Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 1105-1110
Received: 28-03-2019
Accepted: 30-04-2019

MN Agam
Department of Plant Pathology, PGI, MPKV, Rahuri, Maharashtra, India

SB Sable
Department of Plant Pathology, PGI, MPKV, Rahuri, Maharashtra, India

RA Raut
Junior Plant Pathologist, Regional Fruit Research Station, Vengurle, Maharashtra, India

Jejurkar GB
Department of Plant Pathology, PGI, M.P.K.V, Rahuri, Maharashtra, India

Correspondence
MN Agam
Department of Plant Pathology, PGI, MPKV, Rahuri, Maharashtra, India

In vitro evaluation of the fungicides, botanicals and bioagents against *Colletotrichum truncatum* causing anthracnose of soybean

MN Agam, SB Sable, RA Raut and Jejurkar GB

Abstract
Soybean (*Glycine max* (L.) Merill) is one of the world's most important oilseed cum legume crop. The present present research work was conducted at Plant Pathology Section, College of Agriculture, Dhule during 2012-2013. The result revealed that treatments carbendazim @0.1%, Tebuconazole @0.1% and Propiconazole @0.1% were found significantly superior over rest of the treatments, which recorded maximum growth inhibition of 97.67%, 97.67% and 97.28%, respectively of the test pathogen with minimum colony diameter of 2.10 mm, 2.10 mm and 2.45 mm, respectively, all were at par with each other. In case of bioagents and botanicals *Pseudomonas fluorescens* (89.11%), *Trichoderma harzianum* (88.00%), Propineb (87.00%), Neem (84.72%), Chlorothalonil (81.94%) and Copper oxychloride (78.56%), which were recorded mean colony diameter of 9.80 mm, 10.80 mm, 11.70 mm, 13.75 mm,16.25 mm and 19.30 mm, respectively. While, Thiophanate methyl (75.28%), Ziram (66.39%) and Eucalyptus (55.22%) showed least growth inhibition of the test pathogen with mean colony diameter of 22.25 mm, 30.25 mm and 40.30 mm, respectively.

Keywords: Soybean, anthracnose, *Trichoderma harzianum.* Carbendazim, *Pseudomonas fluorescens* and eucalyptus

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. Today, the world production of soybean increased by 4.6% annually from 1961 to 2007 and reached average annual production of 217.6 million tons in 2005-07 and increasing year after year in 21st century. Recently, USA, Brazil, China and Argentina are the four leading countries in soybean production (Tadayoshi and Goldsmith, 2009) [25]. So, there is vast scope for India to improve yield, quality and quantity to capture the world market. 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, good amount of minerals, salts and vitamins (Thiamine and Riboflavin) and is cheapest source of proteins, hence called 'poor man's meat'. It has highest protein content among Leguminous crop (EI-Abady *et al*., 2008) [9].

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. India's share in world production of Soybean is 3%. India is the fifth largest producer of Soybean in the world (Anonymous, 2009-10) [1]. It accounts for 25 per cent of total oilseed produced in the country in a year. It contributes about 0.7 million tons of oil, out of about 7 million tons of vegetable oils currently produced in the country. In India area, production and productivity of soybean during 2011 were 9.60 million ha., 12.74 million t ons and 1327 kg/ha. respectively (Anonymous, 2012) [2]. 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]. More than 100 pathogens are known to affect soybean crop, of which 35 are of economically important (Sinclair and Backman, 1989) [24]. Soybean diseases cause reductions in yield to the tune of 10 to 30% in most of the areas. The most important disease reported to cause economic losses to the soybean crop is anthracnose incited by *Colletotrichum truncatum* (Schw) Andrus and Moore. In soybean about 16-100 per cent losses due to anthracnose incited by *Colletotrichum truncatum* (Schw) Andrus and Moore, frog eye spot (*Cercospora sojina*) cause 15 per cent losses, rust (*Phakospora pachyrhizi*) cause 10-90 per cent losses, downy mildew(*Perenospora monshurica*) cause 8 per cent losses, powdery mildew (*Erysiphae polygoni*) cause 10-35

per cent losses and soybean mosaic cause 25-50 per cent losses (Sinclair, 1992)[23].

