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Pankaj Dunge

a. College of Agriculture,
Jawaharlal Nehru Krishi
Vishwa Vidyalaya, Jabalpur,
Madhya Pradesh, India

b. Department of Agriculture,
Mandsaur University,
Mandsaur, Madhya Pradesh,
India

Dr. Jayant Bhatt

College of Agriculture,
Jawaharlal Nehru Krishi Vishwa
Vidyalaya, Jabalpur, Madhya
Pradesh, India

Chetan Patel

Department of Agriculture,
Mandsaur University, Mandsaur,
Madhya Pradesh, India

Corresponding Author:**Pankaj Dunge**

a. College of Agriculture,
Jawaharlal Nehru Krishi
Vishwa Vidyalaya, Jabalpur,
Madhya Pradesh, India

b. Department of Agriculture,
Mandsaur University,
Mandsaur, Madhya Pradesh,
India

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Management of dry root rot of chickpea through botanical, plant bi-product and bio-control agent

Pankaj Dunge, Dr. Jayant Bhatt and Chetan Patel

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Abstract

Gram or Chickpea (*Cicerarietinum* Linnaeus), a member of family Fabaceae. It is ranked 3rd after common bean (*Phaseolus vulgaris* L.) and pea (*Pisumsativum* L.). Chickpea is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma) from all over the world (Nene *et al.* 1996). The experiment on confirmation of pathogenicity of *Rhizoctoniabaticola* on chickpea was conducted under pot conditions after growing the test fungus on gram straw, soaked in 0.5 per cent dextrose solution and then mixing it with the sterilized pot soil. The pathogenicity of *Rhizoctoniabaticola* on chickpea was confirmed by growing the fungus on gram straw soaked in 0.5 dextrose and then mixing it with pot soil. The symptoms appeared within 13 days and the plants were killed within 16 days after sowing the test fungus was recovered from the roots of diseased plants. The results obtained during the course of investigations that *in vitro* evaluation of plant leaves extracts neem was recorded to be superior in inhibiting the mycelial growth of *R. bataticola*. At all the concentrations under test Eucalyptus and *Jatropha* were also found superior in inhibiting the fungus under laboratory conditions but their efficacies declined as the time lapsed. The wilt symptoms on chickpea appeared within 13 days and the plants were killed 16 days after sowing. The roots of the plants showed typical browning at the color region which advanced further and disintegrated the roots.

Keywords: Complete randomized design, chickpea, bio-control agent, potato dextrose agar

Introduction

Gram or Chickpea (*Cicerarietinum* Linnaeus), a member of family Fabaceae, is an ancient self-pollinated leguminous crop, grown since 7000 BC, in different area of the world (Tekeoglu *et al.*, 2002) [17] but its cultivation is mainly concentrated in semi-arid environments (Saxena, 1990) [8]. It is ranked 3rd after common bean (*Phaseolus vulgaris* L.) and pea (*Pisumsativum* L.).

Chickpea is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma) from all over the world (Nene *et al.* 1996). Some of the serious diseases in order of their importance are wilt *Fusariumoxysporum* f. sp. *ciceri* wet root rot (*Rhizoctoniasolani*), dry root rot (*Rhizoctonia*, *bataticola*) Ascochyta blight (*Ascochyarabiei*) and collar rot (*Sclerotiumrolfsii*).

The Dry root rot (DRR) of chickpea caused by necrotrophic fungus *Rhizoctoniabaticola* (Taub.) Butler has emerged as a serious threat to the chickpea production worldwide (Pande and Sharma, 2010) [6]. Dry root rot generally appears during late flowering and podding stages and the infected plants appear completely dried and are an important component of the disease complex that causes root rots and seedling blight in many grain legumes when they are weakened by other stress factors. In the absence of the host crop, it survives in soil as a competitive saprophyte on available dead organic matter.

Chemical control of dry root rot is not effective as *R. bataticola* has a broad host range and survives in soil for longer periods in the form of sclerotia. The sclerotia can survive up to 10 months even in the absence of the host plants and under prevailing dry soil conditions.

Review of literature

Chickpea is most extensively grown pulse crop in India. It is attacked by air, seed, and soil borne diseases.

Among soil borne diseases dry root rot (*Rhizoctoniabaticola* Taub. Butler) is most important diseases causing heavy losses from flowering to podding stage and disease severity depends

upon the temperature and moisture condition.

Pathogen

The basic characteristics of the species are the formation of sclerotia in the host tissue as well as in culture on the infected plant sclerotia seen in the pith region and outer surface of root just below the bark region. They are brown or jet black, minute, smooth externally, hard, variable in shape, globose, oval oblong, elliptical, curve or even forked and varying in size, but of uniform texture more or less loosely packed (Subramaniam 1971)^[15].

Occurrence and losses

Shrivastava *et al.* (2002)^[13] reported that *Fusarium* sp. was most prevalent (56%) in wilted plant followed by *Rhizoctonia bataticola* (17%) and *Trichoderma* sp. (10%). The incidence of *Rhizoctonia bataticola* also caused withering and drying of plant, which range from 8 to 20 percent.

Jagre (2012)^[2] reported that that highest percent mortality due to dry root rot was observed in the 10th standard week i.e. 2nd week of march 2012 when the temperature range from 11.6 - 30.8°C with 18-76 per cent relative humidity however the disease initiated when the temperature was > 27°C with the decreases in relative humidity.

Symptoms

According to Mehrotra and Aneja (1990)^[4] the first symptom of the disease is yellowing of the leaves within a day or two such leaves drop and in the course of the next two or three days whole plant dies off. The leaves and stem of the affected plants are usually straw coloured. The taproot is dark and quite brittle in dry soil.

Pathogenicity

Katariya *et al.* (2012)^[3] made studies to determine the cultural and pathogenic variation of *Macrophomina phaseolina* isolates collected from infected chickpea from various parts of Rajasthan. Pure cultures of these isolates were found the pathogenic on 40-day-old chickpea cv. C-235 grown in pots.

In vitro evaluation of leaf extracts

Sharma *et al.*, (2005)^[10] have demonstrated the efficacy of plant extract of *Eucalyptus globulus* against *R. bataticola* on gram. There was 85 per cent reduction in sclerotial formation when ten per cent plant extract was used. Leaf extract of neem (*Azadirachta indica*) was found effective in inhibiting the mycelial growth of *R. bataticola* in chickpea. (Hegde and Kalappawavan, (2000), Ramnath *et al.*, (2004), Kshirsagar *et al.*, (2004), Sharma *et al.*, (2005)^[10], Shahraj *et al.*, (2007)^[9]. Tandel *et al.* (2010)^[16] tried phyto-extracts of eleven plant species against *Rhizoctonia bataticola* of gram and revealed that the onion bulb extract produced maximum inhibition (98.14%) followed by extract of acacia, ginger, neem, garlic and karanj.

Influence of botanicals as soil treatment

Raihan *et al.*, (2003)^[7] found that garlic extract gave best control of seedling mortality due to *R. solani* and enhanced the growth of groundnut seedlings. Abbasi *et al.*, (2005) recorded inhibition of *R. solani* under pot conditions with neem amended soil.

