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In vitro evaluation of botanicals and bioagents against Alternaria leaf spot of castor

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Alternaria leaf spot caused by *Alternaria ricini* is one of the major foliar diseases of castor resulting in reasonable yield loss and poor oil quality. Tissue isolation from leaves followed by purification and pathogenicity test of the isolated culture was carried out by artificially inoculating the spore suspension and re-isolation of similar culture from the inoculated plants confirmed positive for Koch's postulate. Cultural and morphological study of *Alternaria* sp. indicated that the growth of mycelia on PDA was slightly fluffy with greyish white to grey colour. The mean length and width of spore of *Alternaria* sp. were 195.65 and 34.35 μm, and 3-7 vertical and 7-13 horizontal septa were observed. Based on the size and septation of spores, it was identified as *Alternaria ricini*. A pathogenicity test of the isolated fungus was conducted in earthen pots. Typical symptoms developed on castor leaves were found to be similar to that of naturally infected castor plants and were found identical and similar to that of the original one. In the present investigation, seven different botanicals were tested for their efficacy against the pathogen and the maximum growth inhibition was recorded in garlic clove extract (71.93%) followed by neem leaves and onion bulb extract with 65.90 and 58.39 % inhibition, respectively. The growth inhibition of *A. ricini* was tested against seven bio-agents. Of them, *Trichoderma viride* proved to be the most inhibitory with maximum growth inhibition (91.07%) of *A. ricini* in comparison with the rest of the bioagents. The next bioagent in order of merit was *T. harzianum* resulting in growth inhibition of 88.36 %. This finding will be helpful for the formulation of bio-intensive management of Alternaria leaf spot in castor.

Keywords: Alternaria leaf spot, Botanicals, Bioagents, Castor, Management

INTRODUCTION

Castor (*Ricinus communis* L.) belonging to the family Euphorbiaceae, is an important non-edible, export-oriented industrial oilseed crop in India, which has been known to mankind from time immemorial. Castor plant serves as an important source of raw material for many industries and is reported to suffer severe losses due to many diseases caused by fungi and bacteria.

Castor plants are attacked by numerous diseases under high relative humidity conditions, but only a few occur in India. Of them, seedling blight (*Phytophthora colocasiae*), leaf spots (*Alternaria ricini* and *Cercospora ricinella*), wilt (*Fusarium oxysporum* f.sp. *ricini*), root rot (*Macrophomina phaseolina*), grey mold rot (*Botrytis cinerea*), powdery mildew (*Leveillula taurica*), etc are very important. In recent years, leaf spot caused by *Alternaria ricini* has become a serious concern in major castor growing areas and affecting yield significantly. Earlier, the status of *Alternaria* leafspots on castor in India was made by Hiremani *et al.* (2012a). Especially in humid areas, extensive fructification of the fungus takes place on the capsules of castor plant with a black, sooty appearance which on shaking gives rise to black

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clouds of conidia. In some fields, the plants are mostly reported to be affected by this disease. All the aerial parts of the plant, i.e., stem, leaves, inflorescence, and capsules were attacked. Infection spots are irregular, scattered, pale brown to dark olivaceous-brown, sometimes with concentric rings of 3-12 mm in diameter. However, the information on the aetiology of this disease and the morphological and physiological characteristics of the pathogen is lacking; thus needs to be explored. Furthermore, the management of this pathogen is another important aspect. Mostly, fungicides are preferred by farmers, but the phytotoxicity effect and chance of fungicide resistance development against the *A. ricini* population is a great concern. Generally, farmers have to spray fungicides onward from the ninety-day age of the castor plant, particularly for Alternaria leaf spot control. It is high time to reduce the use of fungicides by promoting the application of natural products as part of an integrated disease management strategy. Hence, *in vitro* evaluation of botanical and bioagents is felt necessary to manage the disease.