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 (Sinclair, 1992) [23]. The disease has been reported geographically widely distributed on soybean crop especially in tropics (Hepperly *et. al.*, 1983) [15], under warm (20-25 °C) and humid conditions (Sinclair and Backman, 1989) [24]. Infected seeds often show brown discoloration. Samples with upto 30 per cent seed discoloration had up to 70 per cent seed infection of *Colletotrichum truncatum* (Hepperly *et. al*., 1983) [15]. Anthracnosed plants are significantly shorter, with fewer pods and seeds with reduced seed weight compared to non affected plants. 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 disease 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. 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. 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.* Seeds of JS-335 variety of soybean were obtained from the Agriculture College, Dhule for conducting the pathogenicity test. The isolates of *Trichoderma harzianum* and *Psuedomonas flurescence* bioagents obtained from the MPKV Rahuri.

For Pathogenicity test Surface sterilized (0.1% HgCl2) 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. Subsequently reisolation, identification and symptomatology is studied.

a) Experiment details

Design : CRD
Replications : Two
Treatments : Twenty

b) Treatments

Tr. No.	Treatments	Concentration
T_1	Carbendazim	0.1%
T_2	Propiconazole	0.1%
T_3	Mancozeb	0.25%
T_4	Propineb	0.3%
T_5	Copper oxychloride	0.3%
T_6	Chlorothalonil	0.25%
T_7	Hexaconazole	0.1%
T_8	Tebuconazole	0.1%
T_9	Tricyclazole	0.1%
T_{10}	Benomyl	0.1%
T_{11}	Thiophanate methyl	0.1%
T_{12}	Difenconazole	0.1%
T_{13}	Penconazole	0.1%
T_{14}	Carbendazim12% +Mancozeb 63%	0.25%
T_{15}	Ziram	0.25%
T_{16}	Neem	10%
T_{17}	Eucalyptus	10%
T_{18}	*T. harzianum*	-
T_{19}	*Pseudomonas fluorescens*	-
T_{20}	Control	-

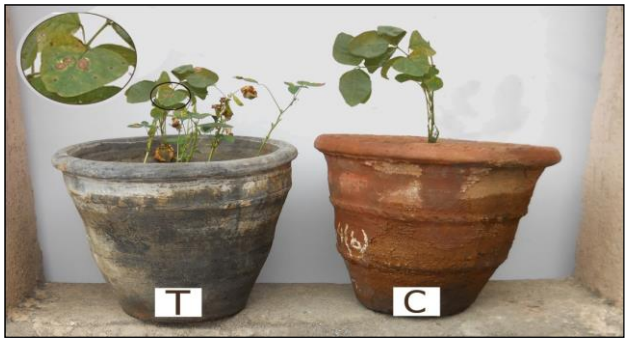


Plate 1: Pathogenicity test of *Colletotrichum truncatum* on soybean. T= Treated C= Control

Poison food technique

The fungicides and plant extracts amended PDA was then poured (15 - 20 ml/plates) in sterilized Petri plates (90 mm dia.) under aseptic conditions. Each treatment with respective concentration was replicated for two times. On solidification of PDA in Petri plats, all treatment plates are inoculated / seeded aseptically by placing in the center with 5.0 mm uniform, mycelial disc obtained from 6-7 days old culture of *C. truncatum* multiplied on agar plates (Nene and Thapliyal, 1993) [20].

Dual culture technique

Disc (5 mm) of *Colletotrichum truncatum* was placed at the center on a petriplates containing solidified PDA medium and disc of *Trichoderma harzianum* was placed at opposite from center. A loopful 24 hour old culture of *Pseudomonas fluorescens* was inoculated at 2 cm just opposite to the pathogen on each plate (Dennis and Webster, 1971) [8].

All the treatment (inoculated) and control petri plates where then incubated at 24 ± 2 °C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen.

Observations on redial mycelial growth of *C. truncatum* where recorded in each treatment and replication and per cent growth inhibition of the test pathogen over control was worked out (Vincent, 1927) [27] as follows.

