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Understanding *Colletotrichum truncatum*, a Pathogen of Soybean Anthracnose, and *In Vitro* Efficacy of Modern Fungicides for it's Management

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ABSTRACT

The present investigation was convened during *kharif*, 2023 (July–December) at J.N.K.V.V., Jabalpur, Madhya Pradesh, India to characterize the fungus isolated from Anthracnose symptomatic sample of soybean, and to assess the fungal growth medium and evaluate the effectiveness of new-generation fungicides against it. During the investigation, apart from morpho-cultural traits, Internal transcribed spacer (ITS) regions of 5.8S ribosomal DNA of pathogenic fungus were amplified using the universal primers ITS 4 and ITS 5 and sequenced. Five readymade and two modified growing media were evaluated for fungal growth and sporulation. Antifungal efficacy of ten fungicides at 10,100 and 250 µg ml^{-1} were tested against pathogens by using the poisoned food technique. PCR amplification of the ITS region produced about 580 bp amplicon, and sequences of Jabalpur isolate (PP930734) showed the highest similarity of 99.12% with *C. truncatum* sequences at NCBI. Soybean leaf extract agar (85.10 mm), corn meal agar (83.13 mm) and potato dextrose agar (81.33 mm) were found to be significantly superior for mycelia growth, and Richard's agar and potato dextrose agar were excellent for sporulation of *C. truncatum*. In vitro testing of fungicides, the percent growth inhibition of *C. truncatum* was significantly higher in thiophanate methyl 45%+Pyraclostrobin 5% FS (91.12%) followed by Carbendazim 12%+Mancozeb 63%WP (88.91%), Carboxin 37.5%+Thiram 37.5% DS (88.01%) and Tebuconazole 25.9 % w/w (77.00%), respectively at 250 µg ml^{-1}. These fungicides were also showed efficacy at lower concentrations (10 and100 µg ml^{-1}) and among all, copper oxychloride 50% WP was least efficient.

KEYWORDS: Soybean, anthracnose, *Colletotrichum truncatum*, ITS, medium, fungicides

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1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the most valuable dual-purpose legume grown across the world (Anand et al., 2024a). It is the most affordable source of high-quality protein (36.1–42.8%), edible oil (16.8–20.2%), carbohydrates (35%), minerals, and several other nutrients (Uikey et al., 2022; Banerjee et al., 2023; Anand et al., 2024b). Soybean spread over the globe faster than any other prominent grain or oilseed crop (Amrate et al., 2024). Biotic and abiotic limitations still exist during production (Amrate et al., 2018; Kumar et al., 2024). Among this, anthracnose disease has been a constraint for soybean production in India since its appearance, and this has now been distributed in all the soybean growing regions in the country (Nene and Shrivastava, 1971; Wrather et al., 2010; Amrate et al., 2023).

The most widely distributed fungus, *Colletotrichum truncatum (Schw.)* Andrus and Moore are majorly associated with the causation of anthracnose disease in soybean (Rajput et al., 2021; Nataraj et al., 2023). The other species, such as *C. cliviae* (=*C. cliviicola*), *C. incanum* and *C. chlorophyti*, have also been associated with developing soybean anthracnose complex (Yang et al., 2012; Yang et al., 2014; Bhatt et al., 2022). *Colletotrichum truncatum* has also been reported to cause anthracnose diseases in tomato (Diao et al., 2014; Saini et al., 2017), garlic (Salunkhe et al., 2018), papaya & pepper (Torres-Calzada et al., 2018; Singhmanini et al., 2022) and several legumes (Pandey *et al.*,2023). Due to its seed-borne nature, the disease can appear in the early stages and may assume destructive forms and reduce soybean yield significantly (Manandhar and Hartman, 1999; Perez et al., 2023). In the infection of Anthracnose, necrotic black lesions and black fungal fruiting (acervuli) are produced primarily on stem and petioles. After that, pods or, in some cases, the whole plant can be affected (Sharma et al., 2011).

The artificial culturing of fungus on a medium facilitates getting information about the target organism, and several in vitro assays are also helpful in managing disease-causing fungus. The mycelial development and sporulation of *C. truncatum* have been found to vary among media and growth conditions (Salotti et al., 2022). Therefore, it could be helpful to determine a suitable readymade and modified media for satisfactory growth and sporulation of the fungus mentioned above. The internal transcribed spacers (ITS) are highly conserved regions in fungal ribosomal DNA (rDNA) segments that usually vary among fungal species, and this can be amplified in PCR (White *et al.,* 1990). The ITS has been successfully utilized to identify and differentiate closely related *Colletotrichum* species (Bailey et al., 1996; Kendall and Rygiewiicz, 2005; Forseille et al., 2011).