MATERIALS AND METHODS

Isolation and purification of pathogen

Castor leaves showing pale brown spots with typical concentric rings were collected and brought to the laboratory. The samples were examined under the microscope for preliminary examination and put into the humid chamber for sporulation on naturally infected castor leaves. Pathogen was isolated from infected leaves by tissue isolation technique on PDA. Diseased leaves were cut into small pieces with the help of a sterilized blade. Pieces were washed with sterilized water and then disinfected with the 1.0 per cent NaOCl solution for 45 seconds. Thus, obtained disinfected pieces were immediately washed thrice with sterilized distilled water and aseptically transferred on PDA plates. Inoculated Petri plates were incubated at 27 ± 2^0 C temperature. Light brownish-black mycelium growth on and around the inoculated pieces was observed after 48 hrs of incubation.

Mycelial growth was observed and fungal culture was further purified by a single spore isolation method. The pure culture was transferred periodically and maintained on PDA slants at 4^0C temperature.

Identification of the pathogen

The isolated culture of the pathogen was grown on a PDA medium and examined visually for cultural and morphology characteristics. The morphological characteristics of the fungus were observed under the microscope after staining the culture with lactophenol. Cultural characters were recorded right from the initiation of growth up to 15 days. The morphological characters *viz.*, length and width of spores, mycelium, *etc.* were measured under high power magnification from 10-days old culture of *Alternaria ricini* and compared with those given in the literature or manual.

Pathogenicity test of isolated fungal culture

The pathogenicity of the isolated fungal culture was tested on the leaves of the castor plant grown in pot-grown plants. Earthen pots (30 cm dia) were filled with sterilized soil and FYM in the ratio of 3:1. Castor seeds were sown in earthen pots covered with plastic to avoid any airborne infection. The pots were labelled and arranged in a line and watered gently up to saturation in the morning. Thirty days old plants were used for inoculation purpose. Before inoculation, the leaves were surface sterilized with 1.0 per cent sodium hypochlorite (NaOCl) solution and washed thoroughly with sterile distilled water to remove the traces. Castor plants were inoculated with a spore suspension (@1×10^4) of *A. ricini* (Nagaraja and Krishnappa, 2016). The spore suspension was prepared in sterilized distilled water by blending 7 days old culture. The blended culture was filtered through a double-layered sterilized cheese-cloth. The desired spore suspension was obtained by adding sterilized distilled water. The castor plants were inoculated by spraying the spore suspension with the help of a hand sprayer. The control was also maintained by spraying the sterilized distilled water only. The inoculated and uninoculated pots were covered with polyethylene bags for 48 hr to provide high humidity. The observations on the disease development on leaves were recorded

periodically from the initiation of the disease. The disease development was studied through visual observation from initiation to the full development of the disease and was compared with control. The pathogen was re-isolated from the artificially inoculated plants showing typical Alternaria leaf spot symptoms by tissue isolation method and the identity of the fungus was confirmed as per the original one. The culture obtained by re-isolation was transferred on PDA slants for comparison with the original culture and further investigations.

In vitro bio-efficacy of different botanicals against associated pathogen Alternaria ricini

The efficacy of seven plant products (botanicals) (Table 1) against the pathogen in the laboratory was tested using poisoned food technique (Dixit *et al.*1995). The freshly collected plant materials were washed thoroughly with tap water and then finally with sterilized distilled water. They were separately ground in sterilized distilled water at the rate of one per cent W/V of the plant parts in a sterilized, chilled pestle and mortar. The extract was first filtered through two-layer of muslin cloth and subsequently filtered through Whatman No. 1 filter paper. This formed the standard plant extract solution (100%). The plant extracts with their three concentrations i.e., 5, 10, and 20 per cent were applied against the pathogen *in vitro*. The medium was poured into the sterilized Petri plates under aseptic conditions and three such plates were maintained for each treatment at each concentration. A five mm disc of seven days old culture of the pathogen was cut using a sterilized cork borer and placed at the centre of the Petri plate. The plates were incubated at 27 ± 2 ^{o}C and growth inhibition of the fungus was recorded after 7 days.