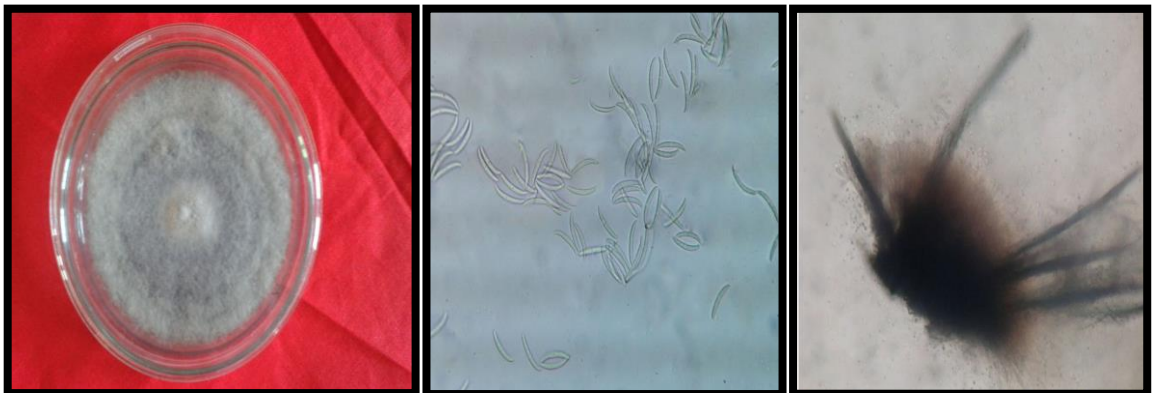
$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,
C = Growth of test fungus (mm) in control plate,
T = Growth of test fungus (mm) in treatment plates

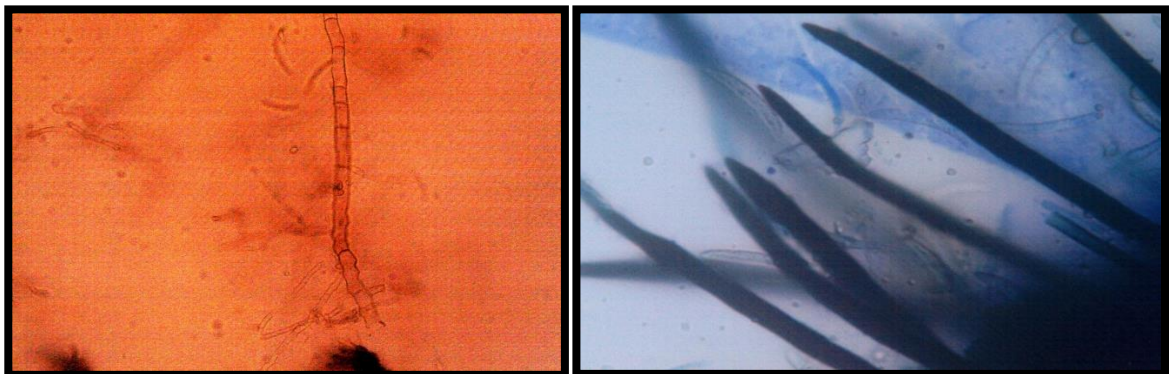
Results

The results obtained are being presented as follows.

Identification of pathogen is done through 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.



Pure culture conidia acervuli



Mycelium Setae

Plate 2: Microphotographs of pathogen *Colletotrichum truncatum* causing anthracnose of soybean

Symptomatology

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.

***In vitro* evaluation of fungicides, botanicals and bioagents**

The result (Table 1 and Fig. 1) revealed that all the treatments exhibited significantly superior over untreated control at their respective concentration tested for the inhibition of mycelial growth of *Colletotrichum truncatum*. The treatments Carbendazim @0.1%, Tebuconazole @0.1% and Propiconazole @0.1% were found significantly superior over rest of the treatments, which recorded maximum growth inhibition of 97.67%, 97.67% and 97.28%, respectively of the test pathogen with minimum colony diameter of 2.10 mm, 2.10 mm and 2.45 mm, respectively, all were at par with each other. The next best treatments were Hexaconazole (95.33%) with the mean colony diameter of 4.20 mm, followed by Difenconazole (94.61%), Tricyclazole (93.50%), Penconazole (92.94%) and Mancozeb (91.89%), with mean colony diameter of 4.85 mm, 5.85 mm, 6.35 mm and 7.30 mm, respectively. It was further observed that Carbendazim 12% + Mancozeb 63% (90.72%) and Benomyl (90.33%) inhibition and recorded mean colony diameter of 8.35 mm and 8.70 mm, respectively and were at par with each other. These were followed by Pseudomonas fluorescens (89.11%), Trichoderma harzianum (88.00%), Propineb (87.00%), Neem (84.72%), Chlorothalonil (81.94%) and Copper oxychloride (78.56%), which were recorded mean colony diameter of 9.80 mm, 10.80 mm, 11.70 mm, 13.75 mm,16.25 mm and 19.30 mm, respectively. While, Thiophanate methyl (75.28%), Ziram (66.39%) and Eucalyptus (55.22%) showed least