Shahraj *et al.*, (2007)^[9] While working with plant extracts and *R. solani* observed that use of aqueous extract of leaves, stem, bark and fruits from *Eucalyptus* sp. at a concentration of five

per cent as soil amendment Increased in germination, shoot and root length and the weights of mungbean plants significantly. They reported that all the plant parts of *Eucalyptus* sp. were equally effective in reducing infection of *R. bataticola* in chickpea.

Influence of botanicals as seed treatment

Chand and singh (2005)^[11] reported efficacy of fungal bio-control agents and plant extracts of eco friendly management of chickpea wilt. The plant extract viz. *Eucalyptus*, *Jatropha* and neem reduced wilt of chickpea.

Shrikrushna and Deshpande (2015)^[12] reported that out of 48 actinobacterial isolates exhibited antagonistic activity against *Rhizoctonia bataticola* and eight isolates demonstrated significant antagonistic activity and plant growth promoting potential.

In vitro effect of bio-control agents

Sharma *et al.*, (2011)^[11] proved the efficacy of *T. harzianum* and *T. viride* in inhibiting mycelial growth of *R. bataticola* in *in vitro* conditions compared with *Pseudomonas maltophilia*, *P. fluorescens* and *Bacillus subtilis*. Kumari *et al.*, (2012) reported that among the tested biocontrol agents, *T. harzianum* was found most effective against the *R. solani* under *in vitro* and in pots conditions followed by *T. viride* and *T. polysporum*.

Influence of oil cake as soil treatment

There was a significant improvement in plant growth in neem cake amended soil infested with *R. bataticola*.

Solanky *et al.*, (2013)^[14] observed efficacy of various botanicals and oil cakes against *Macrophomina phaseolina* (Tassi.) Goid causing dry root rot of chickpea and reported that cakes of neem, castor and mustard were effective in reducing incidence of *R. bataticola* infecting roots of chickpea.

Material and method

Collection of seeds

Chickpea seed (var. JG 226) collected from Breeder Seed Production Unit, Department of Genetics and Plant Breeding, J.N.K.V.V., Jabalpur were used during the course of investigation. The seed were cleaned from inert matter labeled and stored in the paper bags followed by polythene bags.

Preparation of soil composite

Sandy loam soil was collected from the experimental area of J.N.K.V.V., Jabalpur. The soil, so collected, was thoroughly washed with their to four changes of water so as to remove the soluble leachiest and air dried. The soil was mixed with well decomposed dried and pulverized farm yard manure (FYM) in a ratio of 3:1. This soil composite was used throughout the experiments after sterilization.

Medium used

Potato dextrose agar (PDA) was used during the course of study and for isolation and maintenance of culture. The ingredients of the medium are as under.

Potato dextrose agar (PDA)

Potato (peeled and sliced) :	200g
Dextrose :	20g
Agar-agar :	20g
Distilled water :	1000ml.

Isolation and purification of *Rhizoctonia bataticola*

To isolate of *Rhizoctoniabaticola*, the sample were cut into small pieces (5-10 mm long), by sterilized knife. These were then surface sterilized with 1:1000 mercuric chloride (HgCl₂) for 30 seconds followed by these changes of sterilized water before keeping them on Petri plate containing PDA. The inoculated plates were incubated at 25± 1 °c for 6 days and examined for the growth of fungus. The growing colony of *Rhizoctoniabaticola* was identified under research microscopic and after identification, small portion of the fungus was transferred to a petri plate containing PDA and allowed to grow for seven days.

Pathogenicity test

Well pulverized chickpea straw was soaked in 0.5 per cent dextrose solution. The soaked straw was kept in 250 ml flasks and sterilized in an autoclave. After cooling, the flasks were then inoculated with five mm disc of seven days old culture of *Rhizoctoniabaticola* and incubated at 25± 1 °c. On sufficient growth of the fungus on the chickpea straw this was mixed with the sterilized pot soil @ 20g/500g soil (Tripathi 1998). The pots were kept for seven days to allow the multiplication, development and spread of the fungus. Surface sterilized chickpea seed were sown in pots. Each pot received one seed.

Collection of plant leaves

Plant leaves of commonly available trees having medicinal values viz., Neem (*Azadirachta indica*), Karanj (*Pongamiapinnata*), Aak (*Calotropisprocera*), Eucalyptus (*Eucalyptus globulus*), Tulsi (*Ocimumsancatum*), Datura (*Daturasuaveolens*), Mehendi (*Lawsoniainermeris*) and Jatropa (*Jatrophacurcas*) were collected from the botanical garden of College of Agriculture, Jabalpur. The leaves were dried at room temperature.

Botanicals (Leaf extracts)

The leaf extracts of following plants were used in the present Investigations.

S. No.	Common name	Botanical name
1	Neem	<i>Azadirachta indica</i>
2	Karanj	<i>Pongamiapinnata</i>
3	Datura	<i>Daturasuaveolens</i>
4	Aak	<i>Calotropisprocera</i>
5	Tulsi	<i>Ocimumsancatum</i>
6	Mehndi	<i>Lawsoniainermeris</i>
7	Jatropa	<i>Jatrophacurcas</i>
8	Neelgiri	<i>Eucalyptus globulus</i>

Method of extraction and preparation of soil Amendment

Leaf extract was prepared by boiling ten gram powder of each plant leaf in 100ml distilled water and boiling 70°C for 30 minutes. The decoction was filtered through cotton wool to obtain clear extract (Saramangala *et al.*, 1993).

Bioassay

The experiment was conducted under laboratory conditions to evaluate the efficacy of plant leaves against *Rhizoctoniabaticola*. A total nine plant leaves viz. Neem (*Azadirachta indica*), Karanj (*Pongamiapinnata*), Aak (*Calotropisprocera*), Neelgiri (*Eucalyptus globulus*), Tulsi (*Ocimumsancatum*), Datura (*Daturasuaveolens*), Mehendi (*Lawsonia mermen's*), Jatropa (*Jatrophacurcas*) were evaluated using poisoned food technique (Nene *et al.*, 1979). Water deficit potato dextrose agar (PDA) was prepared and

50 ml medium was poured in 150 ml capacity Earlemayer flask.

Efficacy of plant leaf extracts as seed treatment

The experiment was conducted to observe the efficacy of plant leaves extract as seed treatment. Good, Bold and healthy seeds of chickpea were surface sterilized with mercuric chloride (1:1000) and dried in shed. These seeds of them were dipped in 30% leaf extract of each plant for 30 minutes and sown in sterile earthen pots containing mixture of soil and fungus grown on straw.

Efficacy of plant leaf powders as soil treatment

The experiment was conducted in pots under glass house condition. The test fungus *Rhizoctoniabaticola* was grown on chickpea straw and mixed with the soil @ 20g/ kg soil as per the method described earlier. At the same time the leaf powders were also incorporated @ 30g/kg soil along with fungus. The soil mixture was then filled in sterilized pots. Surface sterilized seeds of chickpea were sown in each pot. Each pot received four seeds on germination thinning were done and two seedlings in each pot were maintained. Each treatment was replicated three times and randomized over glass house bench. The pots were irrigated with sterilized tap water as and when required. The glass house temperature during the period of experimentation ranged between 18-30°C. The experiment was allowed to run for 40 days. The data recorded was subjected to statistical analysis following Complete Randomized Design (CRD).

