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Research Article

EVALUATION OF BOTANICALS AND BIO-AGENTS AGAINST *Rhizoctonia bataticola* CAUSING DRY ROOT ROT OF CHICKPEA

PATIDAR JAGDISH KUMAR*, SINGH PRASHANT KUMAR, KASHYAP VIVEK, SINGH REETI AND PATIDAR SATISH
Department of Plant Pathology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, 474002, Madhya Pradesh, India
*Corresponding Author: Email - jdsplantpathology@gmail.com

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Abstract- Nine botanicals and seven isolates of *Trichoderma Spp*. were evaluated by following poison food technique and dual culture technique against *Rhizoctonia bataticola*. Among the botanicals, maximum mycelium inhibition was recorded in *Zingiber officinale* rhizome (47.98 %) followed by *Datura stramonium* leaf (43.35 %), *Allium sativum* clove (39.74 %) and *Eucalyptus Globus* leaf (37.86 %). Out of the seven isolates of *Trichoderma Spp*. tested against *Rhizoctonia bataticola*, T-6 showed highest inhibition percentage (67.32 %) followed by T-7 (63.61%), T-3 (59.72 %) and T-5 (57.50 %).

Keywords- *Botanicals, Trichoderma, Rhizoctonia bataticola, mycelium growth, Inhibition percentage*

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Introduction

Gram or Chickpea (*Cicer arietinum* L.), a member of family Fabaceae, is an ancient self-pollinated leguminous crop, grown since 7000 BC, in different areas of the world [1]. It is the world's second most important food legume next to common bean. About 90% of the global chickpea production contributed by major chickpea producing countries includes India (67.4%), Australia (6.21%), Pakistan (5.73%), Turkey (3.86%), Myanmar (3.74%) and Iran (2.25%) [2]. Chickpea is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma) from all over the world [3]. Some of the serious diseases in order of their importance are wilt *Fusarium oxysborum* f. sp. ciceri) wet root rot (*Rhizoctonia solani*), dry root rot (*Rhizoctonia, bataticola*) *Ascochyta blight* (*Ascocthya rabiei*) and collar rot (*Sclerotium rolfsii*). *Rhizoctonia bataticola* (Taub.) Butler is a nectrotropic fungus caused dry root rot of chickpea which is emerging as a serious threat to the chickpea production worldwide [4]. Botanical extracts are biodegradable [5] and their use in crop protection is a practical sustainable alternative. It reduces environmental contamination and health hazards [6]. Botanical fungicides are unique because they can be produced easily by the farmers and small industries [7]. Different plant extracts also used separately or in combination to control some other fungi by the farmers. Very few studies have conducted using plant extracts and fungicides in the country to control *R. bataticola* causing dry root rot of chickpea.

Materials and methods

In vitro evaluation of botanicals against *Rhizoctonia bataticola*

Nine botanicals *viz*., *Azadirachta indica*, *Azadirachta indica*, *Datura stramonium*, *Eucalyptus globus*, *Calotropis* spp., *Zingiber officinale*, *Allium sativum*, *Parthenium hysterophorus* and *Allium cepa* were tested in vitro by poisoned food technique against *Rhizoctonia bataticola*. Healthy fresh plant parts *i.e*., leaves, bulbs, seeds or rhizomes were taken, washed thoroughly with fresh water and finally rinsed with sterilized distilled water.

Fifty gram of plant parts were cut into small pieces and minced with the help of a grinder by adding 50 ml sterilized distilled water. Double-layered muslin cloth were used to filter botanical extracts in 150ml conical flasks and plugged with non-absorbent cotton. These filtered extracts were autoclaved at 1.2 kg cm^{-2} pressure for 20 minutes. Autoclaved extract was individually added into previously sterilized Potato Dextrose Agar (PDA) plates @ 20 percent and mixed thoroughly at the time of pouring in the previously sterilized Petri plates. Five mm discs of 10 days old culture of *R. bataticola* were inoculated at the centre of Petri plates. Three replications were maintained for each botanical. The plate without botanicals extract served as control. The Petri plates were incubated at 27 ± 2°C temperature till the complete coverage in control plate. The percent growth inhibition (PGI) of the pathogen was worked out by using formula given by [8].