In vitro bio-efficacy of different bio-agents against Alternaria ricini

To study the bio-efficacy of fungal and bacterial biocontrol agents against *Alternaria ricini,* different antagonists (**Table 1**) were tried *in vitro* to test the antagonistic activity against pathogen by dual culture method (Dennis and Webster, 1971). The native isolates of different *Trichoderma* spp., viz., *T. viride*, *T. harzianum*, *T. longibrachyatum T. koningii, T. faciculatum* were isolated in the Department of Plant Pathology, C.P College of Agriculture, S.D. Agricultural University and identified morphologically based on the arrangement of conidiophores, and phialides as given in the standard key (Bissett *et al*. 2015). *Pseudomonas fluorescence* (ATCC 49838), *Bacillus subtilis* (ATCC 19659) were collected from the Department of Microbiology, C.P College of Agriculture, S.D. Agricultural University, Sardarkrushinagar. Sterilized PDA (20 ml) was poured aseptically in 90 mm diameter sterilized Petri plate. Mycelial disc (5mm diameter) from seven days old actively growing culture of bio-agents and A*. ricini* was cut aseptically from the periphery of the colony with the help of sterilized cork borer and placed on solidified PDA plates approximately 70 mm away from each other. The test pathogen was subjected alone for growth and comparison. All inoculated Petri plates were incubated at 27±2ºC temperature in an incubator. Observations on radial growth in each Petri plate were measured periodically and the final observation was recorded when the control plate was fully covered with the growth of the test pathogen. The per cent growth inhibition was calculated by the following formula (Vincent, 1947) and analyzed statistically in CRD.

$$PGI = \frac{C - T}{C} x100$$

where, PGI = Per cent growth inhibition,C = Colony diameter in control (mm), and T = Colony diameter in treatment (mm)

RESULTS AND DISCUSSION

Cultural and morphological study of Alternaria ricini

A fungal culture was isolated from the infected leaf samples of castor (Fig. 1a). The pathogen was initially identified as *Alternaria* sp. by comparing the morphological and microscopic observation of the fungal culture with literatures. After seven days of incubation, the colony diameter was recorded as 78.33mm and the growth of mycelia was fluffy, spreading, septate, and branched with dark brown colour raised margins (Fig. 1b). In microscopic study, the mycelium of the fungi was initially hyaline which

later turned into dark brown to olivaceous brown or smoky. The average length and width of the spores of our culture were 195.65 μm and 34.35μm, respectively. Likewise, the average beak length was 95.89 μm and the beak width was 3.54 μm, with 7-13 transverse septa and 3-7 longitudinal septa (Fig. 1c). In comparison with the standard key of different *Alternaria* sp. (Table. 2), our culture was confirmed that *Alternaria ricini* causing leaf spot of castor. Similar findings were reported by earlier research workers. Hiremani *et al.* (2012b) reported the cultural characteristics of *A. ricini* on different solid media. The maximum mean colony diameter of *A. ricini* was reported in potato dextrose agar (78.33mm). Hiremani *et al*. (2014) studied the morphological and nutritional characteristics of *Alternaria ricini*, and observed the length of conidia varied from 49.35 to 85.50 μm and the number of cross walls varied from 4-14. According to the Woudenberg *et al.* (2013), *A. ricini* comes under the section *Porri* which was characterised by broadly ovoid, obclavate, ellipsoid, large conidia with a simple or branched, long to filamentous beak. Our *A. ricini* also produced obclavate, solitary conidia with long septate beak and conspicuous constriction. This shape and size of conidia is corroborated with the previous reports also.

Pathogenicity of A. ricini

Pathogenicity of the isolated culture was proved on the healthy one month-old plants of castor grown in earthen pots. The result revealed that *A. ricini* was pathogenic to castor causing Alternaria leaf spot (Fig. 1d). After 12-18 days of incubation, typical symptoms of Alternaria leaf spot on the foliage of artificially inoculated castor plants were observed (Fig. 1e,f). However, the control plant sprayed with only sterilized distilled water remained healthy and did not produce any kind of symptoms throughout the observation.
The fungus was re-isolated from the inoculated leaves and was compared with the original culture of the test pathogen. The same was found identical to that of the original culture, thereby confirming the test of pathogenicity. Previously, Nagaraja and Krishnappa (2016) experimented on detection and pathogenicity of *A. ricini* in castor. In this experiment, inoculum sprayed plants showed the symptoms of *A. ricini* in 12-18 days. The symptoms appear on all the aerial plant parts. Similarly, 16.8% of seed infection by *A. ricini* was reported by Nagaraja and Krishnappa (2016). It indicated the seed as a source of inoculum for the spread of the pathogen.

