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Evaluation of botanicals and bioagents against *Colletotrichum truncatum* (SCHW.) Andrus and Moore, causing anthracnose of greengram

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Abstract
Ten botanicals and six bioagents were evaluated *in vitro* against anthracnose of greengram caused by *Colletotrichum truncatum* (Schw.) Andrus and Moore. Azadirachtia @ 10 per cent concentration caused maximum inhibition of growth (63.34%) followed by eucalyptus oil (60.62%) at same concentration. Among the bioagents, *Trichoderma harzianum* was effective in inhibition of growth (64.38%).

Keywords: Greengram, *Colletotrichum truncatum*, botanicals, bioagents and *in vitro*

Introduction
Greengram *(Vigna radiata* L.*)* is one of the important pulse crops of India. It is quite versatile crop grown for seeds, green manure and forage and it is also considered as "Golden Bean" presently in India greengram is cultivated over an area of 32.99 lakh hectares with a production of 13.74 lakh tones. Greengram is a rich source of protein (23-24%), carbohydrate (54-56%), minerals and vitamines. It has high digestibility due to which it is fed to babies, convalescents and elders. Unlike other pulses, it is free from flatulent effects in stomach. It is consumed in many forms including boiled dhal, sprouts, bean cakes, noodles and pudding. Presently, the per capita share of pulses in nutrition supply in India with respect to energy, protein and fat is 117.4 K cal, 6.9 g and 1.0 g per day respectively. An adult male and female requires 80 and 70 g per capita per day, respectively for balanced diet (Anon., 2004) [2]. Greengram crop covers a total world area of 5 m ha with a total production of 3 mt (John, 1991) [5]. It is widely cultivated throughout the South Asia including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Cambodia, Vietnam, Indonesia, Malaysia and South China. India is an important pulse growing country contributing 28 per cent to the global pulse basket from an area of about 37 per cent (Masood Ali and Shivkumar, 2000) [8]. Among the major diseases of greengram, anthracnose caused by *Colletotrichum truncatum* (Schw.) Andrus and Moore is a major disease. It causes both qualitative as well as quantitative losses (Sharma *et al.*, 1971) [12]. The disease severing varies from 18.20 to 86.57 per cent have been reported in northern Karnataka (Laxman. 2006) [6]. Hence an attempt was made to evaluate different botanicals, bioagents and fungicides against the pathogen to manage the disease.

Materials and Methods
***In vitro* evaluation of botanicals:** To evaluate the extracts of different plant species to know the possible presence of fungi toxicant properties against *C. truncatum.* Preparation of plant extracts: Fifty grams of fresh healthy plant parts (leaves/root/bulbs) collected from field were washed with distilled water and air-dried and crushed in 50 ml of sterile water. The crushed product was filtered through muslin cloth and collected the filtrate. The prepared solution gave 100 per cent, which was further diluted to required concentrations of 5.0, 7.5 and 10.0 per cent. The extracts were tested against *C. truncatum* on the cultural media using poison food technique under *in vitro* condition. Details about the botanicals and part used are given below. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) [12]

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

Where,
I: Per cent inhibition
C: Mycelial growth in control
T: Mycelial growth in treatment

Botanicals used for *in vitro* evaluation

S. No.	Plant (common name)	Scientific name	Plant part used
1.	Neem	*Azadirachta indica*	Kernel
2.	Eucalyptus	*Eucalyptus citridora*	Oil
3.	Cynodon	*Cynodon dactylon*	Plant
4.	Bellary jali	*Prosophis juliflora*	Leaf
5.	Parthenium	*Parthenium historophorus*	Plant
6.	Garlic	*Allium sativum*	Bulb
7.	Neem	*Azadirachta indica*	Readymade herbal product
8.	Onion	*Allium cepa*	Bulb
9.	Turmeric	*Curcuma longa*	Rhizome
10.	Ginger	*Zingiber officinale*	Rhizome

* Values in parenthesis are arcsine transformed values

Table 1: *In vitro* evaluation of bioagents against *Colletotrichum truncatum*

Sl. No.	Bio-agents	Per cent inhibition
1	*Gliocladium virens*	58.47 (49. 88)*
2	*Trichoderma koningii*	54.37 (47.51)
3	*Trichoderma viride*	50.46 (45.26)
4	*Trichoderma harzianum*	64.38 (53.35)
5	*Pseudomonas fluorescens*	26.56 (31.02)
6	*Bacillus subtilis*	35.44 (36.54)
	S.Em±	0.13
	CD at 1%	0.53

* Values in parenthesis are arcsine transformed values

***In vitro* evaluation of bioagents:** Antagonistic microorganisms like *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, *T. viride*, *T. koningii* and *Gliocladium virens* were evaluated for their antagonistic properties against *C. truncatum* by dual culture technique.