$$PGI = \frac{CT}{C} \times 100$$

Where,

PGI = Percent growth inhibition
C = Growth in control
T = Growth in botanical

In vitro evaluation of bio-agents against *Rhizoctonia bataticola*

The antagonistic potential of the *Trichoderma* isolates against *R. bataticola* was evaluated by dual culture method on potato dextrose agar medium. Five mm discs of *R. bataticola* was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, antagonistic fungi were placed on the other side *i.e*., at an angle of 180^0. Plates with no antagonists placed served as control for the pathogen. The plates were incubated at 27 ± 2^0C for seven days. Each treatment was replicated thrice. Growth of *Trichoderma* Spp. and *R. bataticola* were measured after recording full growth of the *R. bataticola* in control plate. The percent inhibition of fungal plant pathogens was calculated using formula:

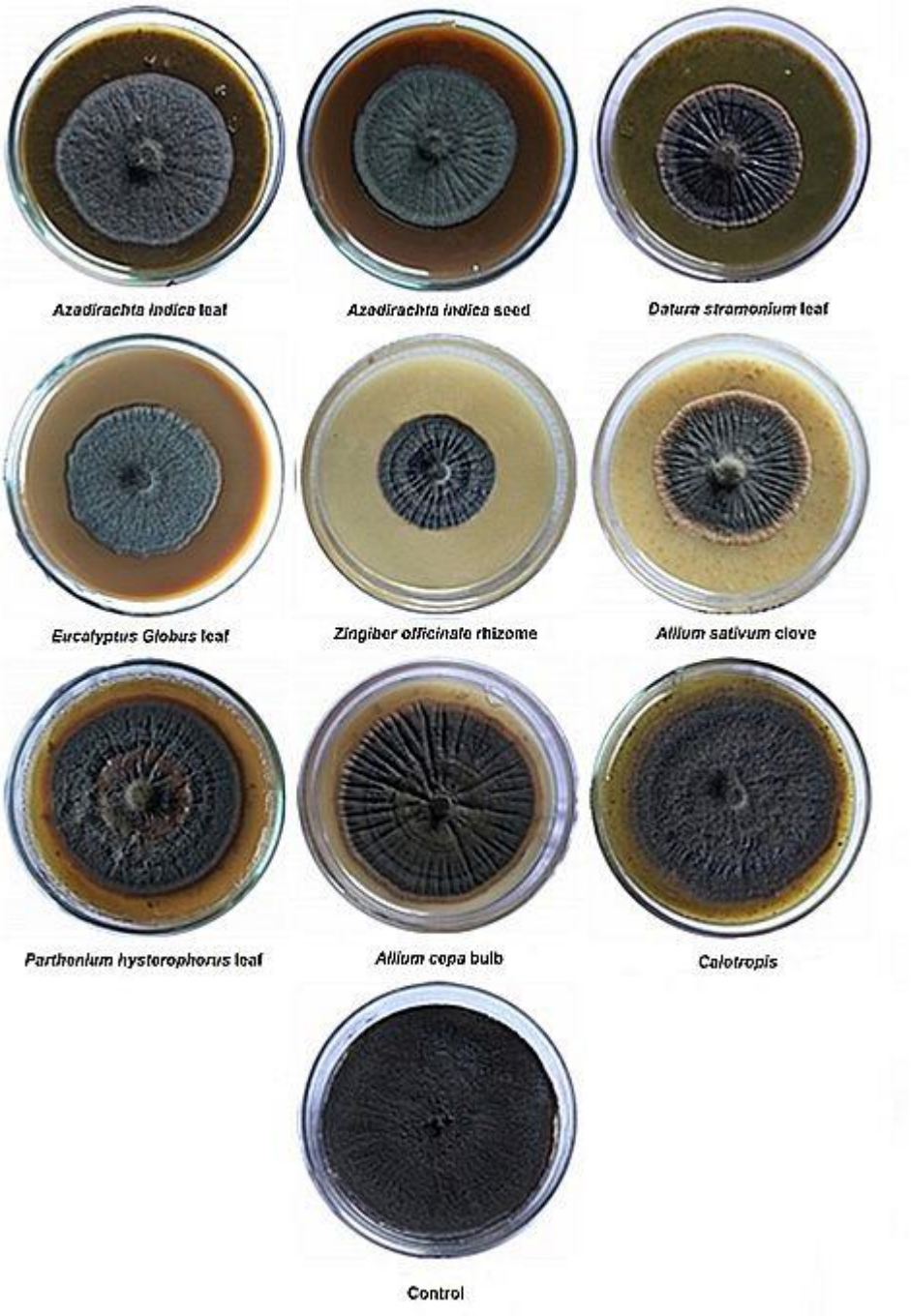

Plate-1 Effect of botanicals on the mycelium growth of *Rhizoctonia bataticola*

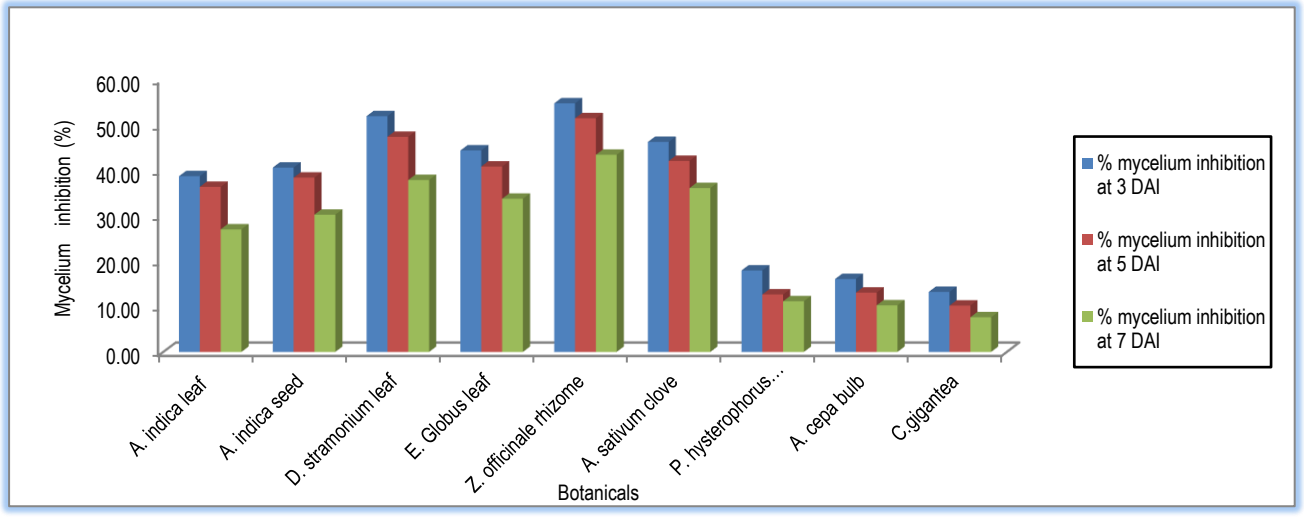

Fig-1 Percent mycelium inhibition of *R. bataicola* by different botanicals at different days after inoculation

