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Evaluation of the fungicides, botanicals and bioagents against *Colletotrichum truncatum* causing anthracnose of Soybean in pot culture

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Abstract
Soybean (*Glycine max* (L.) Merill) is one of the world's most important oilseed cum legume crop. Soybean (Glycine max (L.) Merill) is one of the world's most important oilseed cum legume crop. It has highest protein content among leguminous crops belonging to the family Leguminaceae. Among several diseases, Anthracnose caused by *Colletotrichum truncatum* (Schw) Andrus and Moore is one of the most destructive disease of soybean crop, causing great losses and the disease has attained serious proportion in soybean growing areas of Khandesh. Therefore, present investigations were carried out on the aspects viz. isolation of the pathogen, pathogenicity, identification of the pathogens, in vitro evaluation of the fungicides, botanicals and bioagents against the pathogen and in vivo (pot culture) evaluation of the fungicides. The present research work was conducted at Plant Pathology Section, College of Agriculture, Dhule during 2012-2013. Result reveal that treatments carbendazim @ 0.1% recorded maximum disease control (76.78%) and was found significantly superior over rest of the fungicidal treatments. The next best treatments were Tebuconazole @ 0.1% (63.35%), followed by the Propiconazole @0.1% (60.05%) and Hexaconazole @ 0.1% (49.71%) of disease control. While, Difenconazole @ 0.1% showed minimum (40.61%) disease control.

Keywords: Soybean, anthracnose, *Trichoderma harzianum*, carbendazim, *Pseudomonas fluorescens*

Introduction
Soybean (*Glycine max* (L.) Merill) is one of the world's most important sources of oil and protein. It has highest protein content among Leguminous crops belonging to the family Leguminaceae. Besides its economic value, it is highly nutritious food to human being as its 40 per cent protein content, 20 per cent carbohydrates and 23 per cent oil, holds a great promise in meeting most of the need in human diet (Chandel, 2002) [7]. Soybean also contains valuable amino acids. In addition, it contains a good amount of minerals, salts and vitamins (Thiamine and Riboflavin) and is cheapest source of proteins, hence called 'poor man's meat'. The protein quality of soybean is equivalent to that of meat, milk and eggs. It has highest protein content among Leguminous crop (EI-Abady *et al*., 2008) [8]. Moreover, soybean ranks first as source of vegetable oil. It is highly nutritious and used for preparation of bread, biscuits, cake, baked bean, green bean, flour, soya milk, etc. It is being used extensively for paints and varnishes, soap, candies, glycerine, printing ink and also in fibre and plastic industries. Additionally, soybean is able to leave residual nitrogen effect for succeeding crop equivalent to 35-40 kg nitrogen/ha and serve as a good intercrop or mixed crop with maize, sorghum, pigeon pea, etc. In India area, production and productivity of soybean during 2011 were 9.60 million ha., 12.74 million tons and 1327 kg/ha. respectively (Anonymous, 2012) [1]. Soybean growing major states in the country are Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Rajasthan, Gujrat, Uttar Pradesh, Punjab and Haryana (Bhatnagar, 1997) [4]. Among the major fungal diseases of soybean, anthracnose (pod blight) caused by *Colletotrichum truncatum* (Schw) Andrus and Moore, has been reported as the major constraint in the successful cultivation of soybean (Khan and Sinclair, 1992 and Mittal *et al*., 1993) [16, 21]. The disease has been reported geographically widely distributed on soybean crop especially in tropics (Hepperly *et. al.*, 1983) [13], under warm (20-25 °C) and humid conditions (Sinclair and Backman, 1989) [23].
The soybean crop is susceptible to *Colletotrichum truncatum* at all stages of development particularly from bloom to pod fill. The most prominent symptom occurred on foliage were brown coloured patches with gray coloured centre on upper surface and scorched appearance on the lower surface. The disease cause considerable damage by reducing plant stand, seed quality, seed germination and yield. Foliar symptoms include necrosis of leaf veins, leaf

rolling, petiole canker and premature defoliation. However, pod blight phase is most damaging (Vyas *et al.*, 1997) [25]. Symptoms of the disease typically appear at the early reproductive stages on stem, pods and petioles as irregularly shaped brown lesions (Sinclair and Backman, 1989) [23] often as a result of latent infection (Cerkauskas, 1988) [5]. Considering, the importance of anthracnose disease of soybean and losses incurred in the farmer's field, it was felt necessary to investigate on anthracnose disease problem in Khandesh region of Maharashtra.

