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In vitro evaluation of fungicides, botanicals and bio-agents against *Alternaria alternata* causing leaf spot disease of niger

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Abstract
Niger [*Guizotia abyssinica,* family:compositae] is commonly known as ramtil. It is minor oilseed crop. Under favourable condition niger is attacked by various diseases. Out of which, leaf spot caused by *Alternaria alternata* is one of the predominant disease of niger causes yield loss. The present investigation was carried out to test the efficacy of fungicides, botanicals and bio-agent *in vitro* against the *Alternaria alternata*. Among the fungicides tested carboxin + thiram was found most effective arresting 100% mycelial growth inhibition while carbendazim + mancozeb 93.15%, copper oxychloride 92.22%, propineb 90.00% and pyraclostrobin 80.37%. Bioefficacy of botanicals is tested by poisoned food technique and result revealed that maximum mycelial growth inhibition was recorded in turmeric rhizome extract 54.92% followed by neem leaf extract, garlic bulb extract, tulsi leaf extract and eucalyptus leaf extract 35.83%, 26.38%, 18.47% and 14.72% respectively. Among the bio-agents tested by dual culture technique *Trichoderma reesei* was found superior recorded 75.00% mycelial growth inhibition of *Alternaria alternata* followed by *Pseudomonas fluorescens, bacillus subtilis* and *Trichoderma asperellum* recorded 65.36%, 60.36% and 58.88% respectively.

Keywords: *Alternaria alternata,* fungicides, botanicals, bio-agents

Introduction
Niger [*Guizotia abyssinica,* family:compositae] is commonly known as ramtil. It is important minor, edible, traditional oil seed crop in India. It is mainly cultivated in tribal pockets of Gujarat, M.P, Orissa, Maharashtra, Bihar, Karnataka and Andhra Pradesh in India. (Gupta. 2018) [3]. India ranks first in area, production and export of niger in the world (Rai *et al*. 2016). It provides about 3 per cent of the edible oil requirement of the country (Getinet and Sharma, 1996) [2]. Niger contains 34-36% quality oil with 18-20 % protein in the seed (Rai *et al*. 2016) [10]. Niger oil has good keeping quality and has < 70% unsaturated fatty acids free from toxin. linoleic acid 45-66%, oleic acid 13-39 % and palmitic acid 8.2-9.6%.
Niger is suffering from various fungal disease among them Alternaria leaf spot is distributed throughout the Niger growing areas of India. Leaf blight of niger caused by *Alternaria alternata* is considered to be a major devastating disease to the Niger in India and also reduce the yield and oil quality. (Nagaraja and Krishnappa 2016) [9]. The disease is favoured by warm and humid climate. The accidental rain at flowering stage leads the expansion of alternaria leaf spot incidence and results in the poor seed set and seed yield (Sandipan *et al*. 2014) [11]. Diseases cause heavy damage upto 35-40 per cent to this crop and reduce its seed yields upto 20-30 per cent. Now the crop is gaining importance and its sustainable cultivation is being standardized including disease management. Considering the increasing importance of niger. The present study was focused on *in vitro* evaluation of fungicides, botanicals and bio-agents employed in the management of leaf spot of niger.

Material and Methods
Isolation and pathogenicity
The culture of *Alternaria alternata* used in this study was isolated from infected leaves of niger plants collected from the different places. In order to isolate pathogen infected leaf sample were cut along with healthy leaf and surface sterilized with sodium hyphochlorite solution for one minute and washing with three times by sterilized distilled water. The bits

were placed in petriplates containg PDA medium. All the above operations were carried out in sterilized condition (under laminar air flow unit). The plates were incubated at 27±2 ^{0}C for 7 days. The isolated fungi were identified as *Alternaria alternata* on the basis of morphological characters and published literature. The fungal growth, which developed around each bit, was then transferred to PDA medium slant for sub culturing. The isolated fungus was purified by single spore isolation method. The isolate obtained were maintained on PDA slant by sub culturing. The culture was stored in refrigerator at 10 ºC and sub cultured at subsequent interval as and when required. The inocolums was prepared and spray (1×10 ^{6}spore/ ml) on niger plants within 6-12 days typical leaf spot symptoms were observed. The pathogen was reisolated on the PDA medium from the inoculated plants for confirmation of Koch's postulates.