In vitro bio-efficacy of different botanicals against A. ricini

Using the poisoned food technique, seven different botanicals were evaluated *in vitro* at concentrations of 5, 10, and 20% to determine their effectiveness against *A. ricini*. The *in vitro* growth of *A. ricini* was inhibited by all the botanicals to varying degrees; at different doses, the mean growth inhibition ranged from 41.07 to 71.93% (Table 3). In particular, five botanicals, *viz*., garlic (*Allium sativum*), onion (*Allium cepa*), calotropis (*Calotropis procera*), tulsi leaves (*Ocimum tenuiflorum*), and neem leaves (*Azadirachta indica*) were found to specifically suppress the growth of *A. ricini* by more than 55% (Fig. 2).

Of them, garlic clove extract was found to be superior in mean per cent growth inhibition (71.93%), followed by neem leaf extract (65.90%) and onion bulb extract (58.39%), respectively. Whereas, datura and naffatia, calotropis and tulsi leaf extracts have the least inhibitory effect on the mycelial growth of *A. ricini*. Furthermore, the interaction effect of botanicals and concentration was found significant. The highest inhibition in the growth of *A. ricini* to the tune of 72.93 and 78.73% at 10 and 20% concentrations was recorded only in garlic clove extract at all the concentrations. This was followed by neem leaf extract with recorded 71.18 per cent radial growth inhibition at 20 per cent concentration. Further, it was noted that besides garlic, more than 50 per cent growth inhibition was recorded at all the concentrations in onion bulb and neem leaf extracts, while calotropis, and tulsi leaf extracts recorded more than 50% growth inhibition only at a higher concentration of 20%.

Previously, many researchers also reported similar findings. Six plant extracts were tested against *A. alternata* by Bochalya *et al.* (2012), who found that the Neem leaf extract (74.26%) and garlic extract (80.50%) were shown to be the most

Table 1: List of botanicals and bioagents tested against *Alternaria ricini*

Botanicals	Bio-agents
Neem (*Azadirachta indica*) leaf extract	*Trichoderma viride* (Native isolate)
Tulsi (*Ocimum tenuiflorum*) leaf extract	*Trichoderma harzianum* (Native isolate)
Onion (*Allium cepa*) bulb extract	*Trichoderma longibrachyatum* (Native isolate)
Garlic (*Allium sativum*) clove extract	*Trichoderma koningii* (Native isolate)
Datura (*Datura stramonium*) leaf extract	*Trichoderma faciculatum* (Native isolate)
Calotropis(*Calotropis procera*) leaf extract	*Pseudomonas fluorescence* ATCC 49838
Naffatia (*Ipomea carnea*) leaf extract	*Bacillus subtilis* ATCC 19659

Table 2: Comparison of the parameter of *A. ricini* standard key with our culture

Parameter	*A. ricini* (According to standard key)	Our culture	Remarks
Spore morphology	Conidia obclavate, light olive-brown	Obclavate, light olive-brown	(+)
Method of conidiogenesis	Solitary, rarely in chain of 2-3	Solitary, no chain	(+)
Spore size	168-344.4×29.4-42.7µm	195.65×34.35µm	(+)
No. of horizontal septa	5-12	7-13	(+)
No. of vertical septa	2-9	3-7	(+)
Beak length	Long beak with septate- aseptate (75.6-231×3.15-4.2µm)	Long beak with septate(95.89×3.54µm)	(+)