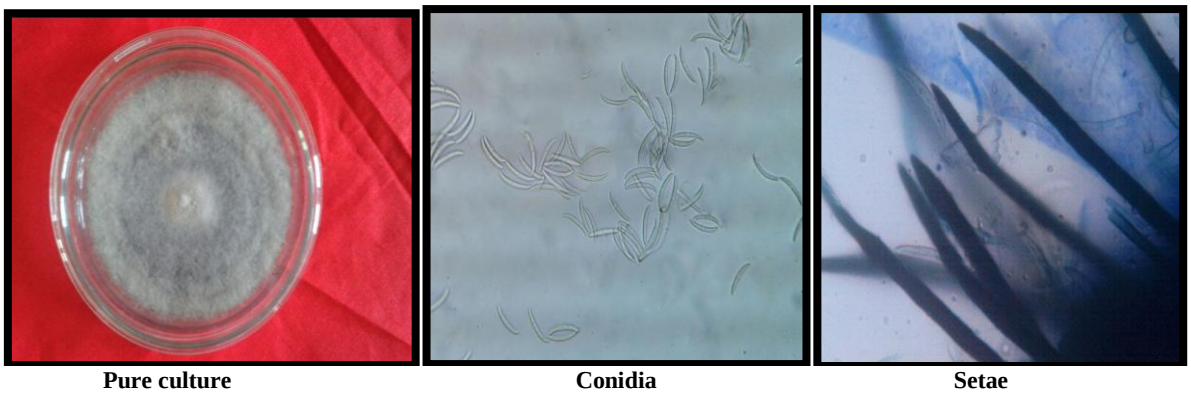
Material and Methods

The details of the materials used and methods followed for various experiment are described here in the following paragraphs.

Leaves and pods exhibiting typical symptoms of anthracnose and pod blight diseased sample were collected separately from the field-grown soybean plants from the farm of Agriculture College, Dhule and farmers fields in the vicinity of the College. Potato dextrose agar (PDA), the common laboratory culture medium was used as basal medium for isolation, multiplication and maintenance of the pure culture of *C. truncatum.* Diseased leaves (anthracnose) and pods (blighted) of soybean collected from various fields were brought to the laboratory and washed thoroughly in running tap water. These diseased specimens (leaves, pods) were blot dried and cut with sharp sterilized blade into small bits (5mm) keeping half healthy and half diseased portion intact. These pieces were surface sterilized with 0.1% aqueous solution of mercuric chloride ($HgCl_2$) for two minutes and then washed by giving three changes with sterile distilled water to remove traces of mercuric chloride and blot dried. The surface sterilized diseased pieces were then inoculated on the solidified and cooled PDA (Potato dextrose Agar) medium in petri plates under aseptic conditions of Laminar-air-flow cabinet. Inoculated plates were then incubated in BOD incubator at 24±2 °C temperature. Three to four days of incubation, the well-developed mycelial growth free from any contaminant was obtained. Following single hyphal-tip technique, the fungus was transformed / subcultured aseptically onto the PDA slant in test tubes. Through frequent sub - culturing, the fungus was purified and pure culture was maintained on agar slants in test tubes stored in refrigerator for further studies.

Seeds of JS-335 variety of soybean were obtained from the Agriculture College, Dhule for conducting the pathogenicity test. Pathogenucity is cinducted by surface sterilized (0.1% $HgCl_2$) seeds of anthracnose succeptible soybean Cv. JS-335 were sown (@ 10 seeds /pot) in the earthen pots (25 cm dia) filled with steam sterilized potting mixture of soil : sand : FYM (2:1:1). Five healthy growing soybean seedlings per pot were maintained, watered regularly and kept in the screen house for further growth. The test pathogen *(C. truncatum)* was mass multiplied on the basal culture medium PDA in petri dishes. Spore suspension of the test pathogen was prepared by harvesting freshly sporulating 7-8 days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore-cum-mycelial suspension was filtered through double-layered muslin cloth and filtrate obtained was suitably diluted with sterile distilled water to get inoculum concentration of 3-5 x 10^6 spores/ml. Thirty days old seedlings of soybean Cv. JS-335 were artificially inoculated by spraying with automizer the conidial suspension (3-5 x 10^6 conidial/ ml) of the test pathogen. Seedlings sprayed with sterile water (without inoculum) were also maintained as suitable control. Inoculated plants were incubated in the screen house where high humidity (>80%) and optimum temperature (24±2 °c) were maintained for further development of anthracnose symptoms.