Efficacy of bio-control agents against *Rhizoctonia bataticola*

Apparently healthy surface sterilize seeds of chickpea were coated with bio agents viz., *Trichoderma harzianum*, *T. viride*, *Basillus subtilis* @ 4 g/ kg seed and *Pseudomonas fluorescens* @ 8 g/kg seed separately. Ten coated seeds were sown in each pot filled with mixture of sterilized soil and fungus. Fungus inoculated pots without treatment served as check. The experiment was allowed to run for 40 days.

Efficacy of oil cakes against *Rhizoctonia bataticola*

The experiment was conducted in pots under glass house condition. The test fungus *Rhizoctoniabaticola* were also grown on chickpea straw and mixed with the soil @ 50g/ kg soil as per the method described earlier. At the same time the oil cakes (Linseed, Cotton, Castor, Mustard) was incorporated (@ 30g/kg soil) along with fungus. The pots were irrigated with sterilized tap water as and when required. The glass house temperature ranged between 18-30°C during the period of experimentation. The experiment was allowed to run for 40 days. The pots were observed for seed germination, seedling mortality and appearance of wilt symptoms. The data so obtained was analyzed following Complete Randomized Design.

Results

Test of Pathogenicity

The experiment was conducted under glass house conditions using infested soil. The fungus, *Rhizoctoniabaticola*, was grown on gram straw, soaked in 0.5 per cent dextrose solution. Ten days old fungal culture grown on this medium was mixed with the sterile pot soil and sterile seeds of chickpea were sown and allowed to grow till the appearance of symptoms.

The symptoms appeared 13 days after sowing of seeds in the infested soil. The symptoms further developed and plants were wilted within 16 days after sowing. The test fungus was

recovered from the roots of diseased plants and was found to be highly pathogenic, (Plate 1 A, B).

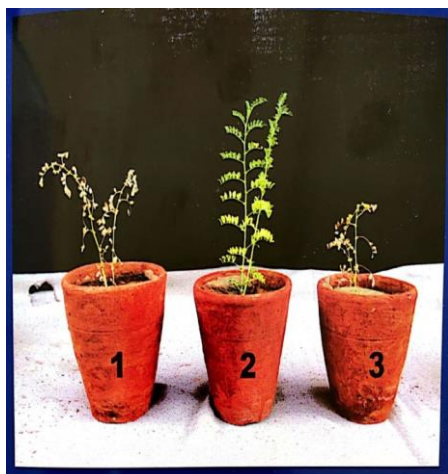


Plate 1 (a): Test of Pathogenicity of *Rhizoctonia bataticola* under pot condition.

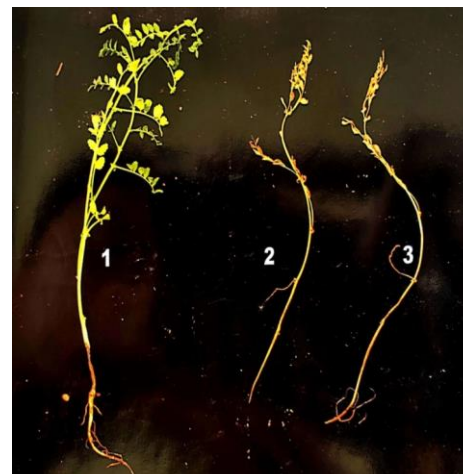


Plate 1 (b): Test of Pathogenicity of *Rhizoctonia bataticola* (Uprooted Condition).

Effect of leaf extract on the mycelial growth of *R. bataticola*

The experiment was conducted under laboratory conditions in plates containing Potato Dextrose Agar. Observations were recorded 48, 96, and 144 hours after inoculation employing ten, twenty, and thirty per cent concentrations of leaf extracts. It is evident from the data presented in the Table-1, Plate-2 and Fig.1 that ten per cent concentration of Neem (*A. indica*) was observed superior over control and rest of the treatments after 48 hours of inoculation. Minimum (10.35 mm) mycelial growth was recorded in this treatment against maximum in control (22.16mm). This was followed by *Jatropha* (*Jatropha curcas*) where 14.17 mm mycelial growth was noted. This concentration did not show any significant mycelial inhibition when the observations were recorded.

During 96 hours, fungal growth on the leaf extract amended medium (22.58mm) of radial growth was recorded in ten per cent concentration of Neem (*A. indica*) leaves followed by *jatropha curcas* (25.38) and *Pongamia pinnata* (25.41mm) in control (41.25mm).

At ten per cent concentration Neem (*A. indica*) leaf extract amended medium showed 39.28 mm radial growth of the test fungus that was minimum against maximum (60.24 mm) in control. *P. pinata* and *O. sanctum* leaf extracts recorded 42.11 mm and 43.26 mm radial growth of the test fungus respectively. Neelgiri (*E. globulus*) stood second in order of efficacy where 41.38 mm radial growth was recorded. Rest of the treatments however less effective in mycelial growth inhibition but superior over control after 144 hrs of test fungus inoculation.

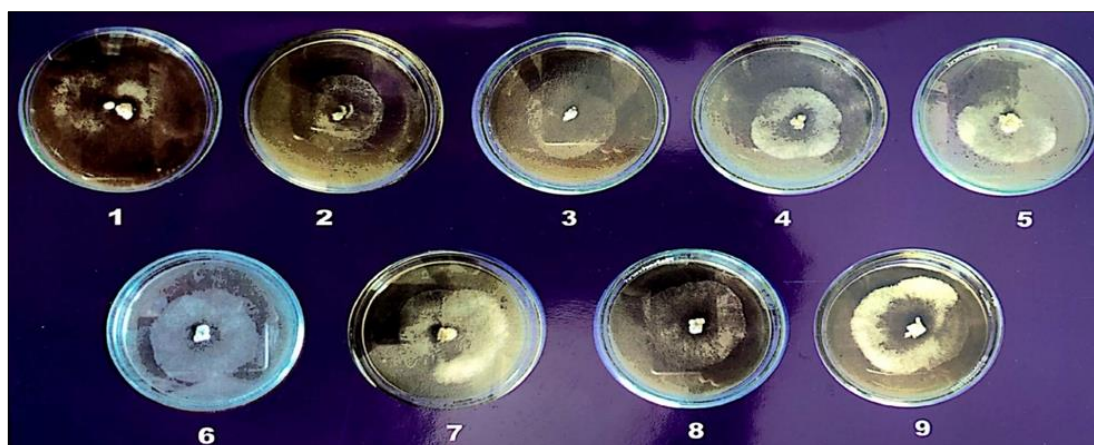


Plate 2: Effect of leaf extracts against *Rhizoctonia bataticola*

Table 1: Effect of leaf extracts on mycelia growth of *Rhizoctonia bataticola* at 10% concentration.