India's soybean production directly depends on developing high-yielding, anthracnose-resistant cultivars; however, most high-yielding varieties and germplasm are sensitive to Anthracnose (Nataraj et al., 2020). In central India, Anthracnose is a major disease that occurs yearly in most soybean genotypes. Mega varieties like JS 93-05 and JS 95-60 are highly affected by this disease (Amrate et al., 2023; Amrate, 2024). Modern fungicides might effectively reduce the severity and spread of Anthracnose in susceptible genotypes. Several researchers have demonstrated that carbendazim, mancozeb (Jagtap et al., 2013) trialzoles like hexaconazole, propiconazole, difenconazole (Kale and Barhate, 2016) and strobilurin group fungicides such as Trifloxystrobin, Picoxystrobin, Pyraclostrobin (Mandloi et al., 2023) are potential inhibitors of *C. truncatum*. New-generation combination fungicides have also shown efficacy in vitro and in vivo against *Colletotrichum* spp. (Bulat et al., 2019; Rajput et al., 2022). Looking at all the facts, the present investigation was undertaken to characterize the pathogenic agent and to determine the effective fungicides for its management.

2. MATERIALS AND METHODS

2.1. Isolation, identification and pathogenicity of test pathogen

The diseased sample of soybean showing symptoms of

Figure 1: Anthracnose necrotic lesion covering different parts of soybean plant: leave and veinlet (a and b), stem, leaves and petioles (c), developing pod (d), developed pods (e and f), only petioles and stem (g), only pods (h) and entire plant of soybean (i), respectively.

anthracnose was collected from the breeder seed production unit, unit (FC)- I, Department of Plant Breeding and Genetics, JNKVV, Jabalpur, Madhya Pradesh, India 2023 during *kharif* (July–December) (latitude 23°12'30"N and longitude 79°56'40"E) (Figure 1). Meanwhile, anthracnose symptoms were recorded on different parts of a soybean variety JS 93-05. The pathogen was isolated using a standard isolation technique on potato dextrose agar at 26±1°C (Bhatt et al., 2022; Mandloi et al., 2023). Initial fungal growth from the infected sample was obtained after four days of inoculation. After that, a five mm mycelia disc was again transferred, incubated and examined for the presence of specific traits of the target pathogen, such as acervuli, setae and conidia, under low (10X) and higher (40X) power magnification. The target pathogen was identified by matching the standard key suggested by previous researchers (Sinclair and Backman, 1989; Sharma et al., 2011). The pathogenicity of the fungus was tested on soybean pods of JS 93-05 (R5-R6 stage). The conidial suspension of 15–20 conidia per microscopic field 5 μl^{-1} drop was inoculated on pin-pricked pods and kept in a moist chamber in Petri plates.

2.2. Molecular identification of pathogen

2.2.1. Extraction of fungal DNA and PCR amplification of ITS region of pathogen

A pure culture of the test pathogen (three to four mycelial bits) was transferred in a 200 ml sterile potato dextrose broth (PDB) medium for multiplication for 10–12 days at 26±1°C. The mycelial mat was harvested using pre-sterilized Whatman filter paper, washed with sterile distilled water, and blotted dry. The mycelial mat was then transferred in mortar, frozen in liquid nitrogen, and crushed to fine powder by pestle. Total genomic DNA was extracted from the targeted isolate using a slight modification of the CTAB method (Lee and Taylor, 1990). The extracted fungal DNA was subjected to amplification of ITS region in Polymerase Chain Reaction by using fungal-specific primers ITS4 (R) (TCCTCCGCTT ATTGATATGC) and ITS5 (F) (GAAGTAAAAGTCGTAACAAGG) (White et al., 1990). PCR master mix from Gene-i Lab Private Ltd. was employed to carry out Polymerase Chain Reaction (PCR). PCR master mix comprised Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimum concentrations. In PCR amplification, a final reaction volume of 25 µl was prepared, which includes 1X PCR master mix (12.5 µl), Primers ITS 4 and 5 (1.0 µl each), Nucleus Free Water (8.5 µl) and in the last 100 ng of template DNA (2 µl). The mixture was subjected to 30 cycles of amplification using the following cycling parameters in a programmable thermal cycler: initial denaturation (95°C for 5 min once), 30 cycles of denaturation (95°C for 1 min), annealing (56°C for 1 min) and extension (72°C for 1 min), and final elongation (72°C for 7 min once). The PCR product (10 µl) and gel loading dye (2 µl) were mixed and loaded in 1.6% agarose gel containing ethidium bromide and 100 bp ladder and electrophoresed at 70 volts until the bands resolved. The banding pattern was visualized on a gel documentation system.