The isolates of *Trichoderma harzianum* and *Psuedomonas flurescence* bioagents obtained from the MPKV Rahuri. Based on microscope observations of spores and mycelium of the fungus (Lenne 1992; Sinclair and Backman, 1989) [17, 23], the fungus was identified upto species level with the help of published literature (Barnet and Barry, 1972) [2] and confirmed.



Pure culture **Conidia** **Setae**

Plate 1: Microphotographs of pathogen *Colletotrichum truncatum* causing anthracnose of soybean

Table 1: fungicides, botanicals and bioagents were used for in vivo (pot culture experiments) conducted during present studies.

Sr. No.	Common name	Trade name	Manufacturer
Fungicides			
1	Carbendazim	Bavistin 10 WP	BASF India Ltd. Mumbai
2	Propiconazole	Tilt 25 EC	Syngenta Agrochemicals Mumbai
3	Mancozeb	Dithane M45 75WP	Dow Agrosciences, Mumbai
4	Propineb	Antracol 70 WP	Bayer Crop Science Ltd. Mumbai
5	Copper oxychloride	Blitox 50 WP	Rallis India Ltd. Mumbai
6	Chlorothalonil	Kavach 75 WP	Syngenta Agrochemicals Mumbai
7	Hexaconazole	Contaf 5 SC	Rallis India Ltd, Mumbai
8	Tebuconazole	Folicur 250 EC	Bayer Crop Science Ltd. Mumbai

9	Tricyclazole	Spyker 75 WP	Coromendel Agrico Pvt. Ltd. UP
10	Benomyl	Benofit 50 WP	Corommendel Fertilisers Ltd.
11	Thiophanate methyl	Roko 70 WP	Biostadt India Ltd. Mumbai
12	Difenconazole	Score 25 EC	Syngenta Group Company
13	Penconazole	Topas 10 EC	Syngenta Group Company
14	Carbendazim12% +Mancozeb 63%	Saaf 75 WP	United Phosphorous Ltd. Gujarat
15	Ziram	Cuman'L 27 SC	Syngenta Group Company
Botanicals			
1	Neem	*Azadirachta indica*	
2	Eucalyptus	*Eucalyptus spp*.	
Bioagents			
1	*T. harzianum*		
2	*Pseudomonas fluorescens*		

a) Pot culture experiment details

Design : CRD
Variety : JS-335
Replications : Three
Treatments : Six

b) Treatments

T1 : Carbendazim @ 0.1%
T2 : Tebuconazole @ 0.1%
T3 : Propiconazole @ 0.1%
T4 : Hexaconazole @ 0.1%
T5 : Difenconazole @ 0.1%
T6 : Control

Spraying

Twenty five days after sowing (DAS) artificial inoculation was done by spraying spore suspension to develop disease on plants. On the fifth day of inoculation disease was developed. In all three sprayings were undertaken at intervals of 15 days. One treatment was maintained as unsprayed control without applying any fungicides.

Observations

Observations on foliage anthracnose disease were recorded before and after 15 days of each sprayings. Five plants per treatment in each replication were selected randomly and tagged for recording the observations. Three leaves at bottom, middle and top from main branch on each plant were selected for recording observations and per cent anthracnose disease intensity / index (PDI) and percent disease control (PDC) was worked out by using following scale and formula.

The anthracnose intensity was recorded by following

Table 2: Disease Rating Scale scale 0 – 9. (Mayee and Datar, 1986) [19].

Category	Description
0	No symptoms on leaves
1	Small pin-head size lesions covering 1% or less leaf area
3	Small pin-head size lesions covering 1-10% of leaf area
5	Lesions big but not coalescing, covering 11-25% of the leaf area
7	Lesions on leaves covering 26-50% of leaf area. Cankers on stem and pod infection
9	Lesions on leaves covering 51% or more of leaf area. Defoliation of leaves, deep cankers on stem and pods, blighting of plant occurs

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{Total no. of leaves examined / plant x Maximum disease grade}} \times 100$$

$$\text{Percent Disease Control} = \frac{\text{PDI in control pot - PDI in treatment pot}}{\text{PDI in control pot}} \times 100$$

Results

Present studies on the anthracnose *(Colletotrichum truncatum* (Schw.) Andrus and Moore of soybean (Glycine max L. Merill) were undertaken in Pathology section of Agriculture College, Dhule during Kharif, 2012 on the aspect viz., isolation, pathogenicity test, evaluation of fungicides, botanicals and bioagents evaluation of fungicides in vivo (Pot culture). The results obtained are being presented as follows.