Dual culture test**:** Bioagents were evaluated for their efficacy through dual culture technique. The bioagents and the test fungus were inoculated side by side on a single petridish containing solidified PDA medium. Three replications were maintained for each treatment with one control by maintaining only pathogen and bioagent separately. Inoculated plates were incubated at 27 $\pm$ 1^0C for eight days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) [12].

Result and Discussion

***In vitro* evaluation of botanicals:** Ten plant extracts were evaluated at three concentrations in the laboratory for their efficacy against *C. truncatum* through poison food technique as detailed in Material and Methods. The data are presented in Table 1.

Table 2: *In vitro* evaluation of botanicals against *Colletotrichum truncatum*

S. No	Botanicals	Percent inhibition of radial growth over control			
		5%	7.5%	10%	Mean
1.	Bellary Jali (leaf)	35.55 (36.62) *	42.58 (40.75)	46.76 (43.16)	41.63 (40.18)
2.	Cynodon (plant)	40.64 (39.62)	45.62 (45.51)	50.42(45.26)	45.56 (42.46)
3.	Parthenium (plant)	40.48 (39.53)	47.31 (43.48)	52.47 (46.44)	46.75 (43.15)
4.	Garlic (bulb)	43.37 (41.21)	49.25 (44.59)	59.44 (50.46)	50.69 (45.42)
5.	Onion (bulb)	39.28 (38.83)	46.61 (43.08)	52.33 (46.36)	46.07 (42.75)
6.	Neem (kernel)	42.17 (40.51)	48.33 (44.06)	56.63 (48.83)	49.04 (44.47)
7.	Eucalyptus (oil)	47.37 (43.51)	50.23 (45.15)	60.62 (51.16)	52.74 (46.61)
8.	Azadirachtin (herbal product)	47.45 (43.56)	51.39 (45.82)	63.34 (52.76)	54.06 (47.38)
9.	Turmeric (rhizome)	39.47 (38.94))	44.36 (41.78)	49.46 (44.71)	44.43 (41.81)
10	Ginger (rhizome)	37.48 (37.77)	44.47 (41.84)	48.56 (44.20)	43.50 (41.27)
	Mean	41.33 (40.01)	47.02 (43.31)	54.00 (47.33)	
		S.E m ±		C.D at 1%	
	Botanicals (B)	0.14		0.52	
	Concentration (C)	0.08		0.30	
	B × C	0.24		0.90	

Table 1 revealed that amongst the ten plant extracts evaluated, azadirachtin at 10 per cent concentration was found to be best in inhibiting the mycelial growth of *C. truncatum* (63.34%) and found significantly superior over all the other extracts, followed by eucalyptus oil (60.62%), garlic (59.44%) and neem seed kernel extract (56.63%) at 10 per cent. Least inhibition of mycelial growth of *C. truncatum* was recorded in bellary jali (35.55%) at 5 per cent concentration (Plate 1).

***In vitro* evaluation of bioagents:** Six bioagents were evaluated for their efficacy against *C. truncatum* through dual culture technique as explained in Material and Method. The results of the study are presented in Table 1. *Trichoderma harzianum* gave highest growth inhibition (64.38%) followed by *Gliocladium virens* (58.47%), *T. koningii* (54.37%) and *T. viride* (50.46%). The least growth inhibition of the fungus was observed in *Bacillus subtilis* (35.44%) and *Pseudomonas fluorescens* (26.56%) (Plate 1).