2.2.2. Sequencing and blasting

The amplified product was sent to Progene Life Sciences Pvt Ltd., Pune, for sequencing and ITS-rDNA sequences of the test pathogen was obtained. The NCBI GenBank nucleotide sequence database was queried using megablast NCBI-BLAST (Basic Local Alignment Search Tool-http://blast.ncbi.nlm.nih) searches to identify the closest matching of obtained sequences in the database (Altschul et al., 1997). The sequences were further submitted to NCBI GenBank.

2.3. Effect of different medium on growth and sporulation of C. truncatum

Seven different solid media were evaluated for growth and sporulation of *C. truncatum*, including potato dextrose agar, corn meal agar, oatmeal agar, rose bengal agar, soybean seed extract, soybean leaf extract, and Richard's agar. The required quantity of readymade Oatmeal Agar (7.25 g), Corn meal Agar (1.7 g), Rose Bengal Agar (3.16 g), Richard's agar (8.25 g), potato dextrose agar (3.9 g) was dissolved in 100 ml. Soybean seed extract agar was prepared by boiling 20 g of soybean seed in 100 ml distilled water, extracting and adding 2 g agar. Similarly, Soybean Leaf extract agar was prepared by boiling 8 g soybean leaves in 100 ml distilled water, extracting and adding 2 g agar. In the experiment, twenty ml sterilized medium was poured into 90 mm petri plates; after solidification, the medium was inoculated with test fungus and incubated at 26±1°C. Each treatment was replicated three times. The pathogen's radial growth and colony appearance were measured at regular intervals. The sporulation of the pathogen was checked by dissolving the two mycelia mats (5 mm) in a test tube containing 5 ml of distilled water. One drop of suspension (about 5µl) was put on a glass slide and examined for spore count under a compound microscope. The sporulation was categorized into grades excellent (≥40), good (20–40), fair (10–20), poor (0–10) and no sporulation, respectively, based on the number of conidia present in each diluted conidial suspension.

2.4. In vitro fungicidal efficacy against C. truncatum

Antifungal efficacy of ten fungicides i.e. Carbendazim 12%+Mancozeb 63% WP, Kresoxim methyl 15%+Chlorothalonil 56% WG, Carboxin 37.5%+Thiram 37.5% DS, Propineb 70%WP, Copper oxychloride 50% WP, Penflufen 13.28% w/w+Trifloxystrobin 13.28% w/w, Tebuconazole 25.9% w/w, Fluxapyroxad 167g

l^{-1}+Pyraclostrobin 333 g l^{-1} sc, Tebuconazole 10%+Sulphur 65% WG, Thiophanate methyl 45%+Pyraclostrobin 5% FS was evaluated at 10 µg ml^{-1}, 100 µg ml^{-1} and 250 µg ml^{-1} concentrations by using poisoned food technique (Nene and Thapliyal, 1982). A hundred times higher strength stock solution of each fungicide concentration was prepared in 10 ml sterile water. One ml of stock solution of respective concentration was added to 99 ml of PDA to make the required concentration of the poisoned medium. Twenty millilitre poisoned medium was poured into a sterilized petri plate under aseptic conditions with four replications of each concentration and solidified. The PDA plate without fungicides was also maintained as a control plate. Five mm of mycelia disc of freshly growing culture of *C. truncatum* was placed in the centre of the plate. The plates were incubated in a BOD incubator at 26±1°C. The radial growth of the pathogen was measured on the 7th and 13th day of inoculation.

The percent inhibition (PI) was calculated by using the formula of Vincent (1947).

$$PI = \frac{C-T}{C} X\ 100$$

Where,

C=Growth of test fungus in control plate (mm)

T=Growth of test fungus in treated plate (mm)

2.5. Data analysis

The per cent data of experimental results were angular transformed and analysed using the Opstat online statistical data analysis package.