The test pathogen was isolated from infected leaves and pods (blighted) of soybean on potato dextrose agar (PDA) medium. The colonies of the fungus developed on PDA were creamy to blackish-grey, with thin mat of mycelium (Plate 1).

Symptoms were developed on inoculated leaves after 8-10 days of inoculation (Plate 2). The leaves showed characteristic brown coloured patches with grey colored centre on upper surface and scorched appearance on lower surface as those observed under natural infection. However, the plants those were under control, only sprayed by sterilized distilled water only, they did not produce any kind of symptoms throughout the period of observation.

Pathogen is identified by a piece of sporulating mycelium was mounted with lactophenol cotton blue and observed under the light microscope. Based on typical symptoms on foliage and pods, cutural characteristic of the fungus on PDA and microscopic observations recorded such as, mycelium -

hyaline, septate and branched. Acervuli - the acervuli were oval to conical and appeared single. It was dark brown to black in colour and measured 181.0 X 275.5µ in size, with numerous black, needle like intermixed long and short setae. Conidia - single celled, smooth, hyaline, curved and measured 21 to 23.5 X 3.8 to 4.1 µ in size. Conidiophore- simple and elongate. The measurements were recorded with the help of stage and occular micrometer. The fungus has been identified and confirmed with the help of available literature as *Colletotrichum truncatum* (Schw) Andrus and Moore, causing anthracnose of soybean.

Initial symptoms of anthracnose on foliage were noticed at 25-30 DAS on soybean crop. The most prominent symptoms occurred on foliage were brown coloured patches with gray coloured centre on upper surface and scorched appearance on the lower surface. In advance stage necrosis of leaf vein, leaf rolling, petiole canker, and defoliation occurred. Typical symptoms observed on the pods were reddish brown spots which later turns black. Acervulli on infected pods resembled small pinkish coloured patches surrounded by the minute blackish brown setae. Infected pods finally dried out prematurely with shrivelled and moldy seeds.

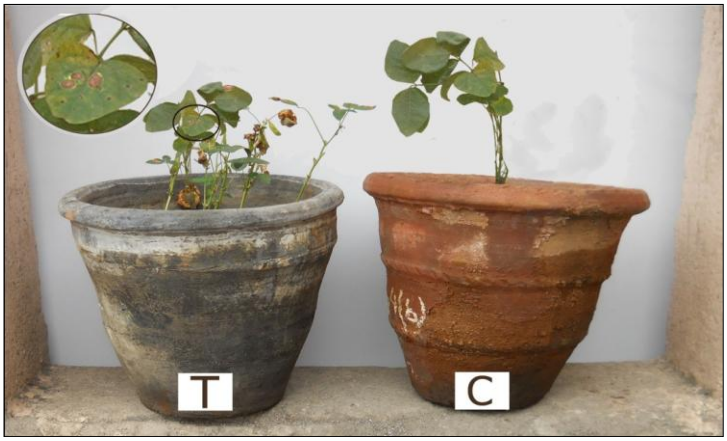


Plate 2: Pathogenicity test of *Colletotrichum truncatum* on soybean. T= Treated C= Control

The organism was reisolated on PDA medium and was compared with original culture of test pathogen. The same was found identical to that of the original culture, thereby confirming the test of pathogenicity.

Among the fungicides tested five fungicides which were found best in in vitro were evaluated in vivo (pot culture) against anthracnose of soybean using susceptible cultivar JS-335 during Kharif, 2012. Results obtained revealed that all 5 fungicides tested were found significantly effective and reducing the disease intensity over unsprayed control. Results obtained in respect of disease intensity are presented as follows.

After 1st spray

Results (Table 3 and Fig 1) revealed that the treatment Carbendazim was significantly superior over all other treatments, which recorded 18.18% percent disease index (PDI) and maximum percent disease control (PDC) 36.98%. The next best treatments were Tebuconazole which recorded 20.22% PDI and 29.91% PDC, followed by Hexaconazole, Propiconazole and Difenconazole which recorded PDI of 22.72%, 23.18% and 25.44% and 21.24%, 19.65% and 11.81% PDC, respectively. The maximum PDI was observed in control 28.85%.