***In vitro* evaluation of fungicides by poisoned food technique**

Poisoned food technique was used to evaluate the efficiency of six fungicides against pathogens. Potato dextrose agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flask, autoclaved 1.05kg/cm2 for 15 min. Then before solidification of media different fungicides with desired concentration were incorporated aseptically in different flasks. These flasks shaken to facilitate uniform mixture of fungicides thoroughly and poured in Petri plate's 20 ml/plate likewise three plates for each treatment were poured. One set of three plates was poured without any fungicides to serve as a control. After solidification of medium, the plates inoculated with seven days old pathogens separately. Five mm diameter mycelial disc selected from peripheral growth of the plate by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelial growth touch the surface of medium. The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated by using following formula (Vincent, 1927) [12].

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

Where,
PI = Per cent Inhibition
C = Growth of fungi in control (mm)
T = Growth of fungi in treatment (mm)

***In vitro* evaluation of botanicals by poisoned food technique**

Aqueous leaf extracts of the test botanicals were obtained by grinding the washed rhizome and leaves (100 g) in mortal and pestle with equal volume (100 ml) of sterilized distilled water. The macerate obtained was filtered through the folds of muslin cloth and the filtrate obtained formed 100% phytoextracts, which were evaluated by poisoned food method. Twenty ml of poisoned medium was poured into each stetrile petriplates. Five mm diameter mycelial disc selected from periphery of actively growing culture were cut out by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelial growth touch the surface of medium. of each agar plate. Control were also maintained by growing the pathogen on PDA plates. The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated by using above formula.

***In vitro* evaluation of bio-agents by dual culture method**

The lawn culture of test fungi and bio-agents *viz*., *Trichoderma asperellum* and *Trichoderma reesei* were prepared. Autoclaved, melted potato dextrose agar was poured in petri plates and allowed to solidify for obtaining leveled surface. The plates were inoculated with the culture of test fungi and bio-agents after solidification of media and then plates were incubated at room temperature for seven days.

Bacterial bio-agents, *Bacillus subtilis* and *Pseudomonas fluorescens* were prepared by inoculating a loopful culture in sterilized conical flask containing 100 ml of nutrient broth. Broth culture was incubated at room temperature for three days. Five mm disc of one week old test fungus and bio-agent lawn culture was cut with the help of sterilized cork borer lifted and transferred in petri plates, containing autoclaved solidified PDA medium. In each petri plates, four discs of bio-agents were inoculated at four peripheral points of the plates and the test fungi was placed in the center of petri plates. In case of *Pseudomonas fluorescens* and *Bacillus subtilis*, a three days old culture was streaked around the disc of test fungus. The test fungi grown in same condition on potato dextrose agar without bio-agents served as control. All these plates were incubated at room temperature for seven days. After an expiry of seven days incubation period the mycelial inhibition was calculated as per formula mentioned in the poisoned food method.

Result and Discussion

In vitro* evaluation of fungicides against *Alternaria alternata

Fungi toxic activites of different fungicides was assayed against *Alternaria alternata* and observed in (Table1. and Plate1.) indicated that, Carboxin + Thiram @ 0.3% inhibit 100% mycelial growth followed by Carbendazim+Mancozeb 93.15%, Copper oxychloride 92.22%, Propineb 90.00%, Pyraclostrobin 80.37%. Azoxystrobin were found least effective as recorded only 49.07% inhibition. The present findings are in agreement with Hariprasad *et al*. (2017) [4] who reported 78.83 % mean mycelial growth inhibition of *Alternaria tenuissima* by Carboxin + Thiram. Waghe *et al*. (2015) [13] also recorded highest 90.36% mycelial growth inhibition by Carbendazim + Mancozeb against the *Alternaria solani* followed by Azoxystrobin 72.96%. Similarly complate mycelial growth inhibition of *Alternaria macrospora* with Carbendazim + Mancozeb and with Copper oxychloride 75.44% inhibition was recorded by Mohan *et al*. (2018) [8].