3. RESULTS AND DISCUSSION

3.1. Symptomatology of anthracnose

Soybean plants were critically observed during the growing season in the research field. The first symptom of anthracnose was noticed in varieties JS 95-60 and JS 93-05 during the last week of July, 2023. Initially, a brown necrotic lesion was found on the stem, leaves and veinlets (Figure 1 a,b,c). Leaves exhibited yellowness, followed by necrotic lesions around the veinlets and the margins (figure 1 a,b). The leave veinlets also showed blackening. In some plants, where the infection spread rapidly in the early stage, plant parts like stem, petiole, and veinlets became black, and leaves were blighted (Figure 1c). Due to blackening and necrotic lesions on the plant parts, the plant appeared to have died. However, the root system was robust, and the plant did not uproot easily (Figure 1c). In the reproductive stage, during pod formation, pods were affected, became brownish-black and shrivelled. Seed formation was ultimately aborted in affected pods (Figure 1d). Partial infection in the pod was

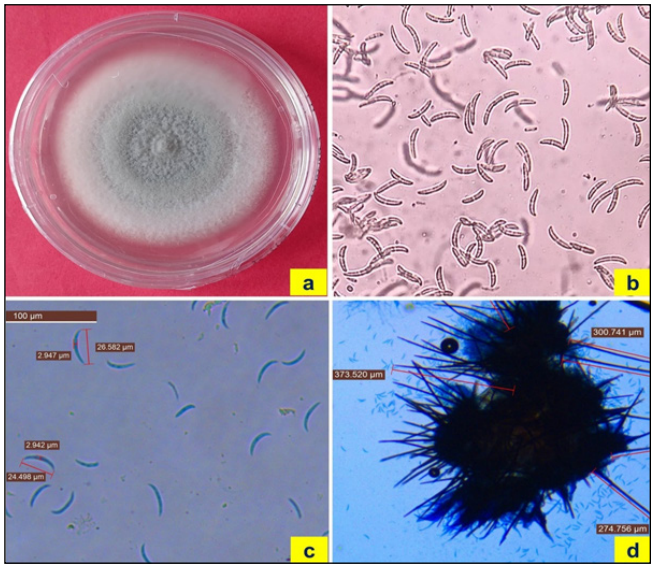

Figure 2: Pure culture plate (a), and measurement of Conidia and sitea (b, c and d) of *C. truncatum*

also noticed with numerous small black fungal bodies called acervulus (Figure 1e). In some cases, either stem/petioles or pods were affected with black, irregular, long lesions (figure 1 f,g,h). In severe infection, the whole plant, including pods, became brownish-black and blighted (figure 1i). The symptoms of pod blight, in the form of dark brown lesions, also appeared in inoculated pods after 7–10 days of inoculation in the lab. Other researchers also reported Anthracnose necrotic lesions and black fruiting with tiny black spines (setae) on infected tissue of different parts prominently at advanced stages of disease development (Sharma et al., 2011; Amrate et al., 2023)

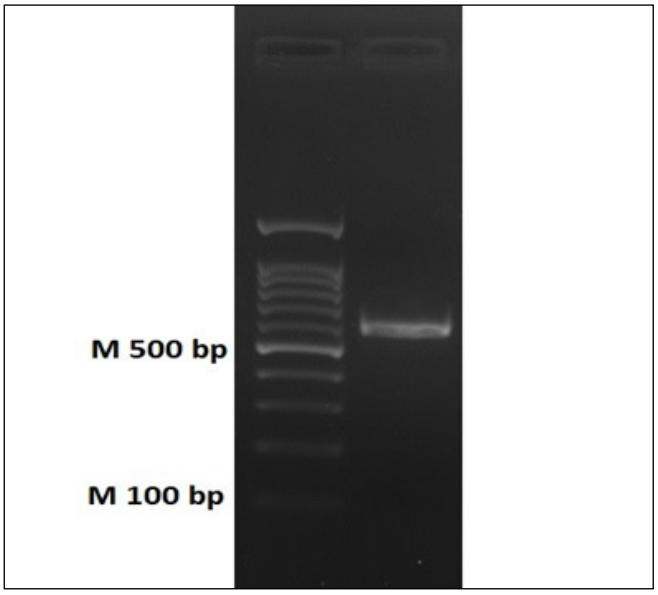

Figure 3: PCR amplification of ITS region of *C. truncatum* by using ITS4 and ITS 5 primer