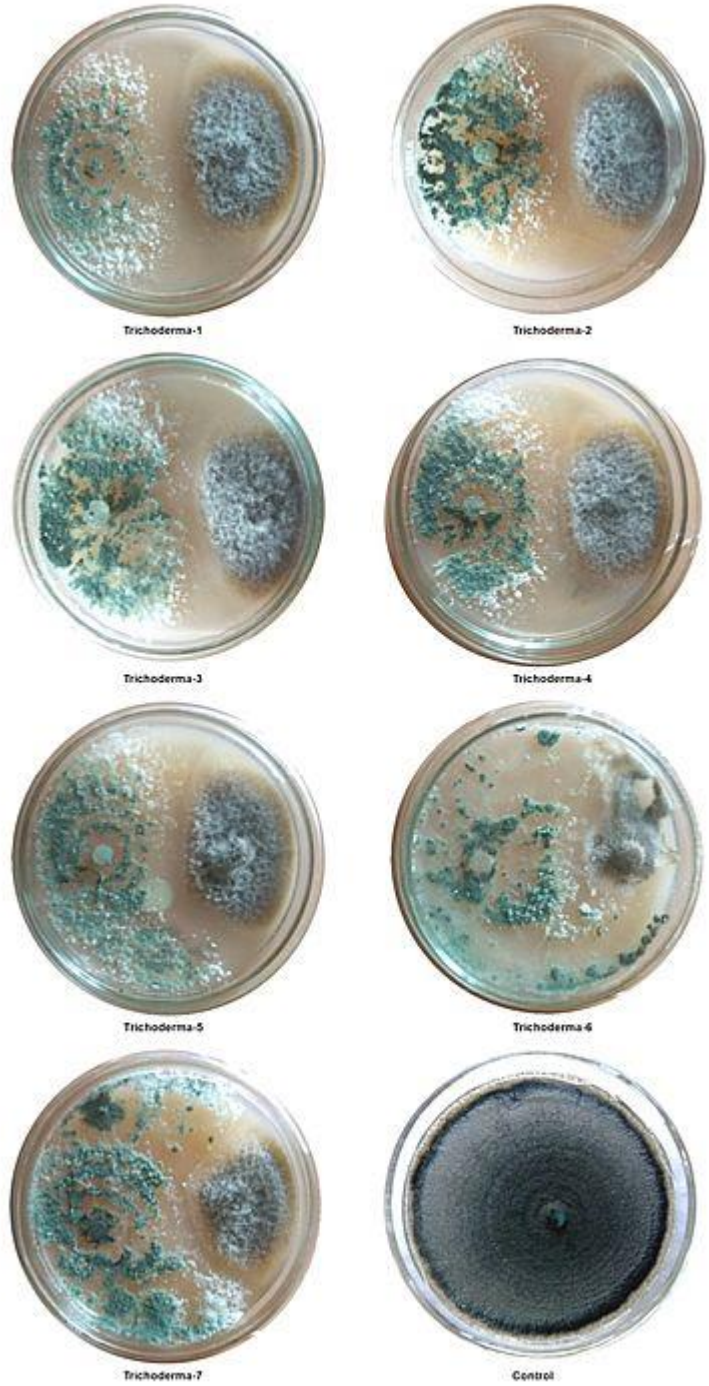

Plate-2 Interaction of *Rhizoctonia bataticola* with different strains of *Trichoderma*

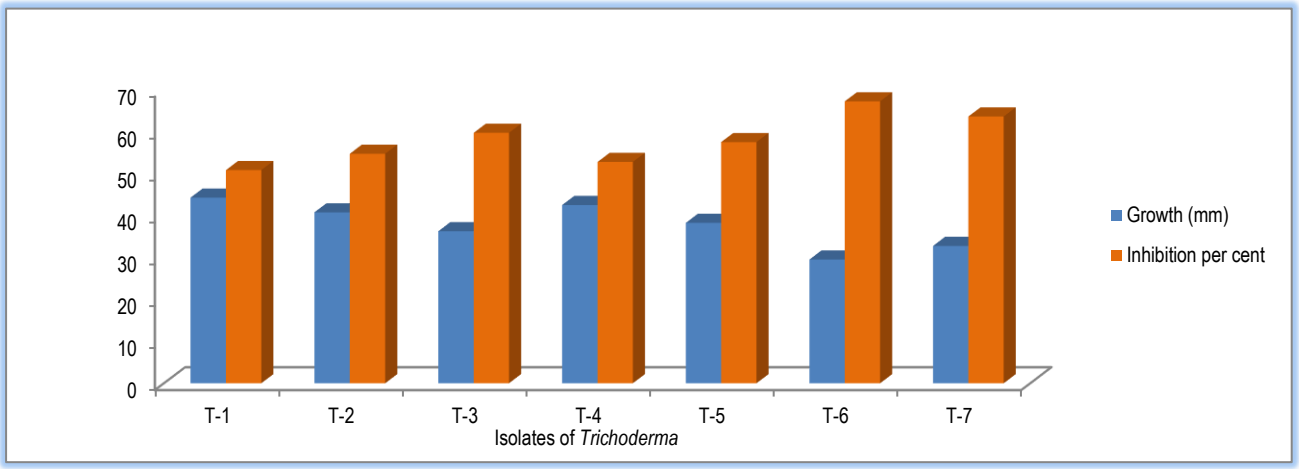

Fig-2 Mycelial growth and percent mycelium inhibition of *R. bataicola* by different isolates of *Trichoderma*