S. No.	Treatment	Mean radial growth (mm)*					
		48 hours		96 hours		144 hours	
		10% Conc.	Inhibition%	10% Conc.	Inhibition%	10% Conc.	Inhibition %
1	Datura	14.73	33.52	28.26	31.49	48.16	20.05
2	Karanj	17.26	22.11	25.41	38.4	42.11	30.09
3	Neem	10.35	53.29	22.58	45.26	39.28	34.79
4	Eucalyptus	16.28	26.53	27.27	33.89	41.38	31.3
5	Mehandi	16.15	27.12	27.36	33.67	45.28	24.83

6	Jetropha	14.17	36.05	25.38	38.47	44.3	26.46
7	Aak	15.23	31.27	26.51	35.73	44.36	26.36
8	Tulsi	16.29	26.48	27.28	33.86	43.26	28.18
9	Control	22.16		41.25		60.24	
	SEm±	0.074		0.158		0.184	
	CD at 5%	0.155		0.333		0.387	

Each value is mean of three replication.

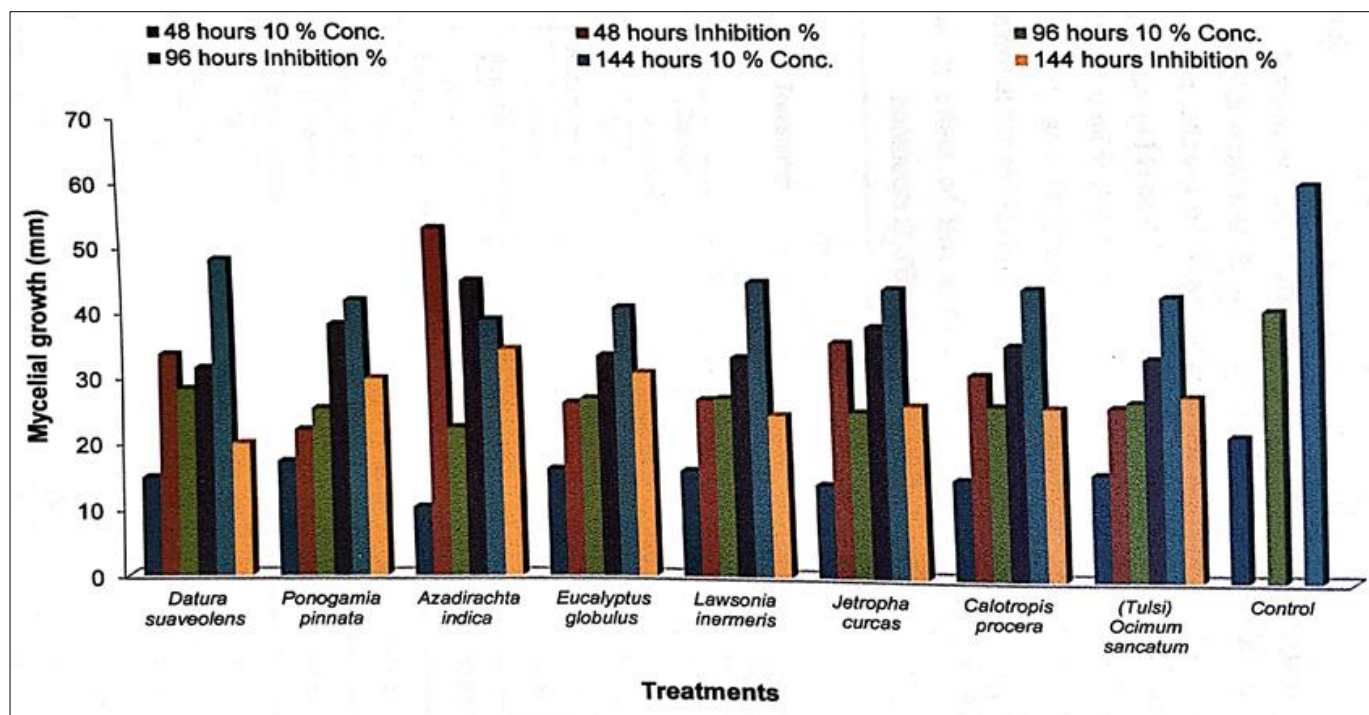


Fig 1: Effect of leaf extracts on mycelial growth of *Rhizoctonia bataticola* at 10% conc. concentration

At 20 per cent concentration of leaf extracts minimum (11.23 mm) radial growth of the test fungus was noted in Neem (*A. indica*) followed by *Lawsonia inermis* (13.35mm). Maximum (22.12 mm) radial growth was recorded in control. *Jatropha curcas* (14.33mm) stood next in order of efficacy *Eucalyptus globulus* recorded 15.25 mm of mycelial growth of test fungus.

At 20 per cent concentration Neem (*A. indica*) remained effective and superior over rest of the treatments and control. Minimum (23.55 mm) radial growth of the *R. bataticola* was

recorded in this treatment as against maximum (41.30mm.) radial growth in control. Twenty per cent concentration of Neem (*A. indica*) leaf extract inhibited the mycelial growth of *R. bataticola* significantly followed by Neelgiri (*E. globulus*). Radial growth of mycelium of the test fungus was 40.26mm in neem and 40.58mm in Eucalyptus respectively against maximum (60.15mm) in control. This was followed by, *O. sanctum* (43.43mm), and *Pongamia pinata* (44.23). Rest of the treatments remained at par with each other but superior over control.

Table 2: Effect of leaf extracts on mycelial growth of *Rhizoctonia bataticola* at 20% concentration.

S. No.	Treatment	Mean radial growth (mm)*					
		48 hours		96 hours		144 hours	
		20% Conc.	Inhibition %	20% Conc.	Inhibition %	20% Conc.	Inhibition %
1	Datura	16.3	26.31	28.41	31.21	45.3	24.68
2	Karanj	16.33	26.17	27.4	33.65	44.23	26.46
3	Neem	11.23	49.23	23.55	43.25	40.26	33.06
4	Eucalyptus	15.33	30.69	25.22	38.93	40.58	32.53
5	Mehandi	13.35	39.64	25.35	38.61	45.41	24.5
6	Jetropha	14.33	35.21	27.33	33.82	46.11	23.34
7	Aak	15.45	30.15	27.3	33.89	45.4	24.52
8	Tulsi	15.25	31.05	25.28	38.78	43.43	27.79
9	Control	22.12		41.3		60.15	
	S.Em±	0.042		0.059		0.047	
	CD at 5%	0.088		0.124		0.099	

Each value is mean of three replication.