3.2. Morpho-cultural characterization of pathogen

In potato dextrose agar, the colony's growth was whitish in the initial days, and it turned greyish later with numerous conidia (figure 2). The conidia appeared colourless, crescent-shaped, with blunt ends. An oil globule was also noticed in the centre of the conidia. In the later growth stage, dark brown colour setae were formed in the culture, which was like a spine more comprehensive at the base (figure 2). Ten conidia's average length and width ranged from 20–28 µm and 2.94 µm, respectively, at 13-day-old culture. The average length of setae was 215.95 µm (figure 3). Accordingly, the inciting agent of anthracnose was identified as *C. truncatum*. Several researchers also reported similar morph-cultural traits of *C. truncatum* associated with soybean anthracnose (Sharma et al., 2011; Shinde et al., 2016).

3.3. Molecular pattern of pathogen

PCR amplification of ITS-5.8S rDNA region of *C. truncatum* (Jabalpur isolate) by using the ITS 4 and ITS 5 primer, the isolate produced about 580 bp amplicon (figure 3). The sequences of amplified regions were obtained and aligned using the BLASTn sequence alignment tool at NCBI. Our sequences showed the highest similarity

Table 1: Mycelial growth (mm) and sporulation of *C. truncatum* on different medium

Treatments	Media	Colony growth (mm)			Sporulation	Colony appearance
		3rd day	7th day	11th day		
T_1	Potato dextrose agar	21.10	57.93	81.33	++++	Slightly fluffy
T_2	Corn meal agar	20.00	53.30	83.13	++	Appresed
T_3	Oat meal agar	22.67	34.50	59.33	+++	Fluffy
T_4	Rose bengal agar	17.66	43.17	79.17	+++	Slightly fluffy
T_5	Soybean seed extract agar	20.10	48.27	62.33	++	Appresed
T_6	Soybean leaf extract agar	26.33	65.33	85.10	+++	Appresed
T_7	Richards agar	23.50	64.00	76.67	++++	Appresed
	SEm±	0.62	0.74	0.88	-	-
	CD (p=0.05)	1.91	2.28	2.77	-	-

++++ =excellent, +++ =good, ++ =fair

of 99.12% (KX621963.1), (MN202701.1) followed by 98.95% (KY799045.1), KY799043.1), (KY799042.1) with *C. truncatum* sequences previously submitted at NCBI. The sequence of Jabalpur isolate of *Colletrichum* spp was submitted to NCBI with Gene bank accession number PP930734. Previous to this, Bhatt et al. (2022) also found 480–610 bp amplicon (mostly at 560 bp) of ITS-5.8S rDNA of 24 isolates of *Colletotrichum* spp causing pod blight/anthracnose disease in soybean by using ITS4 and ITS5 primers. Kavanashree et al. (2022) also found *C. truncatum* isolates amplified at 550 bp. Other researchers also successfully characterized *C. truncatum* or spp. associated with anthracnose in soybeans by using ITS primers (Forseille et al., 2011; Marmat et al., 2017).

3.4. Effect of different media on pathogen's growth and sporulation

The radial growth of test fungus was significantly varied from 17.66 (Rose Bengal Agar) to 26.33 mm (Soybean leaf extract agar) and 34.50 (Oat meal agar) to 65.33 mm (Soybean leaf extract agar) on 3rd and 7th day of inoculation, respectively (Table 1). On the eleventh day of inoculation, soybean leaf extract demonstrated significant

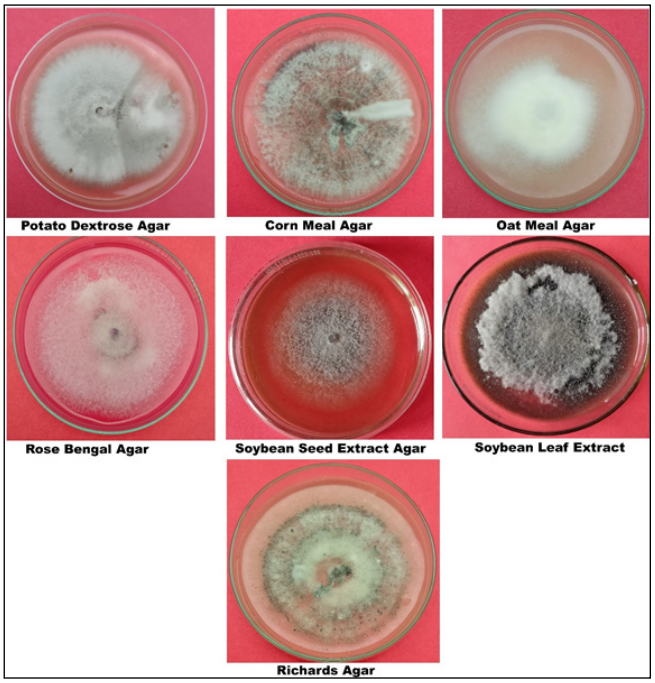

Figure 4: Effect different fungal growing media on the mycelial growth and colony development of *Colletotrichum truncatum*