Table-1 Efficacy of botanicals on mycelium growth of *Rhizoctonia bataticola*

Botanicals	Mycelium growth (mm) and per cent mycelium inhibition at							
	3 DAI		5 DAI		7 DAI		Overall mean	
	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)
Azadirachta indica leaf	16.25	38.68 (38.39)*	39	36.33 (37.06)*	62.25	26.98 (31.28)*	39.17	32.08 (34.50)*
Azadirachta indica seed	15.75	40.57 (39.52)	37.75	38.37 (38.26)	59.5	30.21 (33.32)	37.67	34.68 (36.08)
Datura stramonium leaf	12.75	51.89 (46.09)	32.25	47.35 (43.48)	53	37.83 (37.95)	32.67	43.35 (41.18)
Eucalyptus Globus leaf	14.75	44.34 (41.74)	36.25	40.82 (39.70)	56.5	33.72 (35.49)	35.83	37.86 (37.97)
Zingiber officinale rhizome	12	54.72 (47.71)	29.75	51.43 (45.82)	48.25	43.40 (41.20)	30	47.98 (43.84)
Allium sativum clove	14.25	46.23 (42.82)	35.5	42.04 (40.42)	54.5	36.07 (36.90)	34.75	39.74 (39.07)
Parthenium hysterophorus leaf	21.75	17.92 (24.92)	53.5	12.65 (20.71)	75.75	11.14 (19.48)	50.33	12.72 (20.84)
Allium cepa bulb	22.25	16.04 (23.51)	53.25	13.06 (21.16)	76.5	10.26 (18.50)	50.67	12.14 (20.34)
Calotropis	23	13.21 (21.21)	55	10.20 (18.52)	78.75	7.62 (15.82)	52.25	9.39 (17.80)
Control	26.5	-	61.25	-	85.25	-	57.67	-
SEm ±		1.54		1.07		1.06		0.67
C.D. at 5 %		4.47		3.1		3.07		1.96

$$I = \frac{CT}{C} \times 100$$

Where, I = Percent inhibition.

C = Growth of fungal plant pathogens in control (mm).

T = Growth of fungal plant pathogens in dual culture plate (mm).

Results and discussion

Effect of botanicals on the growth *R. bataticola*

Botanical extracts may be used as an alternative source for reducing incidence of soil-borne diseases because they have a rich source of bioactive substance [9, 10, 11]. Botanical extracts are eco-friendly, show structural diversity and complexity and infrequently comprise halogenated atoms [12]. In the present investigation, efficacy of nine different botanicals was evaluated on the growth of *R. bataticola*. Data presented in [Table-1], revealed that all the botanicals significantly inhibited the growth of *R. bataticola*. Significantly maximum mycelium inhibition was recorded in *Zingiber officinale* rhizome (47.98 %) followed by *Datura stramonium* leaf (43.35 %), *Allium sativum* clove (39.74 %) and *Eucalyptus globus* leaf (37.86 %), however minimum mycelium inhibition was recorded in *Calotropis* spp. leaf (9.39 %) followed by *Allium cepa* bulb (12.14 %) and *Parthenium hysterophorus* leaf (12.72 %). At three days after inoculation, all the botanical significantly inhibited the mycelial growth of *R. bataticola* over control. Among the botanicals, maximum mycelium inhibition was recorded in *Zingiber officinale* rhizome (54.73 %) followed by *Datura stramonium* leaf (51.89 %), *Allium sativum* clove (46.23 %) and *Eucalyptus globus* leaf (44.34 %), however minimum mycelium inhibition was recorded in *Calotropis* spp. leaf (13.21 %) followed by *Allium cepa* bulb (16.46 %) and *Parthenium hysterophorus* leaf (17.92 %). At five days after inoculation, all tested botanicals significantly inhibit and reduced mycelial colony diameter over the control. Data presented in [Table-1] showed that significantly maximum mycelium growth inhibition was recorded in *Zingiber officinale* rhizome (51.43 %) followed by *Datura stramonium* leaf (47.35 %), *Allium sativum* clove (42.04 %) and *Eucalyptus globus* leaf (40.82 %), whereas minimum mycelium inhibition was recorded in *Calotropis* spp. leaf (10.20 %) followed by *Parthenium hysterophorus* leaf (12.65 %). and *Allium cepa* bulb (13.06 %). Data recorded on mycelium inhibition of *R. bataticola* at seven days after inoculation showed that all the screened botanicals significantly inhibited and reduced mycelial colony diameter over the control. Significantly maximum mycelium growth inhibition was recorded in *Zingiber officinale* rhizome (43.40 %) followed by *Datura stramonium* leaf (37.83 %), *Allium sativum* clove (36.07 %) and *Eucalyptus globus* leaf (33.72 %), whereas minimum mycelium inhibition was recorded in *Calotropis* spp. leaf (7.62 %) followed by *Allium cepa* bulb (10.26 %) and *Parthenium hysterophorus* leaf (11.14 %) [Table-1], [Plate-1], [Fig-1]. Mandhare and Suryawanshi, (2009) [13] reported that *Azadirachta indica* extract inhibited the growth of the fungus by *Allium sativum* and *Azadirachta indica* found effective against *R. bataticola*, which was inhibited the mycelium growth by 77.77 and 64.44% respectively. The other extracts were not effective. Kane, *et al*., (2002) [14] reported that crude extract of *A. sativum, Eucalyptus globulens* L. and *Zingiber officinale* L. were effective in inhibiting the mycelial growth of the *R. Solani* to the extent of cent percent. Ammajamma, *et al*., (2009) [15] evaluated botanicals and reported that eupatorium was highly effective at 10 percent followed by garlic and neem leaf extract (50 & 16.67% respectively). Mallesh, *et al*., (2008) [16] observed the maximum inhibition of mycelial growth of *F. solani* and *R. Solani* with 10% garlic bulb and neem leaf extracts. Mandhare and Suryawanshi, (2008) [17] reported that *Allium sativum* (15%) inhibited the growth of *R. bataticola* by 88.8%. More and Parate, (2016) [18] reported that *Azadirachta indica* at 20 percent concentration inhibits 85.22 percent growth of *Rhizoctonia bataticola*.