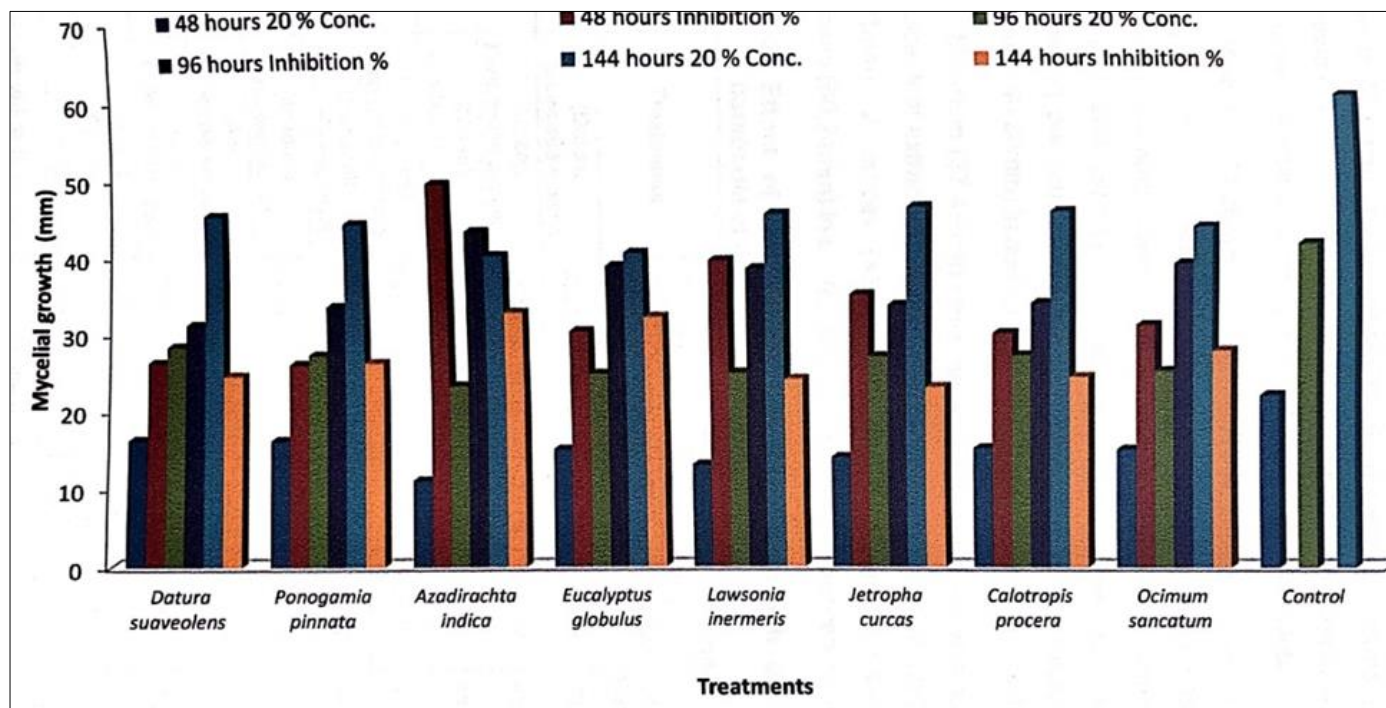


Fig 2: Effect of leaf extracts on mycelial growth of *Rhizoctonia bataticola* at 20% conc.

At 30 per cent concentration, Neem (*Azadirachta indica*) observed to be significantly superior over rest of the treatments and control. This treatment showed minimum (9.13 mm) radial growth of *R. bataticola* followed by *Lawsonia inermis* (14.26 mm). Rest of the treatments however, remained at par with each other but superior over control at 48 hours after inoculation

Minimum 21.36 mm radial growth of *R. bataticola* was again observed at 30 per cent concentration of Neem (*A. indica*) leaf extract followed by *P. pinnata* (23.05 mm), *Ocimum sanctum*

(25.26 mm), *Jatropha curcas* (25.46 mm) and *Eucalyptus globules* (26.13 mm). Rest of the treatments remained at par with each other in the inhibition of test fungus as against maximum (40.90 mm) in control. (Plate 4).

Minimum (37.43 mm) radial growth of *R. bataticola* was noted in Neem (*A. indica*) leaf extract followed by *Datura suaveolens* (40.46 mm), *E. globulus* (41.25 mm), *J. curcas* (42.51 mm) and *P. pinnata* (43.33 mm) against maximum (60.24 mm) in control after 144 hours at 30 per cent concentration.

Table 3: Effect of leaf extracts on mycelial growth of *Rhizoctonia bataticola* at 30% concentration

S. No.	Treatment	Mean radial growth (mm)*					
		48 hours		96 hours		144 hours	
		30% Conc.	Inhibition %	30% Conc.	Inhibition %	30% Conc.	Inhibition %
1	Datura	15.25	31.49	26.33	35.62	40.46	32.83
2	Karanj	15.28	31.35	23.05	43.64	43.33	28.07
3	Neem	9.13	58.98	21.36	47.77	37.43	37.86
4	Eucalyptus	15.25	31.49	26.13	36.11	41.25	31.52
5	Mehandi	14.26	35.93	26.26	35.79	45.13	25.08
6	Jetropha	14.51	34.81	25.46	37.75	42.51	29.43
7	Aak	15.35	31.04	27.13	33.66	44.56	26.02
8	Tulsi	16.31	26.72	25.26	38.23	42.3	29.78
9	Control	22.26		40.9		60.24	
	S.Em±	0.073		0.079		0.053	
	CD at 5%	0.153		0.167		0.112	

Each value is mean of three replication

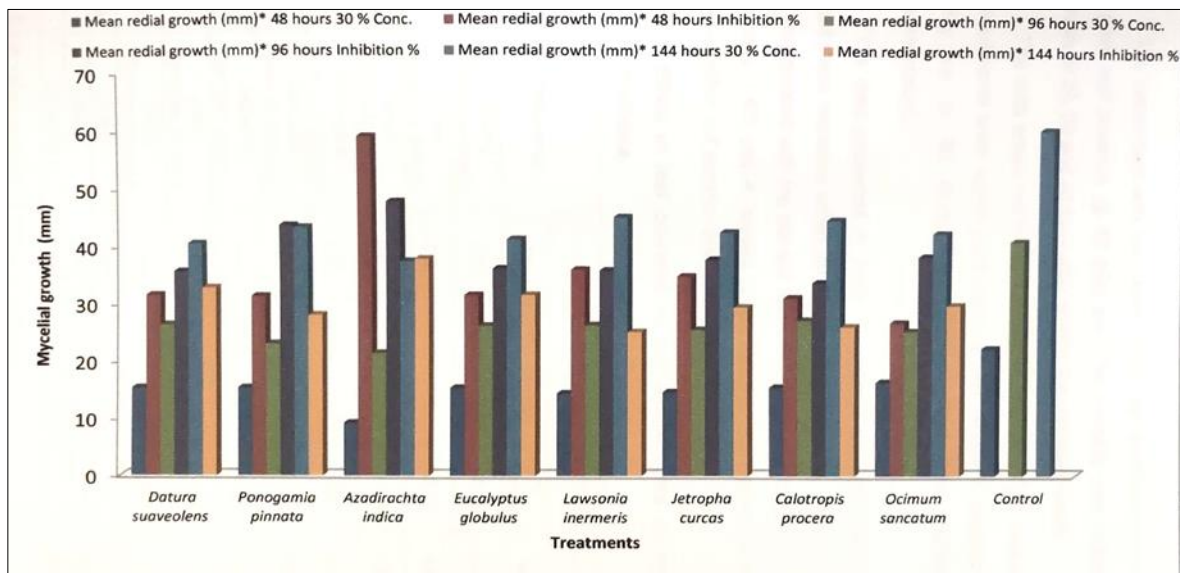


Fig 3: Effect of leaf extracts on mycelial growth of *Rhizoctonia bataticola* at 30% conc.

Effect of leaf powders as soil amendments against *R. bataticola*.

The experiment was conducted under pot conditions by incorporating the plant leaf powders @ 30 g/kg soil. The mortality was recorded at the intervals of 20, 30 and 40 days after sowing the chickpea seeds.