maximum mycelia growth (85.10 mm), followed by corn meal agar (83.13 mm) and potato dextrose agar (81.33 mm), respectively. Oatmeal agar (59.33 mm) and soybean seed extract agar (62.33) showed the least significant development of mycelia growth. Richard's agar and potato dextrose agar showed excellent sporulation of *C. truncatum*. Soybean leaf extract agar, which showed high growth, also exhibited good sporulation. In contrast, fair sporulation was recorded in the case of Soybean seed extract agar and corn meal agar. The colony appearance was slightly fluffy in PDA and rose bengal agar; however, in corn meal agar, soybean seed extract agar, and soybean leaf extract agar, the colonies were appressed. Additionally, there was a variation in colony colours such as light grey in PDA, intermixed light grey and brown in corn meal agar, dull white in rose Bengal agar, grey in soybean seed extract agar, dark grey in soybean leaf extract agar, whitish in oatmeal agar, and greyish brown in Richard's agar (figure 4). Shirshikar (1995) reported that PDA, soybean leaf extract agar and soybean seed extract agar were suitable medium for the colony development of *C. truncatum*. Corn meal agar, Potato dextrose agar and Richard's agar medium also found suitable for the mycelial growth of *Colletotrichum spp* (Sardhara et al., 2016).

3.5. In vitro testing of new-generation fungicide

The radial growth of pathogen varied from 25.15 (Thiophanate methyl 45%+Pyraclostrobin 5% FS) to 83.00 mm (Copper oxychloride 50% WP), 17.38 (Carbendazim 12%+Mancozeb 63% WP) to 73.63 mm (copper oxychloride 50% WP) and 7.88 mm (thiophanate methyl 45%+Pyraclostrobin 5% FS) to 66.13 mm (copper oxychloride 50% WP) at 10, 100 and 250 µg ml^{-1}, respectively in comparison to control plate (>88.00 mm) at 13 days of inoculation (figure 5). The per cent growth inhibition of fungus was significantly affected by all three concentrations of fungicide at 7 and 13 days after inoculation (Table 2). The significant highest and lowest growth inhibition was recorded by thiophanate methyl 45%+Pyraclostrobin 5% FS and Copper oxychloride 50% WP, respectively, at 7 days of inoculation in the case of all three concentrations. However, at 13th days of inoculation, at 250 µg ml^{-1}, the per cent growth inhibition was significantly higher in thiophanate methyl 45%+Pyraclostrobin 5% FS (91.12%) followed by Carbendazim 12%+Mancozeb 63% WP (88.91%), Carboxin 37.5%+Thiram 37.5% DS (88.01%) and Tebuconazole 25.9% w/w (77.00%), respectively. At 100 µg ml^{-1}, the significantly maximum inhibition was recorded from Carbendazim 12%+Mancozeb 63% WP (80.34%) followed by Thiophanate methyl 45%+Pyraclostrobin 5% FS (79.09%), Carboxin 37.5%+Thiram 37.5% DS (70.72%) and Tebuconazole 25.9% w/w (67.90%), respectively. At 10 µg ml^{-1}, the per cent growth inhibition was significantly higher in Thiophanate methyl 45%+Pyraclostrobin 5%