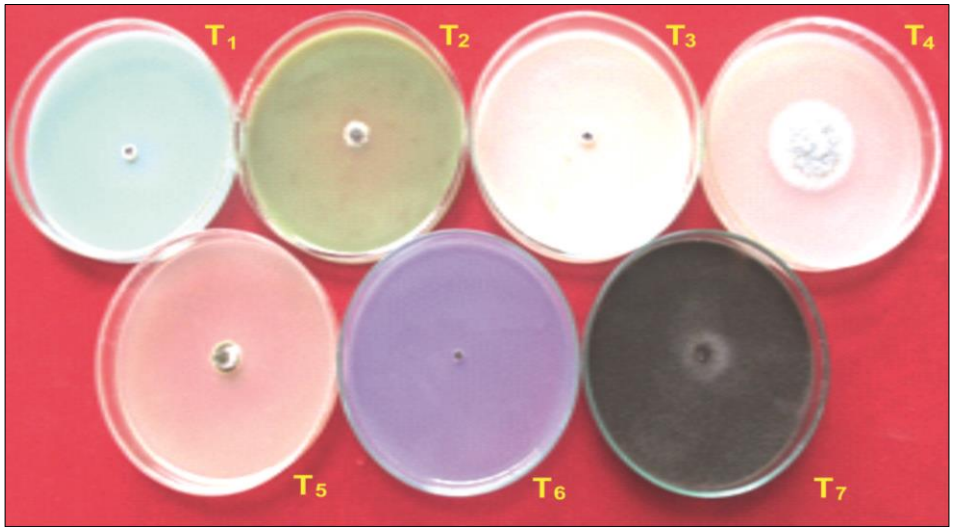
In vitro* evaluation of botanicals against *Alternaria alternata

The results presented in the (Table 2. and Plate 2.) indicated that all the tested botanicals showed significant differences compared with control. Among the plant extracts, Turmeric rhizome extract @ 10% recorded maximum inhibition 54.92% of mycelial growth of test fungus and was significantly superior to rest of the treatments. This was followed by neem leaf extract and garlic bulb extract recorded 35.83 % and 26.38% mycelial growth inhibition of *A. Alternata* respectively. Rest of the plant extract *viz*. tulsi leaf extract and eucalyptus leaf extract recorded 18.47% and 14.72% mycelial growth inhibition which was least effective against test pathogen. The present result of turmeric rhizome

extract 54.92% are in accordance with Kansara and sabalpara (2015) [7] who reported mycelial growth inhibition of *Alternaria alternata* causing leaf spot disease of niger with turmeric rhizome extract upto 54.37%. Kadam *et al*. (2018) [6] also recorded 60.19% mycelial inhibition of *Alternaria alternata* isolated from pomegranate with turmeric rhizome.

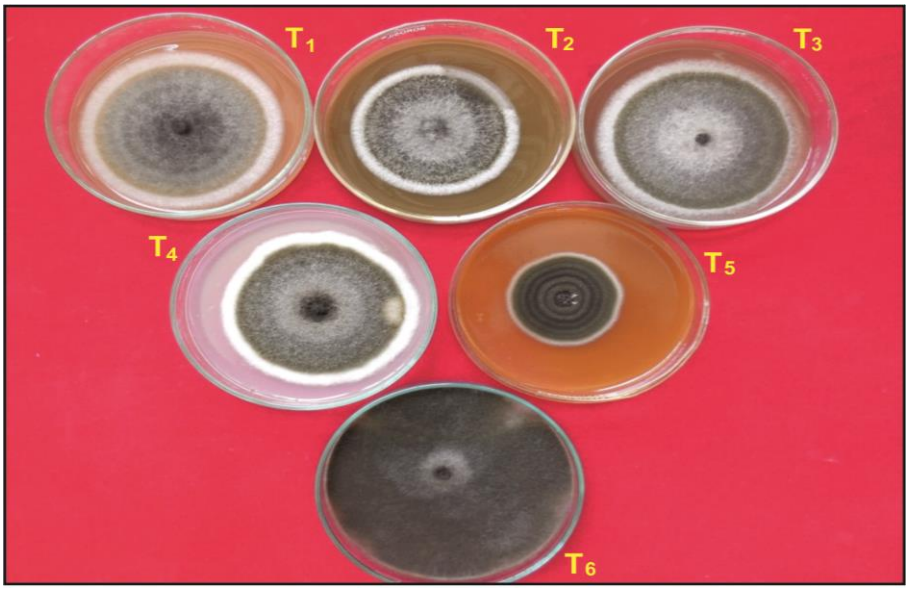
In vitro* evaluation of bio-agents against *Alternaria alternata