(+) positive relation

effective at inhibiting the mycelial growth compared to the control. According to Barman *et al.* (2016), among a variety of phyto-extracts tested, the ethanolic leaf extract of *Allium sativum* and *Polyalthia longifolia* had the best results against Alternaria leaf blight of tomato with 100% growth inhibition. Furthermore, Kumar and Singh (2017) examined six different plant extracts against *A. solani in vitro* and observed that *Euphorbia hirta* exhibited the least mycelium growth suppression at 5%, while *Allium sativum* (@5% concentration) and *Crotalaria juncea* (@5% concentration) inhibited mycelium growth to the greatest extent (45.15% and 44.40%, respectively). According to research by Rani *et al.* (2018), garlic clove extract at 10% was the most effective, showing 84.31% inhibition against *A. alternata*. In contrast, *A. tenuissima* showed 82.18% reduction in mycelial growth, which was followed by onion and neem.

Plant extracts typically include a range of secondary metabolites with diverse antimicrobial properties. These chemicals may be the reason behind the botanicals' efficacy against the growth inhibition of fungal pathogens, particularly *A. ricini* in this particular investigation. Onions include oleic acid, alpha and beta tocopherol, and allyl propile di-sulphide, whereas garlic additionally contains di-allyl di-sulphide and di-allyl thiosulfinate (allicin). These sulpher-based compounds have potential antimicrobial properties responsible for the inhibition of fungal mycelial growth by targeting the plasma membrane and cell wall (Aala *et al.* 2014; Sánchez-Hernández *et al.* 2023). Garlic also contains Ajoene, antifungal compound inhibiting the spore germination of different *Alternaria* sp. Interestingly, garlic has three times more amount of sulphur-based compounds like allicin than onions do (Tagoe *et al.* 2011). Thus, garlic extracts were found to have more effective antimicrobial activity than onions. This is supported with by the previous finding of Khounganian *et al*. (2023). Furthermore, azadirachtin and nimonol found in neem leaves (Mahmoud *et al*. 2011) and atropine present in

Table 3: *In vitro* bio-efficacy of different botanicals against *A. ricini*

Phytoextracts	Growth inhibition (%) Concentration (%)			Mean
	5	10	20	
Neem leaves	58.37* (49.82^{g**})	67.87 (55.47^{c})	71.18 (57.53^{b})	65.90 (54.27^{b})
Tulsi leaves	47.45 (43.54^{j})	54.82 (47.77^{hi})	64.35 (53.34^{d})	55.60 (48.21^{d})
Onion bulb	49.17 (44.52^{j})	61.13 (51.43^{f})	64.67 (53.53^{d})	58.39 (49.83^{c})
Garlic clove	63.48 (52.82^{de})	72.93 (58.65^{b})	78.73 (62.54^{a})	71.93 (58.01^{a})
Datura leaves	30.56 (33.56^{m})	40.65 (39.61^{l})	47.21 (43.40^{j})	41.07 (38.86^{g})
Calotropis leaves	45.54 (41.37^{k})	54.59 (46.49^{i})	65.71 (51.61^{ef})	55.18 (46.49^{e})
Naffatia leaves	43.67 (39.40^{l})	52.60 (43.49^{j})	61.44 (48.52^{h})	52.60 (43.80^{f})
Mean	47.53 (43.57^{c})	56.94 (48.99^{b})	63.66 (52.93^{a})	
	Phytoextracts	Concentration		Phytoextracts × Concentration
S. Em. ±	0.34	0.22		0.59
C. D. at 5%	0.96	0.63		1.28
C.V. %		2.09		

*Figures in parentheses are arc-sin transformed values; **Treatment means with the common letter(s) are not significant by DNMRT at 5% level of significance

Table. 4: *In vitro* bio-efficacy of different bio-agents against *Alternaria ricini*

Bio-agents	Mean colony diameter (mm)	Growth Inhibition (%)
Trichoderma viride	49.46^{f}	91.07 (72.61^{a})*
Trichoderma harzianum	50.13^{ef}	88.36 (70.05^{b})
Trichoderma longibrachyatum	52.75^{de}	78.46 (62.35^{c})
Trichoderma koningii	53.79^{cd}	74.78 (59.66^{d})
Trichoderma faciculatum	57.20^{b}	63.71(52.96^{f})
Pseudomonas fluorescence	55.85^{bc}	67.95 (55.52^{e})
Bacillus subtilis	57.82^{b}	61.84 (51.85^{g})
Control	90.00^{a}	-
S. Em.±	0.93	0.79
C.D. at 5%	2.915	2.71
C.V. %	2.885	2.20