growth inhibition of the test pathogen with mean colony diameter of 22.25 mm, 30.25 mm and 40.30 mm, respectively, as against 90 mm in untreated control.

Hence, it was revealed that the fungicides viz. Carbendazim (0.1%), Tebuconazole (0.1%) and Propiconazole (0.1%) were proved most effective in inhibiting the growth of *Colletotrichum truncatum*.

Table 1: *In vitro* effect of fungicides, botanicals and bioagents on radial growth of *C.truncatum.*

Treat. No.	Treatments	Concentration (%)	Mean colony diameter(mm)*	Inhibition (%)
T1	Carbendazim	0.1%	2.10	97.67
T2	Propiconazole	0.1%	2.45	97.28
T3	Mancozeb	0.25%	7.30	91.89
T4	Propineb	0.3%	11.70	87.00
T5	Copper oxychloride	0.3%	19.30	78.56
T6	Chlorothalonil	0.25%	16.25	81.94
T7	Hexaconazole	0.1%	4.20	95.33
T8	Tebuconazole	0.1%	2.10	97.67
T9	Tricyclazole	0.1%	5.85	93.50
T10	Benomyl	0.1%	8.70	90.33
T11	Thiophanate methyl	0.1%	22.25	75.28
T12	Difenconazole	0.1%	4.85	94.61
T13	Penconazole	0.1%	6.35	92.94
T14	Carbendazim12% +Mancozeb 63%	0.25%	8.35	90.72
T15	Zirum	0.25%	30.25	66.39
T16	Neem	10%	13.75	84.72
T17	Eucalyptus	10%	40.30	55.22
T18	*T. harzianum*	-	10.80	88.00
T19	*P. fluorescence*	-	9.80	89.11
T20	Control	-	90.00	0.00
	S.E.+	-	0.13	-
	C.D. (at 0.05%)	-	0.38	-

*Mean of two replications. Figures in parenthesis indicates Arc sin transformed value

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) [26], Chacko and Khare (1978) [5], Gizard (1979), Mathur and Tyagi (1982) [19], Bhardwaj and Singh (1986), Hartman *et al.* (1986), Manandhar *et al.* (1988) [18] and Khan and Sinclair (1992) [23]. 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) [17] for C. dematum on Rauvolfia serpentina by Janardhanan *et al.* (1972) [16] for *C. dematium* on periwrinkle by Gotmare (1981) [12] for C. capsici on chilli by Rizvi and Ahmad (2005) [21] for C. capsici on Chlorophytum borivilianum.

Among the different treatments tested, Carbendazim @ 0.1%, Tebuconazole @ 0.1% and Propiconazole @ 0.1% recorded maximum growth inhibition of 97.67%, 97.67% and 97.28% of the test pathogen, respectively, which were statistically at par with each other. The next best treatments were Hexaconazole (95.33%), followed by Difenconazole (94.61%), Tricyclazole (93.50%), Penconazole (92.94%) and Mancozeb (91.89%), Carbendazim 12% + Mancozeb 63% (90.72%) and Benomyl (90.33%) which were at par with each other. The treatments *Pseudomonas fluorescen*s (89.11%), *Trichoderma harzianum* (88.00%), Propineb (87.00%), Neem (84.72%), Chlorothalonil (81.94%) and Copper oxychloride (78.56%) were also found effective. While, Thiophanate methyl (75.28%), Ziram (66.39%) and Eucalyptus (55.22%) showed least growth inhibition of the test pathogen. These results are in agreement with those reported by Gawade D. B. (2009) who found Carbendazim and Propioconazole to be effective fungicides followed by Hexaconazole and Difenconazole. These results are in accordance with those reported earlier by many workers viz. Chakraborty and Shyam (1988) [6], Gupta *et al.* (2005) [13] in French bean. Shukla and Singh (1993) [22], in soybean.