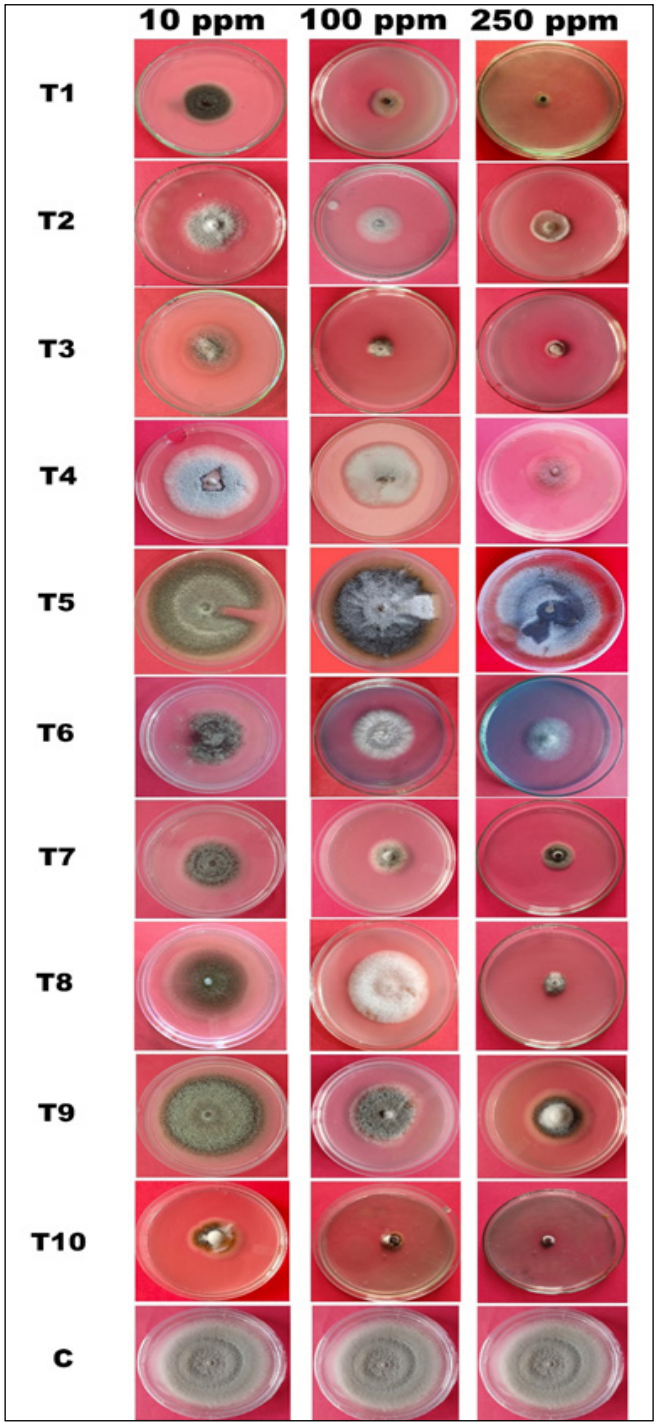

Figure 5: Effect of different fungicidal concentrations on mycelial growth of *C. truncatum* i.e. T_1: Carbendazim 12%+Mancozeb 63% WP, T_2: Kresoxim methyl 15%+Chlorothalonil 56% WG, T_3: Carboxin 37.5%+Thiram 37.5% DS, T_4: Propineb 70% WP, T_5: Copper oxychloride 50% WP, T_6: Penflufen 13.28% w/w+Trifloxystrobin 13.28% w/w FS, T_7: Tebuconazole 25.9% w/w, T_8: Fluxapyroxad 167 g l^{-1}+Pyraclostrobin 333 g l^{-1} SC, T_9: Tebuconazole 10%+Sulphur 65% WG, T10: Thiophanate methyl 45%+Pyraclostrobin 5% FS; C: Control, sequentially at 13th day of Incubation

Table 2: Per cent mycelial growth inhibition of *C. truncatum* by modern fungicides