*Figures in parentheses are arcsin transformed values; Treatment means superscript with the common letter(s) are not significant by DNMRT at 5% level of significance

datura (Gul *et al*. 2012) may have contributed to their antifungal property. As plant extracts have been known for their potential antifungal and antibacterial properties for a long time, there is a need to extract, identify and test their active compounds as a safe alternative to chemical pesticides.

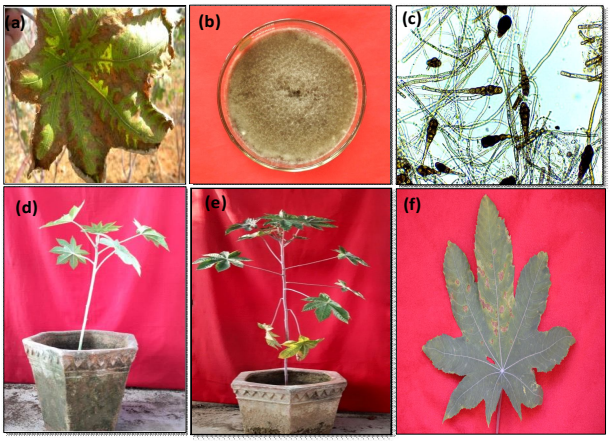

Fig.1: Symptomatology, isolation and pathogenicity of *Alternaria ricini*. (a) Leaf showing typical symptom of Alternaria leaf spot (b) Pure culture of *Alternaria ricini* showing brown coloured colony (c)Dictyospores of *A. ricin with* long beak(d) Control pot having healthy castor plant (e, f) Pathogenicity of *A. ricini* showing typical leaf spot symptoms starting from the margin.

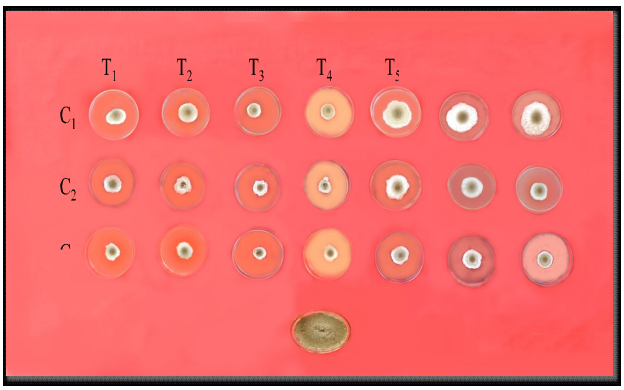

Fig.2: *In vitro* bio-efficacy of different botanicals against *Alternaria ricini*. The botanicals tested are neem leaves (T_1), Tulsi leaves (T_2), Onion bulb (T_3), Garlic clove (T_4), Datura leaves (T_5), Calotropis leaves (T_6), Naffatia leaves (T_7) at three different concentrations C_1- 5%, C_2-10% and C_3-20% respectively

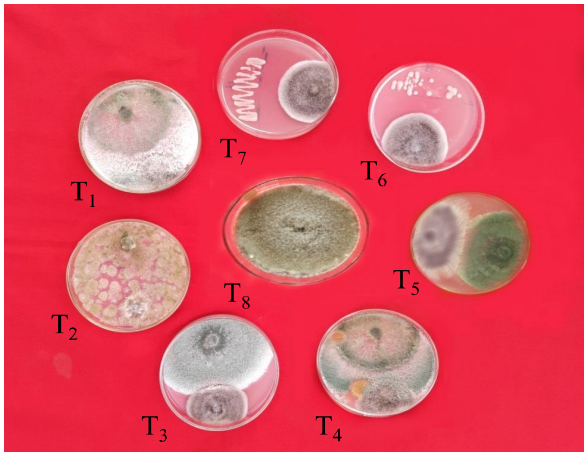

Fig 3: *In vitro* bio-efficacy of different bioagents against *Alternaria ricini*. The bio agents tested are *Trichoderma viride* (T_1),*Trichoderma harzianum* (T_2),*Trichoderma longibrachyatum* (T_3),*Trichoderma koningii* (T_4),*Trichoderma faciculatum* (T_5), *Pseudomonas fluorescence* (T_6),*Bacillus subtilis* (T_7),and Control (T_8)