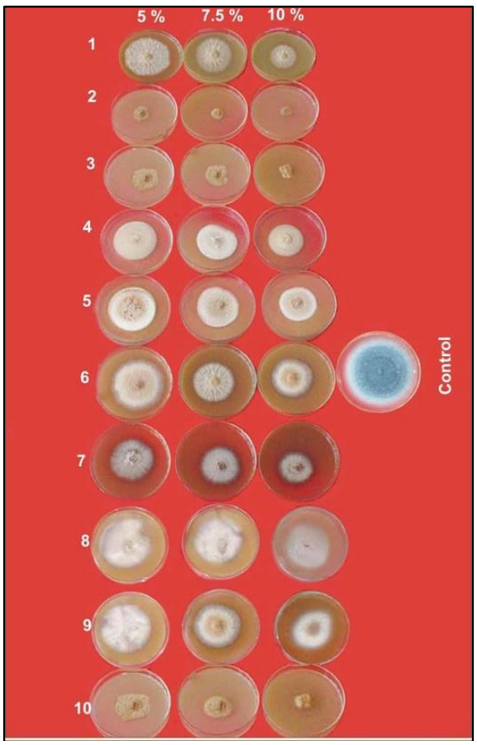

Plate 1: In vitro evaluation of botanicals against Co *Lletotrichum cruncatztnz*

1. Cynodon
2. Azadirachtin
3. Eucalyptus oil
4. NSKE
5. Onion
6. Turmeric
7. Parthenium
8. Bellary Jali
9. Ginger
10. Garlic

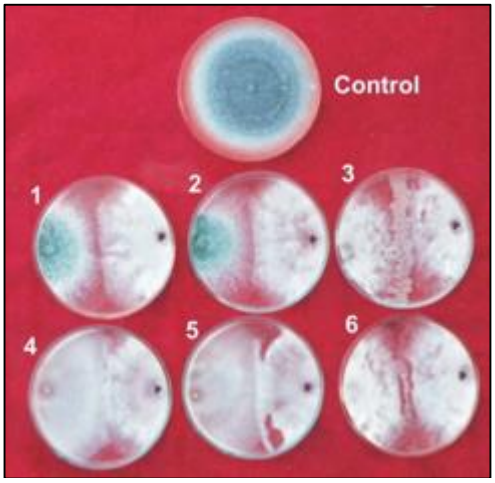

Plate 2: In vitro evaluation of bioagents against *Colletotrichum truncatum*

1.Trichoderma Wilda
2. Trichoderma koningli
3. Pseudomonas fluorescens
4. Trichoderma Wrens
5. Trichoderma harzlanum
6. Bacillus subtilis

In vitro evaluation of botanicals: At present, plant extracts are gaining importance in plant disease management practices. These are the cheaper and safer means of disease management which reduce not only toxicity hazards but also present ecofriendly approach in nature. In the present investigation though the complete inhibition of the fungus was not observed in any of the ten botanicals used, considerable amount of inhibition of growth was noticed in some of the botanicals. Herbal products *viz.*, azadirachtine was found to be effective followed by eucalyptus oil and to some extent garlic bulb extract. The fungicidal spectrum of neem (*Azadirachta indica*) has already been investigated by Singh and Pande (1966) [15] and reviewed in detail by Praveen and Alam (1993) [10]. Further, Shivapuri *et al.* (1997) [13], found neem, garlic and *Datura stramonium* most effective against *C. capsici*. Similarly, Angadi (1999) [1] reported that nimbicidine and NSKE showed considerable amount of inhibition of *C. capsici*. Later, Laxman (2006) [6] observed the effectiveness of eucalyptus oil, garlic and neem against *C. truncatum* in greengram.

In vitro evaluation of bioagents: Biological control through the use of antagonistic microorganisms is a potential non-chemical means of controlling plant disease by reducing inoculum levels of the pathogens. Such a management would help in preventing the pollution and also health hazards. In the present investigation, the antagonistic effect of different bioagents was assessed against *C. truncatum* by dual culture technique. Among the different bioagents evaluated *Trichoderma harzianum* has inhibited the growth of fungus with maximum extent followed by *Gliocladium virens* and *T. koningii*. Gupta *et al.* (1991) [4] reported that *Gliocladium virens*, *T. harzianum* and *T. viride* significantly inhibited growth of *C. lindemuthianum in vitro*. The present investigations are in agreement with Varaprasad (2000) [16], who found effectiveness of *Trichoderma* sp. against *Colletotrichum dematium*, whereas Laxman (2006) [6] against *C. truncatum*. This could be obviously due to several possibilities of existence of microbial interactions such as stimulation, inhibition, mutual intermingling of growth of antagonistic isolate over test pathogen *etc.* have been enumerated by many workers (Porter, 1924, Ghaffar, 1969 and Naik and Sen, 1995) [11, 3, 9].

Conclusion

Among ten botanicals evaluated *in vitro* azadirachtin (63.34%), eucalyptus oil (60.62%) and garlic (59.44%) were found most promising ones which showed higher mycelial growth inhibition at 10 per cent concentration. *In vitro* evaluation of bioagents revealed that, *Trichoderma harzianum* inhibited the growth of fungus with maximum extent followed by *Gliocladium virens* and *T. koningii*.

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