All the bio-agents tested showed the significant effect compared with control in (Table 3. and Plate 3.) Antagonist *Trichoderma reesei* gave the better effect against *Alternaria alternata* forming maximum per-cent mycelial inhibition 75.00% and decreased the mycelial growth from 90 to 22.5mm. *Pseudomonas fluorescence* was next best recorded 65.36 % inhibition while the least mycelial inhibition was observed in *Bacillus subtilis* 60.36% and *Trichoderma asperellum* 58.88%. In present experiment fungal antagonist found effective than bacterial known antagonist. Their findings are conformity with the results of Jakatimath *et al.* (2017) [5] who reported that *Trichoderma spp*. was most effective against *Alternaria alternata* as compared *Pseudomonas fluoroscens* and *Bacillus subtilis*. The similar observation was also reported by Chaitali (2014) [1] who recorded that *Trichoderma harzianum* was most effective against *Alternaria spp*. with significant mycelial inhibition 83.30% followed by *Trichoderma viride* 81.40%, *Pseudomonas fluorescence* 66.00% and *Bacillus subtilis* 67.80%.



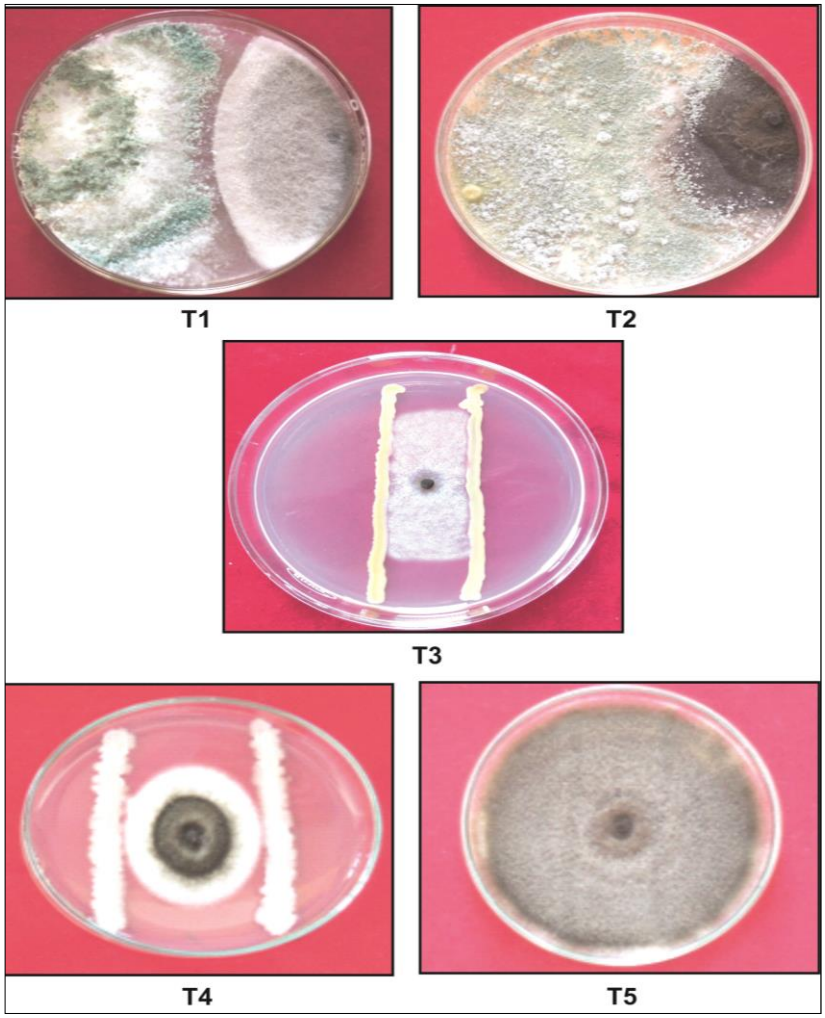
T1Carbendazim+Mancozeb (0.25%) T2: Copper oxychloride (0.25%) T3: Propineb (0.3%)
T4: Azoxystrobin (0.1%) T5: Pyraclostrobin (0.1%) T6: Carboxin37.5% +Thiram 37.5% DS
T7: Control