Conclusions

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 *in vitro* revealed that carbendazim (0.1%), Tebuconazole (0.1%) and Propiconazole (0.1%) as most inhibitory to the test pathogen, followed by Hexaconazole (0.1%) and Difenconazole (0.1%).

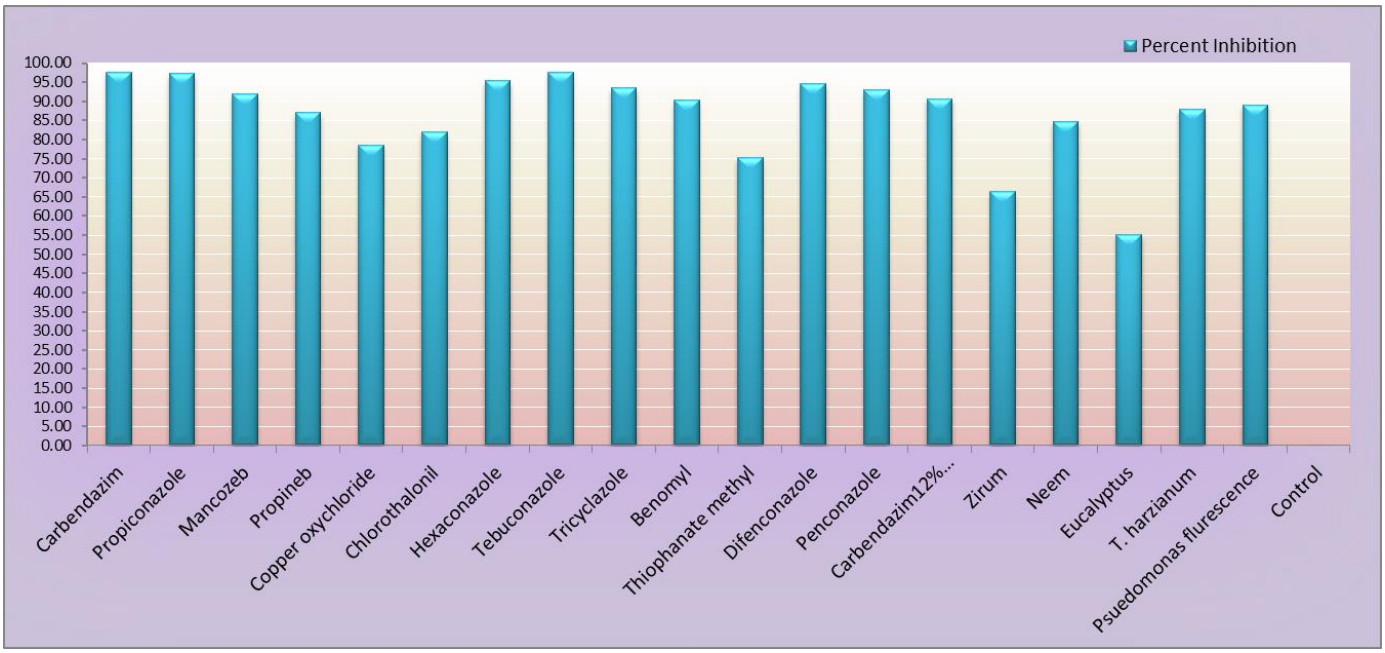


Fig 1: Effect of fungicides, botanicals and bioagents on growth inhibition of *Colletotrichum truncatum.*