The data presented in the Table-4, Plate-3 A,B and Fig.4 revealed that all the treatments were significantly superior

over control in keeping the plants healthy up to 40 days, the maximum time limit involved in the experimentation.

The data presented in Table 4 indicated that minimum (0.67) plant mortality was recorded within 20 days where Neem(*A. indica*) leaf powder was incorporated with the pot soil followed by *O. sanctum* (1.00) a treatments followed by *P. pinnata*(2.00mm) and *Daturasuaveolens*(2.33). Rests of the treatments were at par with each other but superior over control.



Plate 3(a): Effect of leaf powders as soil amendments on mortality of chickpea



Plate 3(b): Effect of leaf powders as soil amendments on mortality of chickpea

Table 4: Effect of leaf powder as soil amendments on mortality of chickpea.

S. No.	Treatment	No. of plants mortality					
		Days					
		20	% control	30	% control	40	% control
1	Datura	2.33	36.51	3.00	30.71	3.67	21.41
2	Karanj	2.00	45.50	2.67	38.33	4.00	14.34
3	Neem	0.67	81.47	1.67	61.43	3.00	35.76
4	Eucalyptus	2.67	27.24	3.33	23.09	4.33	7.28
5	Mehandi	1.67	54.49	2.33	46.18	4.00	14.34
6	Jetropha	3.00	18.25	4.00	7.62	4.33	7.28
7	Aak	2.33	36.51	3.67	15.24	4.33	7.28
8	Tulsi	1.00	72.75	2.00	53.81	3.33	28.69
9	Control	3.67		4.33		4.67	
	S.Em±	0.222		0.192		0.222	
	CD at 5%	0.466		0.404		0.466	

Each value is mean of three replication.

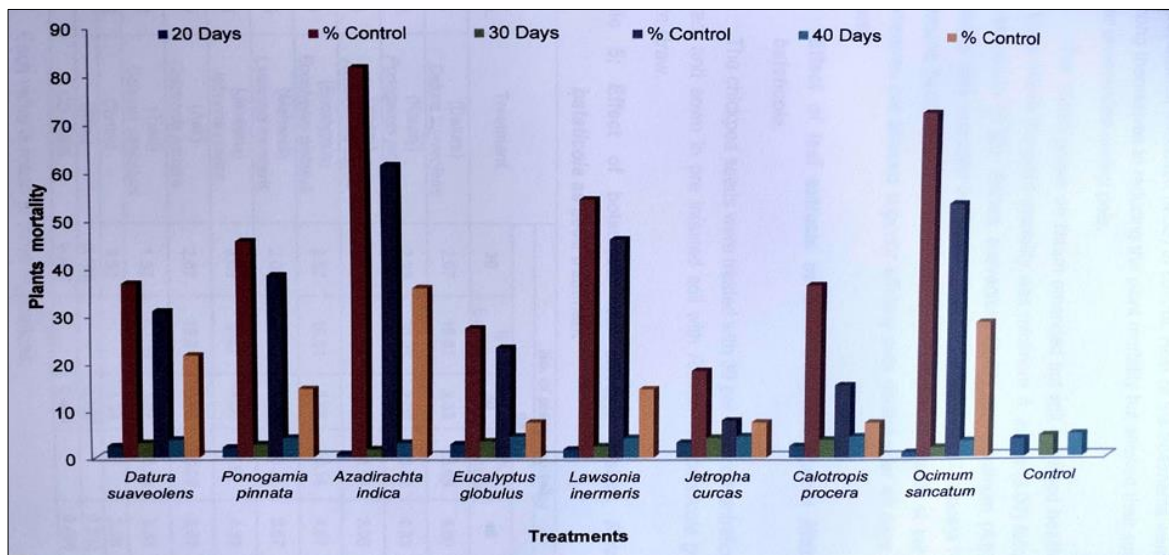


Fig 4: Effect of leaf powder as soil amendments on mortality of chickpea

During 30 days of plant growth minimum plant mortality was recorded with *A. indica* (1.67) followed by *O. sanctum* (2.00), *Lawsonia inermis* (2.33) against maximum (3.67) in control. Rest of the treatments were at par among themselves in reducing the plant mortality but showed their superiority over unamended control pots.

The plants grown on Neem amended pot soil remained healthy up to 40 days where the plant mortality was minimum *A. Indica* (3.00) followed by *O. sanctum* (3.33), *Datura suaveolens* (3.67) and maximum (4.67) plant mortality was recorded in control. *J. curcas* (4.33) and *C. procera* (4.33) *L. inermis* (4.00) and *P. pinnata* (4.00) remained statistically at par among themselves but showed superior efficacy over

control after 40 days of plant growth.

Effect of leaf extracts as seed treatment against *R. bataticola*

The chickpea seeds were treated with 30 per cent concentration of leaf extract and sown in pre infested soil with *Rhizoctonia bataticola* grown on gram straw.

The data presented in Table-5, Plate-4 and Fig 5 revealed that after 20 days of plant growth *O. sanctum* showed its efficacy where minimum (1.33) mortality was recorded. This was followed by *A. indica* (1.67), and *L. inermis* (2.00) which were remained at par among themselves. Maximum mortality was (3.33) recorded in control.



Plate 4: Effect of leaf extracts against *Rhizoctonia bataticola* as seed treatments.

Table 5: Effect of botanicals leaf extracts against *Rhizoctonia bataticola* as seed treatment.

S. No.	Treatment	No. of plants mortality					
		Days					
		10	% control	20	% control	30	% control
1	Datura	2.67	19.81	3.33	23.09	4.00	20
2	Karanj	2.33	30.03	3	30.71	4.33	13.4
3	Neem	1.67	49.84	2.33	46.18	3.00	40
4	Eucalyptus	2.67	19.81	3.67	15.14	4.67	6.6
5	Mehandi	2.00	39.93	3	30.71	3.67	26.6
6	Jetropha	3.00	9.90	4	7.62	4.33	13.4
7	Aak	2.67	19.8/1	3.33	23.09	3.67	26.6
8	Tulsi	1.33	60.06	2.67	38.33	3.33	33.4
9	Control	3.33		4.33		5.00	
	S.Em±	0.169		0.192		0.222	
	CD at 5%	0.356		0.404		0.466	

Each value is mean of three replication.