In vitro bio-efficacy of different bio-agents against *Alternaria ricini*

The growth inhibition of *A. ricini* by all seven different bio-agents was tested and found to range from 61.84 to 91.07 per cent (Fig. 3). Among all seven bioagents tested, *Trichoderma viride* was proved as the most inhibitory with maximum growth inhibition (91.07%) of *A. ricini* followed by *T. harzianum* with a growth inhibition of 88.36% per cent. *T. longibrachyatum*, *T. koningii,* and *P. fluorescens* were good with recorded growth inhibition of 78.46, 74.78, and 67.95 per cent, respectively and were statistically at par with each other. Although *T. faciculatum* and *B. subtilis* were found to be comparatively less effective, with recorded growth inhibition of only 63.71 and 61.84 per cent, respectively (Table 4).

Previously, similar findings were reported by various workers. Of them, Hiremani *et al.* (2014) tested different bioagents against *A. ricini* and reported that inhibition of radial growth was maximum in the case of *T. viride* (56.63%), followed by *T. harzianum* (46.83%). But *B. subtilis* (28.47%), and *P. fluorescens* (28.44%) were less effective in inhibiting the radial growth of the pathogen. Whereas, Varma *et al.* (2008) studied the biological control of *A. solani* using fungal and bacterial antagonists significantly reduced the per cent disease index compared to inoculated control. The lowest disease intensity (27.52%) was recorded with foliar spray of *Trichoderma* followed by *Bacillus subtilis* with 33.35% disease intensity. Similarly, Kiran *et al.* (2018) studied the antagonistic potential of bioagents against *A.brassicicola*, the incitant of Alternaria leaf spot of cabbage. Among different bioagents, the *Trichoderma viride* gave the highest percentage of mycelial inhibition of *A. brassicicola*. Further, different bio-agents and botanicals were tested against *Alternaria alternata* of pomegranate by Kadam *et al.* (2018); among them *Trichoderma viride* was found most effective with the highest mycelia inhibition of 86.85% of the test pathogen, followed by *Trichoderma hamatum* and *Aspergillus niger*. Aldiba and Escov (2019) also studied biological control of early blight on potato caused by *Alternaria solani*, and reported that among different bioagents, *Trichoderma* sp. gave the best result with minimum mycelial growth

(0.55 mm) followed by *Pseudomonas brassicacearum* (0.74 mm) against *Alternaria solani*. Recently, Gadhi *et al.* (2020) checked the *in-vitro* effectiveness and sustainability of a bio-control agent *Trichoderma viride* against *Alternaria alternata* of chickpea. Of different bioagents, *Trichoderma viridae* is a well-known bio-control agent which reduces disease incidence in plants either through direct inhibition mechanism (antibiosis and hyper-parasitism), or through induced higher levels of host defense mechanism.

CONCLUSION

Based on the present findings, the aetiology of Alternaria leaf blight in castor was confirmed as *Alternaria ricini*. The pathogen produced typical concentric, necrotic leaf spot symptoms when artificially inoculated on plants. Seven different botanicals and bioagents were tested *in vitro*. Among botanicals, the maximum mycelial growth inhibition was recorded in garlic clove extract followed by neem leaves and onion bulb extract. Further, *Trichoderma viride* followed by *T. harzianum* showed maximum growth inhibition of *A. ricini* as compared to other bioagents. Further field evaluation of these botanicals and bioagants is necessary; so that, a sustainable integrated management strategy can be formulated by combining all these strategies effective against castor leaf blight.

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DECLARATIONS

Conflict of interest. Authors declare no conflict of interest.

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