References

1. Anonymous. Indian commodity exchange. 2009-10. (WWW.icexindia.com).
2. Anonymous. The report of the Soybean Processors Association of India, 2012. (http://sopa.org).
3. Bhardwaj CL, Singh BM. Strain variation in Colletotrichum dematium f spp, trunchatum from four leguminous hosts. Indian J Mycol. Pl. Pathol. 1986; 16(2):139-141.
4. Bhatnagar PS. An overview of soybean in India strategies for augmenting productivity and production with special reference to combating soybean rust, in: global focus on soybean and crop outlook for India. 1997; Soybean kharif, SOPA, Indore, 1997-98.
5. Chacko S, Khare MN. Reaction of soybean varieties to C. dematium f. sp. Truncate. JNKVV, Res. J. 1978; 12:138-139.
6. Chakraborty PK, Shyam KR. Evalation of systemic fungitoxicant against *C. lindemuthianum*, the incinant of French bean anthracnose. Indian Phytopath. 1988; 6(1):67-70.
7. Chandel AS, Soybean. Cited from textbook of field crops production edited by Dr. Rajendra Prasad, ICAR. 2002, 372-396.
8. Dennis C, Webster J. Antagonistic properties of species-group of Trichoderma and hyphal interactions. Trans british myco soc. 1971; 57:363-369.
9. El-Abady MI, Seadh SE, Attia AN, El-Saidy Aml EA. Impact of foliar fertilization and its time of application on yield and seed quality of soybean. The 2th field crops conference. FCRI. AV. Giza, Egypt, 2008.
10. Gawade DB. Studies on soybean anthracnose incited by *C. truncatum* (schw). Thesis M.sc. (Agri.) M.K.V. Parbhani, 2007.
11. Gorawar MM, Hedge VR, Kalappanavar IK, Mathod JC. Management of leaf spot of turmeric caused by *Colletotrichum capsici*. Indian Phytopath. 2005; 58(3):348.
12. Gotmare CS. Studies on some pre-disposing factors and severity of die-back disease of chilli caused by *Colletotrichum capsici* (Syd.) Butler and Bisby. M. Sc. (Agri.) Thesis (unpub.) submitted to Dr. PDKV. Akola. 1981, 44.
13. Gupta S, Kalha CS, Vaid A, Rizvi SE. Studies on management of anthracnose of French bean caused by *C. lindemuthianum*. J Mycol. Pl. Pathol. 2005; 35(3):604.
14. Hartman GL, Manandhar JB, Sinclair JB. Incidence of *Colletotrichum* sp. On soybean and weeds in Illinois and pathogenicity of *C. truncatum*. Pl. Dis. 1986; 70(8):780-782.
15. Hepperly PR, Mignucci JS, Sinclair JB, Mendoza JB. Soybean anthracnose and its seed assay in Puerto. Rico. Seed Sci. Tech. 1983; 11:371-380.
16. Janardhanan KK, Gupta ML, Hussain A. Pythium dieback a new disease of *Catharanthus roseus*. Indian Phytopath. 1972; 30:427-428.
17. Lele VC, Ashram AM. Dieback disease of *Rauvolfia serpentine* caused by *Colletotrichum dematium*. Indian Phytopath. 1968; 53 95-98.
18. Manandhar JB, Hartman GL, Sinclair JB. Soybean garmplasm evaluation for resistance of *C. truncatum*. Pl. Dis. 1988; 72 (1):56-59.
19. Mathur AK, Tyagi NS. Symptomatology of *C. truncatum* leaf spot of moth bean. Indian Phytopath. 1982; 35:135.
20. Nene YL, Thapliyal PN. Evaluation of fungicides In: fungicides in plant disease control (3rd ed.). Oxford, IBH Publishing Co., New Delhi. 1993, 531-532.
21. Rizvi G, Ahmad ST. Management of foliar diseases of safed musli. Agrobios Newsletter. 2005; 7(4):46-47.
22. Shukla AK, Singh DP. Management of fungal disease of soybean by fungicidal sprays. Legume Res. 1993; 16(1, 2):75-76.
23. Sinclair JB. Discoloration of soybean seeds an indicator of quality. Pl. Dis. 1992; 76(11):1087-1091.
24. Sinclair JB, Backman PA. Compendium of soybean diseases. The American Phytopath. Soc. St. Paul. Minnesota, U.S.A, 1989
25. Tadayoshi M, Goldsmith PD. World Soybean Production: Area Harvested, Yield and Long Term Projections. International Food and Agribusiness Management Review. 2009; 12(4):143-162.

26. Verma ML, Upadhyay AR. Studies on incidence of soybean in a fertility inoculum varietal trial. Proc. Indian Acad. Sci. 1973; 78(5):234-239.
27. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors, Nature. 1927, 159-18.