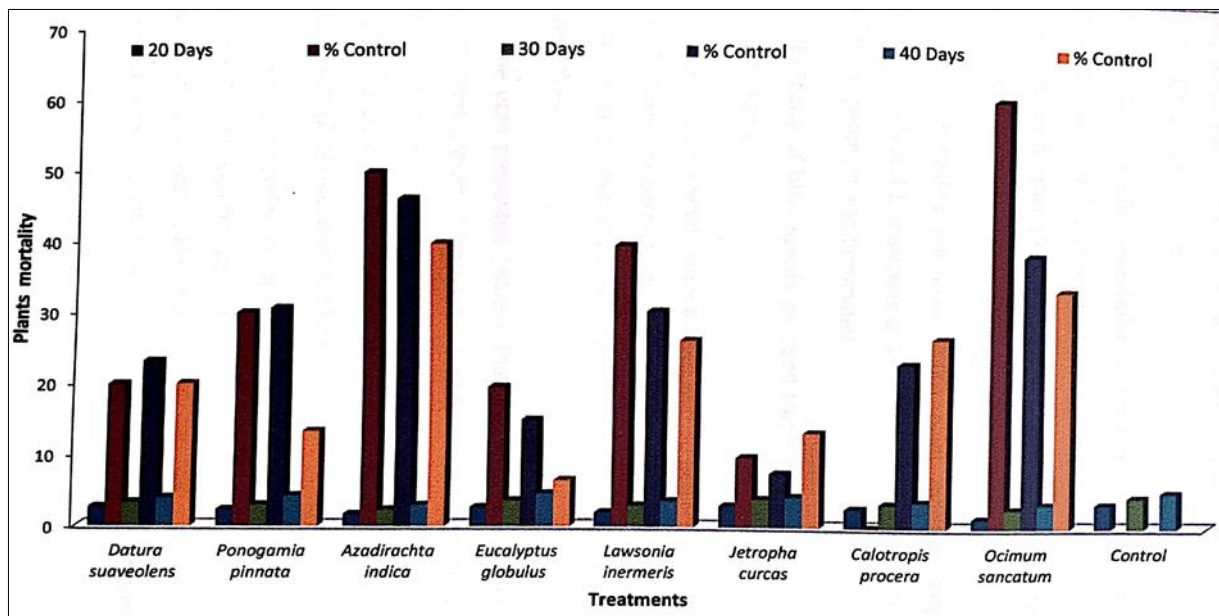


Fig 5: Effect of leaf extracts against *Rhizoctonia bataticola* as seed treatments.

Efficacy of bio- agents as seed treatment against dry root rot of chickpea.

The chickpea seeds were treated with 30 per cent concentration of bio- agent *Trichoderma harzianum*, *T. viride*, *Basillus subtilis* and *Pseudomonas fluorescens* were sown in pre infested soil with *Rhizoctonia bataticola* grown on gram straw.

The data presented Table-6, Plate-5 and fig 6 revealed that after 20 days of plant growth *Trichoderma harzianum*, showed

its efficacy where minimum (1.00) mortality was recorded. This was followed by *T. viride* (1.40) and *Basillus subtilis* (1.80) which were remained at par among themselves. Maximum mortality was (2.60) recorded in control.

Under pot conditions, after 30 and 40 days *T. harzianum* was found most effective in reducing pre and post emergence mortality. *T. viride* and *Basillus subtilis* was also moderately effective, while *P. fluorescens* was least effective in reducing pre and post emergence mortality.



Plate 5: Effect of Bio control agents against *Rhizoctonia bataticola* as soilamendments on plant mortality of chickpea.

Table 6: Efficacy of bio- agents as seed treatment against dry root rot of Chickpea.

S. No.	Treatment	No. of plants mortality					
		Days					
		10	% control	20	% control	30	% control
1	<i>T. viridi</i>	1.40	46.15	2.20	38.88	3.20	30.43
2	<i>T. harzenium</i>	1.00	61.53	1.40	61.11	2.40	47.82
3	<i>B. subtilis</i>	1.80	30.76	2.40	33.33	3.40	26.08
4	<i>P. floresence</i>	2.20	7.69	2.80	22.22	3.80	17.39
5	Control	2.60		3.60		4.60	
	S.Em±	0.089		0.101		0.101	
	CD at 5%	0.186		0.212		0.212	

Each value is mean of three replication.

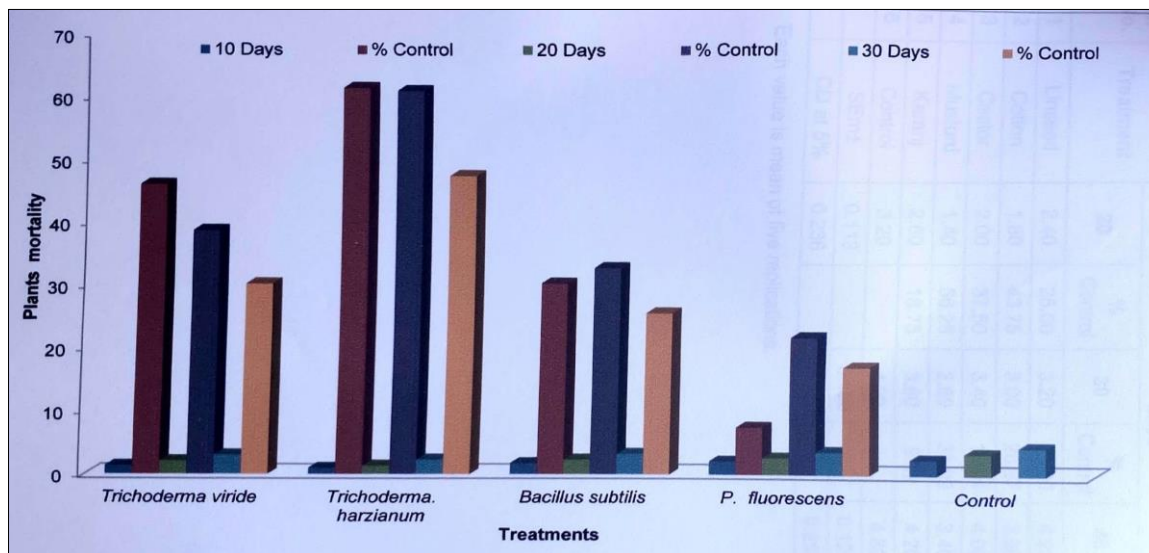


Fig 6: Efficacy of bio agents as seed treatment against dry root rot of chickpea.

Effect of oilcakes as soil amendments on mortality of Chickpea

The data presented in Table-7, plate-6, and fig-7 revealed that after 20 days of plant growth mustard showed its efficacy where minimum (1.40) mortality was recorded. This was followed by castor (2.00) and cotton cakes (1.80) which were remained at par among themselves. Maximum mortality was (3.20) recorded in control.

Similarly 30 days after sowing minimum (2.60) plant mortality was recorded in mustard amended soil whereas maximum (4.20) mortality was recorded in control. Cotton

and castor cake recorded 3.00 and 3.40 wilted plants respectively. Rest of the treatment remained at par among themselves in keeping the plants healthy up to 30 days.

Three wilted plants were recorded in 30 percent concentration of 40 days after sowing in the pots treated with mustard cake. This was followed by mustard (3.40) against maximum (4.80) in untreated control. Linseed and Karanj did not show any significant effect in managing the disease and remained at par with control. Rest of the treatments however, statistically inferior in their efficacies but superior over control 40 days after sowing when the observations were recorded.



Plate 6: Effect of oil cakes against *Rhizoctonia bataticola* as soil amendments on plant mortality of chickpea.

Table 7: Effect of oilcakes as soil amendments on mortality of Chickpea.

S. No.	Treatment	No. of plants mortality					
		Days					
		10	% Control	20	% Control	30	% control
1	Linseed	2.40	25.00	3.20	23.8	4.20	12.5
2	Cotton	1.80	43.75	3.00	28.57	3.80	20.83
3	Castor	2.00	37.50	3.40	19.04	4.00	16.66
4	Musturd	1.40	56.25	2.60	38.09	3.40	29.16
5	Karanj	2.60	18.75	3.80	9.52	4.20	12.5
6	Control	3.20		4.20		4.80	
	S.Em±	0.113		0.109		0.123	
	CD at 5%	0.236		0.228		0.257	

Each value is mean of three replication.