Fig 1: *In vitro* efficacy of fungicides against *Alternaria alternata*



T1: *Oscimum sanctum* T2: *Azardirachta india* T3: *Eucalyptus globules*
T4: *Allium sativum* T5: *Curcuma longa* T6: Control

Fig 2: *In vitro* efficacy botanicals against *Alternaria alternata*



T1: *Trichoderma asperellum* T2: *Trichoderma reesei* T3: *Pseudomonas fluorescens*
T4: *Bacillus subtilis* T5: Control

Fig 3: *In vitro* efficacy bio-agents against *Alternaria alternata*

Table 1: Following fungicides were evaluated for their efficacy against *Alternaria alternata in vitro*

Sr. No.	Fungicides	Conc (%)	Mean radial mycelial growth (mm)*	Mycelial growth inhibition (%)
1.	Carbendazim 12% + Mancozeb 63% WP	0.25	6.16	93.15
2.	Copper oxychloride 50% WP	0.25	7.00	92.22
3.	Propineb 70% WP	0.3	9.00	90.00
4.	Azoxystrobin 23% EC	0.1	45.83	49.07
5.	Pyraclostrobin 20% WG	0.1	17.66	80.37
6.	Carboxin37.5% +Thiram 37.5% DS	0.3	0.00	100.00
7.	Control	-	90.00	-
	'F test	-	Sig	-
	SE(m)±	-	0.53	-
	CD(P=0.01)	-	2.25	-

*Average of three replications.

Table 2: Following botaniclas were evaluated for their efficacy against *Alternaria alternata in vitro*

Sr. No.	Botanicals	Conc (%)	Mean radial mycelial growth (mm)*	Mycelial growth inhibition (%)
1.	*Oscimum sanctum* (Tulsi)	10.00	73.37	18.47
2.	*Azardirachta indica* (Neem)	10.00	57.75	35.83
3.	*Eucalyptus globules* (Nilgiri)	10.00	76.75	14.72
4.	*Allium sativum* (Garlic)	10.00	66.25	26.38
5.	*Curcuma longa* (Turmeric)	10.00	40.57	54.92
6.	Control	-	90.00	-
	'F test	-	Sig	-
	SE(m)±	-	0.94	-
	CD(P=0.01)	-	2.80	-

*Average of four replications

Table 3: Following bio-agents were evaluated for their efficacy against *Alternaria alternata iin vitro*

Sr. No.	Bio-agents	Mean radial mycelial growth (mm)*	Mycelial growth inhibition (%)
1.	*Trichoderma asperellum*	37.00	58.88
2.	*Trichoderma reesei*	22.50	75.00
3.	*Pseudomonas fluorescens*	31.17	65.36
4.	*Bacillus subtilis*	35.25	60.36
5.	Control	90.00	-
	'F test	Sig	-
	SE(m)±	0.26	-
	CD(P=0.01)	1.10	-

*Average of four replications

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