven fungal antagonists tested, *T. asperellum* was found most effective and recorded least linear mycelial growth (22.60 mm) with highest mycelial inhibition (74.88%) of the test pathogen over untreated control (90.00 mm and 00.00%). The second and third best antagonists found were *T. harzianum* and *T. hamatum*, which recorded mycelial growth of 24.40 mm and 25.30 mm, respectively with mycelial growth inhibition of 72.88 and 71.88 per cent, respectively. This was followed by *T. hamatum* (25.30 mm and 71.88%), *V. lecani* (27.80 mm and 69.11%), *M. anisopliae* (29.50 mm and 67.22%), *A. niger* (31.40 mm and 65.11%) and *A. flavus* (35.60 mm and 60.44%), respectively.

The results are in consonance with the earlier reports as with Rajashree *et al.*, (2020) [9] checked *in vitro* efficacy of bio-agents against *C. truncatum*. It was found that, *T. asperellum* was found significantly superior than other bio-agents with mycelial growth inhibition of 75.55 per cent.

Effect of phyto-extracts

At 10 per cent concentration, mycelial growth inhibition of *C. truncatum* was ranged from 36.66% (ghaneri) to 79.66% (garlic) against untreated control. However, significantly highest mycelial growth inhibition was recorded with garlic (79.66%). This was followed by onion (61.00%), tulasi

(54.88%), ginger (53%), neem (48.33%), *Parthenium* (42.66%) and Ghaneri (36.66%), respectively.

At 20 per cent concentration, mycelial growth inhibition of *C. truncatum* was ranged from 42.11% (ghaneri) to 87.33% (garlic) against untreated control. However, significantly highest mycelial growth inhibition was recorded with garlic (87.33%). This was followed by onion (67.33%), tulasi

(62.00%), ginger (61.00%), neem (55.00%), *Parthenium* (53.66%) and Ghaneri (42.11%), respectively. The results obtained on present studies were in consonance with Gurjar *et al.*, (2021) [7], who tested *in vitro* efficacy of different botanicals against *C. truncatum* and among all botanicals used, it was found that, garlic bulb extract showed maximum mycelial growth inhibition.

Table 1: *In vitro* evaluation of systemic fungicides against *C. truncatum*

Tr.no	Treatment	* Mean radial mycelial growth @ 500 ppm	* Mean radial mycelial growth @1000 ppm	Avg. mycelial growth (mm)	% inhibition @500 ppm concn.	% inhibition @1000 ppm concn.
T1	Azoxystrobin	24.30	16.50	20.40	73.00 (58.69)	81.66 (64.33)
T2	Metalaxyl	18.30	13.20	15.75	79.66 (63.19)	85.33 (67.47)
T3	Carbendazim	5.10	3.60	4.35	94.33 (76.22)	96.00 (78.46)
T4	Difencconazole	12.60	8.70	10.65	86.00 (73.21)	90.33 (71.88)
T5	Hexaconazole	7.50	5.10	6.30	91.66 (73.21)	94.33 (76.22)
T6	Propiconazole	5.10	3.60	4.35	94.33 (76.22)	96.00 (78.46)
T7	Tebuconazole	8.10	6.30	7.20	91.00 (72.54)	93.00 (74.65)
T8	Control	90.00	90.00	90	00 (00)	00 (00)
	SE $\pm$ m	0.59	0.07		0.18	0.17
	CD@ 1%	0.24	0.30		0.73	0.69

All the figures are means of three replications.

*figures in parenthesis are arc sin transformed values.

Table 2: *In vitro* evaluation of bio-agents against *C. truncatum*

Tr. no	Treatment	Mean colony diameter of pathogen (mm)	% mycelial growth inhibition
T1	<i>Trichoderma asperellum</i>	22.60	74.88(59.92)
T2	<i>Trichoderma harzianum</i>	24.40	72.88 (58.61)
T3	<i>Trichoderma hamatum</i>	25.30	71.88 (57.97)
T4	<i>Verticillium lecani</i>	27.80	69.11 (56.23)
T5	<i>Metarhizium anisopliae</i>	29.50	67.22 (55.07)
T6	<i>Aspergillus niger</i>	31.40	65.11 (53.79)
T7	<i>Aspergillus flavus</i>	35.60	60.44 (51.02)
T8	Control	90.00	0.00 (0.00)
	S.E. $\pm$ m	0.07	0.16
	C.D.@1%-	0.30	0.66

All the figures are means of three replications.

*figures in parenthesis are arc sin transformed values.

Table 3: *In vitro* efficacy of phyto-extracts against *C. truncatum*

Tr. no	Treatment	*Mean Radial mycelial growth @ 20% (mm)	*Mean Radial mycelial growth @ 20% (mm)	Avg. mycelial growth (mm)	% inhibition @ 10%	% inhibition @ 20%
T1	Neem	46.50	40.50	43.50	48.33 (44.04)	55.00 (47.86)
T2	Garlic	18.30	11.40	14.85	79.66 (63.19)	87.33 (69.14)
T3	Onion	35.10	29.40	32.25	61.00 (51.35)	67.33 (55.13)
T4	Parthenium	51.60	41.70	46.65	42.66 (40.77)	53.66 (47.09)
T5	Ginger	42.30	35.10	38.70	53.00 (46.71)	61.00 (51.35)
T6	Tulsi	40.60	34.20	37.40	54.88 (47.80)	62.00 (51.94)
T7	Ghaneri	57.00	52.10	54.55	36.66 (51.02)	42.11 (40.46)
T8	Control	90.00	90.00	90.00	00 (00)	00 (00)
	SE $\pm$ m	0.09	0.07		0.16	0.14
	CD@ 1%	0.37	0.30		0.65	0.60

*Figures in parenthesis are arc sin transformed values

All the figures are means of three replications

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