Table-2 Mean of growth inhibition of *Rhizoctonia bataticola* by *Trichoderma* Strains

Trichoderma isolates	Mycelium growth (mm) and per cent mycelium inhibition	
	Growth (mm)	Inhibition percent
T-1	44.25	50.83 (45.48)*
T-2	40.75	54.72 (47.71)
T-3	36.25	59.72 (50.61)
T-4	42.50	52.78 (46.59)
T-5	38.25	57.50 (49.32)
T-6	29.50	67.22 (55.09)
T-7	32.75	63.61 (52.90)
Control	90.00	-
SEm ±		0.61
C.D. at 5 %		1.80

In vitro evaluation of *Trichoderma spp.* against *R. bataticola* in dual culture technique

Besides chemical control, biological control is an effective, eco-friendly and alternative approach for any disease management practice. In the present experiment, seven isolates of *Trichoderma* were evaluated against *R. bataticola* by dual culture method. The results presented in [Table-2] reveal that all the isolates of *Trichoderma* suppressed the colony growth of *R. bataticola*. The suppression of the growth pathogen was maximum with T-6 (67.32 %) followed by T-7 (63.61%), T-3 (59.72 %) and T-5 (57.50 %). The minimum inhibition was shown by T-1 (45.56%) followed by T-4 (52.78 %) and T-2 (54.72 %) [Table-2], [Plate-2], [Fig-2]. Bandyopadhyay, *et al*., (2003) [19] agreed with the finding and reported that strain of *Trichoderma* inhibited the growth of *R. bataticola* by 51.1 percent under in vitro conditions. 11 *Trichoderma* isolates evaluated by Paul, *et al*., (2008) [20] and reported that maximum mycelium inhibition of *R. Solani* was recorded in *T. Harzianum* (77 %) under in vitro conditions.

Kaushal, (2008) [21] reported that *T. Harzianum* was effective in inhibiting the mycelial growth of *R. bataticola* the causal organism of chickpea dry root rot. Maruti, *et al*., (2017) [22] reported that *Trichoderma* viride (Tv-B) was found more effective and statistically significant over other bio-control agents in inhibiting the mycelial growth (77.20 %) of *R. bataticola* followed by *Trichoderma virens* (Tvn-B) (75.76 %) and rest of other treatments.

Conclusion

It is concluded that out of nine botanicals *Zingiber officinale* rhizome was found best which was recorded maximum mycelium inhibition of *Rhizoctonia bataticola*. Among isolates of *Trichoderma* Spp. T-6 was found best which was recorded maximum mycelium inhibition of *Rhizoctonia bataticola*.

Application of research: 1. Management of dry root rot of chickpea through botanical extract and bio-agents. 2. Management of plant disease without use of chemicals.

Research Category: Plant Pathology

Abbreviations:

mm: millimetre
^{0}C: Degree centigrade
Spp.: Species
viz : Namely
% : Percent

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***Research Guide: Dr Reeti Singh**
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