Table 3: Effect of fungicides on anthracnose disease intensity in soybean after subsequent spray.

T. No	Treatments	Conc. (%)	Percent Disease Index (Mean)*	Percent Disease Control	Percent Disease Index (Mean)*	Percent Disease Control	Percent Disease Index (Mean)*	Percent Disease Control
			1st spray (30 DAS)		2nd spray (45 DAS)		3rd spray (60 DAS)	
T1	Carbendazim	0.1	18.18 (25.24)	36.98	11.48 (19.80)	61.39	7.30 (15.67)	76.78
T2	Tebuconazole	0.1	20.22 (26.72)	29.91	17.18 (24.49)	42.23	11.52 (19.84)	63.35
T3	Propiconazole	0.1	23.18 (28.78)	19.65	21.70 (27.77)	27.03	12.56 (20.75)	60.05
T4	Hexaconazole	0.1	22.72 (28.47)	21.24	22.22 (28.12)	25.28	15.81 (23.43)	49.71
T5	Difenconazole	0.1	25.44 (30.29)	11.81	24.55 (29.71)	17.45	18.67 (25.60)	40.61
T6	Control	-	28.85 (32.49)	-	29.74 (33.05)	-	31.44 (34.11)	-
S.E±		-	0.09	-	0.13	-	0.09	-
C.D. (at 0.05%)		-	0.29	-	0.41	-	0.27	-

* Mean of three replications. Figures in parenthesis indicates Arc sin transformed values

After 2nd spray

The results indicated, that the treatment Carbendazim showed significantly superior over all other treatments, which recorded least PDI 11.48% and maximum PDC 61.39%. The next best treatment were Tebuconazole recorded 17.18% PDI and 42.23% PDC, followed by Propiconazole, Hexaconazole and Difenconazole with 21.70%, 22.22% and 24.55% PDI and 27.03%, 25.28% and 17.45% PDC, respectively. The maximum PDI was recorded in control 29.74%.sssssss