Treatment	Fungicides	Growth inhibition (%)					
		10 µg ml^{-1} (ppm)		100 µg ml^{-1} (ppm)		250 µg ml^{-1} (ppm)	
		7th day	13th day	7th day	13th day	7th day	13th day
T_1	Carbendazim 12%+Mancozeb 63% WP	66.55 (54.65)	52.62 (46.48)	82.61 (65.54)	80.34 (63.65)	88.83 (70.46)	88.9 (70.52)
T_2	Kresoxim methyl 15%+Chlorothalonil 56% WG	50.88 (45.48)	43.30 (41.12)	66.19 (54.42)	63.23 (52.64)	78.95 (62.70)	74.62 (59.72)
T_3	Carboxin 37.5%+Thiram 37.5% DS	55.55 (48.17)	46.74 (43.11)	72.10 (58.09)	70.72 (57.22)	87.91 (69.63)	88.01 (69.71)
T_4	Propineb 70%WP	21.94 (27.87)	23.53 (28.91)	45.11 (42.17)	36.54 (37.17)	59.94 (50.73)	51.62 (45.90)
T_5	Copper oxychloride 50% WP	4.74 (12.54)	6.98 (15.29)	13.72 (21.65)	16.69 (24.08)	28.31 (32.12)	25.38 (30.22)
T_6	Penflufen 13.28% w/w+Trifloxystrobin 13.28% w/w FS	53.21 (46.83)	34.49 (35.94)	53.04 (46.72)	45.93 (42.64)	66.56 (54.65)	62.05 (51.95)
T_7	Tebuconazole 25.9% w/w	55.88 (48.36)	37.33 (37.64)	77.40 (61.56)	67.90 (55.47)	83.66 (66.16)	77.00 (61.32)
T_8	Fluxapyroxad 167 g l^{-1}+Pyraclostrobin 333 g l^{-1} SC	39.17 (38.72)	25.14 (30.07)	57.07 (49.04)	45.54 (42.42)	80.39 (64.61)	72.51 (58.39)
T_9	Tebuconazole 10%+Sulphur 65% WG	15.21 (22.90)	10.99 (19.34)	49.90 (44.92)	40.87 (39.72)	59.57 (63.16)	52.87 (46.63)
T_{10}	Thiophanate methyl 45%+Pyraclostrobin 5% FS	80.88 (64.06)	71.80 (57.90)	83.08 (65.61)	79.09 (62.78)	100.0 (90.00)	91.12 (72.67)
T_{11}	Control	-	-	-	-	-	-
	SEm±	1.32	0.43	0.55	0.44	0.75	0.62
	CD (p=0.05)	3.84	1.24	1.59	1.27	2.17	1.81

Values in parenthesis are angular transformed

FS (71.80%) followed by Carbendazim 12%+Mancozeb 63% WP (52.62%), Carboxin 37.5%+Thiram 37.5% DS (46.74%) and Tebuconazole 25.9% w/w (37.33%), respectively. Copper oxychloride 50% WP was found to be least effective as recorded lowest growth inhibition of 25.38, 16.69 and 6.98% at 250, 100 and 10 µg ml^{-1}, respectively, on the 13th day of inoculation.

In previous findings, Kale and Barhate (2016) and Jagtap et al. (2013) reported carbendazim as the best growth inhibitor of *C. truncatum*. Similar to our findings, Mandloi et al. (2023) also found significant high inhibition of *C. truncatum* from propiconazole 25% EC and carboxin 37.5%+thiram 37.5% WS and carbendazim 25%+mancozeb 50% at 100, 200, 500 and 1000 ppm. However, in our findings, the fungicides such as Thiophanate methyl 45%+Pyraclostrobin 5% FS, Carbendazim 12%+Mancozeb 63% WP, and Carboxin 37.5+Thiram 37.5% DS showed a high level of significant inhibition even at 10 PPM (10 µg ml^{-1}) concentrations. Our findings also validated Mandloi et al. (2023) report wherein Tebuconazole 10%+Sulphur 65% WG reported as a less effective fungicide in mycelial growth inhibition of *C. truncatum*. In field testing, triazoles and strobilurin, such as Picoxystrobin 7.05%+Propiconazole 11.71% w/w also showed significant efficacy in the management of soybean anthracnose (Rajput et al., 2022). The fungicides showed efficacy against *C. truncatum* such as Thiophanate methyl 45%+Pyraclostrobin 5% FS, Carboxin 37.5%+Thiram 37.5% DS and Penflufen 13.28% w/w+Trifloxystrobin 13.28% w/w FS in this investigation, were also reported effective seed dresser to reducing down the infection of *M. phaseolina* in soybean (Sagarika et al., 2023). Hence, our findings could be valuable, and these highly efficient fungicides may be tested for field applicability in the form of seed treatment and foliar application for prevention and cure of Anthracnose infection (*C. truncatum*) and other fungal agents causing disease in soybean.

4. CONCLUSION

Jabalpur isolate of Anthracnose causal agent (PP930734) showed the highest similarity of 99.12% with *C.*

truncatum. Soybean leaf extract agar (85.10 mm), corn meal agar (83.13 mm) and potato dextrose agar (81.33 mm) were found to be significantly superior for mycelia growth. In fungicidal testing, thiophanate methyl 45%+Pyraclostrobin 5% FS followed by Carbendazim 12%+Mancozeb 63% WP and Carboxin 37.5%+Thiram 37.5% DS exhibited high efficacy in mycelia growth inhibition of *C. truncatum*..

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