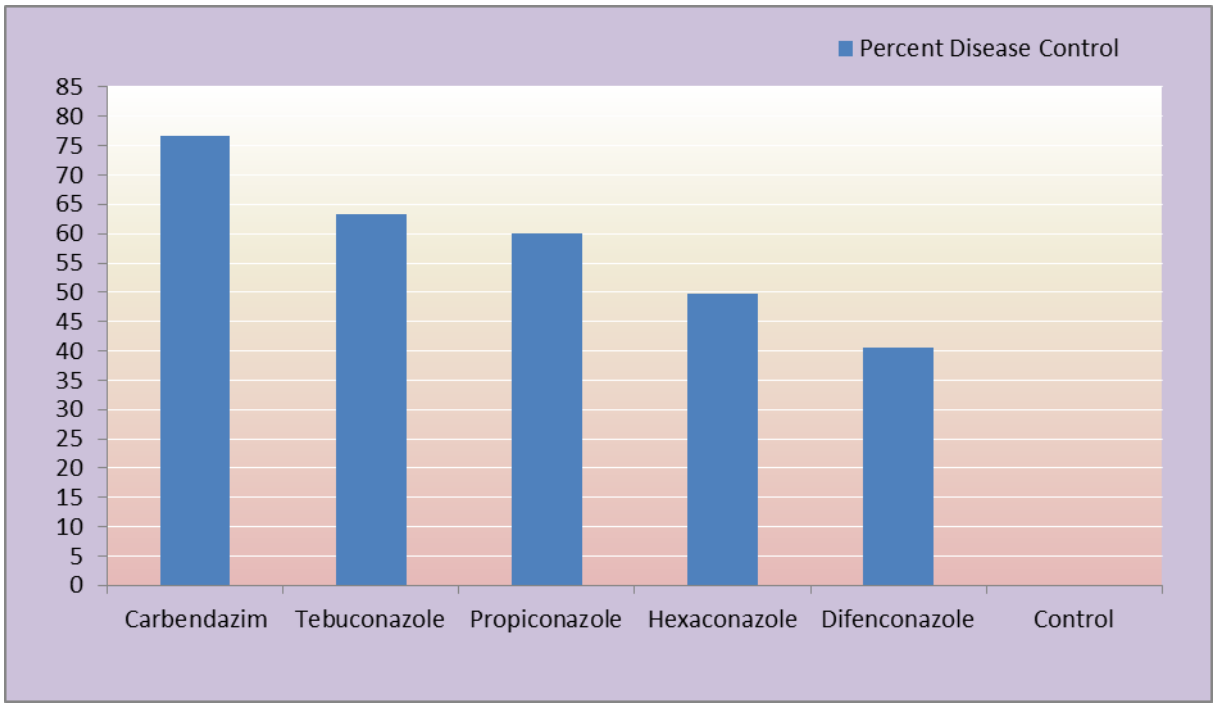


Fig 1: Effect of fungicides on anthracnose of soybean disease in vivo (Pot culture) after 3rd spray (60 DAS).

After third spray

The results revealed that the Carbendazim recorded significantly minimum PDI 7.30% and was significantly superior over the rest of the treatments. The next best treatments were Tebuconazole recorded 11.52% PDI, followed by Propiconazole, Hexaconazole and Difenconazole which recorded 12.56%, 15.81% and 18.67% PDI, respectively over control (31.44%).

The results obtained in respect of percent disease control indicated that the fungicide Carbendazim @ 0.1% recorded maximum PDC (76.78%) and was significantly superior over rest of the fungicidal treatments. The next best treatments were Tebuconazole @ 0.1% (63.35%), followed by Propiconazole @ 0.1% (60.05%), Hexaconazole @ 0.1% (49.71%) and Difenconazole @ 0.1% (40.61%) of disease control over untreated control.

Therefore it was concluded that the fungicide Carbendazim @ 0.1% was proved to be the most effective to control the anthracnose disease of soybean over all other treatments under study.

Discussion

Soybean (*Glycine max* (L.) Merill) is one of the most important oilseed cum legume crop grown in India. The losses caused by the anthracnose are obvious on account of severe loss of the yield. On the other hand foliar diseases affected plants resulting in reduction of the size of pods and seeds. Infected pods finally dried out prematurely with shrivelled and moldy seeds. Hence, studies on isolation, pathogenecity, symptomatology and control measures *in vitro* on anthracnose of soybean were undertaken. The results obtained on these aspects during the present investigations are being discussed below.

Isolations of the pathogen *Colletotrichum truncatum* (Schw) Andrus and Moore and pathogenicity is carried out by many scientist Thus results are mostly in agreement with Verma and Upadhyay (1973) [24], Chacko and Khare (1978) [6], Gizard (1979) [10], Mathur and Tyagi (1982) [19], Bhardwaj and Singh (1986) [3], Hartman *et al.* (1986) [12], Manandhar *et al.* (1988) [18] and Khan and Sinclair (1992) [16]. Who reported *Colletotrichum truncatum*, the cause of anthracnose disease of soybean. However, the symptoms observed in the present investigations are similar to those described by Lele and Ashram (1968) for C. dematum on Rauvolfia serpentina by Janardhanan *et al.* (1972) [15] for *C. dematium* on periwrinkle by Gotmare (1981) [11] for C. capsici on chilli by Rizvi and Ahmad (2005) [22] for C. capsici on Chlorophytum borivilianum. Many workers attempt on evaluation of fungicide, botanicals and bioagens against fungal pathogen such as Jagatap *et al.* (2013) [14] and Wagh (2012) [26]. reported that *Colletotrichum* leaf spot of turmeric and *Colletotrichum* fruit rot of chili were effectively controlled by Propiconazole and Hexaconazole, similarly reported that Carbendazim, Propiconazole, Hexaconazole and Difenconazole effectively controlled the *Colletotrichum* anthracnose of soybean. Also, reported the same fungicides for the control of *Colletotrichum* anthracnose of soybean.

Conclusion

Present research work concluded that the pathogen Colletotrichum truncatum was found to be associated with anthracnose of soybean in the Khandesh, the region of Maharashtra state which producing symptoms viz. brown coloured patches with gray coloured centre on upper surface and scorched appearance on the lower surface of leaves. On the pods reddish brown spots showed which later turns black. Acervuli on infected pods resembled small pinkish coloured patches and finally dried prematurely with shrivelled and moldy seeds also having circular, compact colonies and colour of mycelium was grayish later turn black in colour and also proved Carbendazim (0.1%) as most effective in reducing anthracnose disease intensity, followed by Tebuconazole (0.1%) and Propiconazole (0.1 %).

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