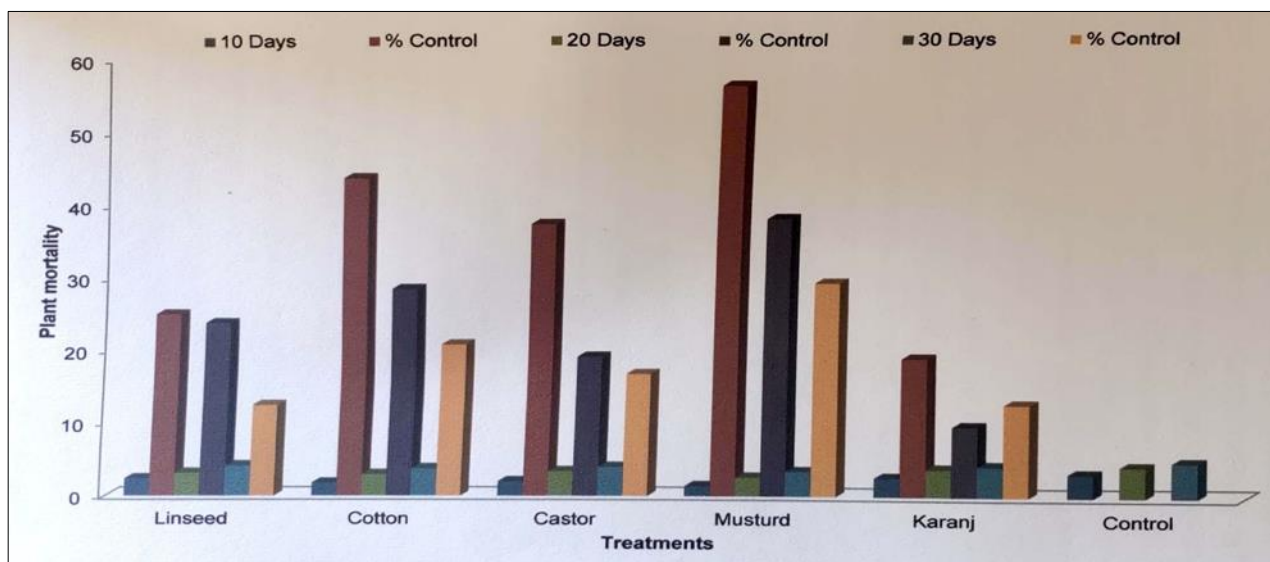


Fig 7: Effect of oilcakes as soil amendments on mortality of chickpea.

Discussion

Test of pathogenicity

The experiment on confirmation of pathogenicity of *Rhizoctoniablatitcola* on chickpea was conducted under pot conditions after growing the test fungus on gram straw, soaked in 0.5 per cent dextrose solution and then mixing it with the sterilized pot soil.

Dry root rot of chickpea caused by *Rhizoctoniablatitcola* a soil borne pathogen is the most important disease and cause enormous losses at podding and maturity stage when the temperature exceeds $> 30^{\circ}\text{C}$. Several workers have reported various types of symptoms produced on plants with varying intensity of the disease in different crops and different regions. Narsimhan (1929) observed that plants infected with *Rhizoctoniablatitcola* initially showed drooping of leaves followed by wilting. The main roots of such plants show blackening of finer roots.

The disease was found to appear in post flowering and pod formation stage. Nema and Khare (1973) reported that chickpea cultivation in Madhya Pradesh was greatly threatened by soil borne disease. Gupta *et al.* (1983) reported the incidence of root rot ranging from 3.2 to 20.6 per cent in 30 villages of northern Madhya Pradesh. Nene *et al.* (1984) also recorded heavy losses to chickpea crop due to dry root rot caused by *Rhizoctoniablatitcola*.

Dry root rot symptoms observed in diseased plants exhibited straw colour appearance and tap root mainly becomes brittle having minute sclerotia. These symptoms separated this disease from the so called "Wilt complex" as stated by various workers in India (Nene *et al.* 1989). Maximum atmospheric temperature up to 35°C favored the disease development. Substantial mortality and losses in chickpea due to *Rhizoctoniablatitcola* had been reported in high ambient temperature 33°C and above in the post flowering stage (Singh and Mehrotra 1982).

Reddy and Hernalsteens (1996), reported that symptoms on *Arapidopsis thaliana*, infected with *Rhizoctoniasolani* appeared as brown lesion at the site of infection within six to seven days. The lesions increased in size and extended in both directions with time. Rotting of the infected part and death of the plant tissue was also observed by these workers. The difference in days of appearance of symptoms may be because of difference in soil type and strain of fungus used during the course of investigations.

In vitro evaluation of leaf extracts

The experiment was conducted under laboratory conditions where the leaf extracts of different plants were amended with water deficit potato dextrose agar (PDA) medium in three concentrations (10, 20 and 30 per cent).

Neem (*Azadirachthaindica*) leaf extract was observed superior in all the concentrations in inhibiting the mycelial growth of the test fungus (*R. bataticola*) up to 144 hours which was very much superior over control and rest of the leaf extracts used during the course of investigation. Neem (*Azadirachthaindica*) leaf extract contains azadirachtin which shows toxic effect against the *R. bataticola* was also demonstrated by Shivpuri *et al.*, (1997), Sindhan *et al.*, (1999), and Sharma *et al.*, (2005) [10]. The Neem (*Azadirachthaindica*) leaf extract contains azadirachtin and monoterpenes which show toxic effect against the *R. bataticola*.

All the plant leaf extracts were found toxic to the mycelial growth of *R. bataticola*, however the difference in their toxication varied from that *Jatropha (Jatropha curcas)* was observed to be inhibitory at ten per cent concentration within 48 hours and later its toxicity declined. Similarly, *Datura (Daturasuaveolens)*, Neelgiri (*Eucalyptus globulus*), Aak (*Calotropisprocera*), Tulsi (*Ocimumsancatum*), and Mehndi (*Lawsoniainermeris*) were noted to be toxic to the mycelial growth but their efficacy declined as the time lapsed. However *Jatropha (Jatropha curcas)* was recorded to be effective in reducing the mycelial growth of the test fungus.

The results are in accord with the findings of Sindhan *et al.*, (1999). The effectiveness of these extracts may be due to the presence of antifungal constituents in the form of phenolic substances and resinous gummy and non volatile substance of unknown nature (Skinner 1995).

Inhibition of mycelial growth of *R. bataticola* in the present study may be attributed to the presence of antifungal properties and inhibitory compounds in the control of root rot diseases.

Influence of leaf powder as soil treatment on plant mortality

The experiment was carried out under pot conditions. In the present investigation leaf powders of plants were used as soil amendment. The result obtained during the course of study indicated that all the treatments were significantly effective in managing *R. bataticola* and increasing the plant stand. Out of

the powders tested *A. indica* showed minimum plant mortality up to 40th day when the experiment was terminated. These results are in the conformity with the findings of Bhattacharya and Pramanik (1998) who reported that neem was found toxic when applied as soil drench and reduced the club rot severity significantly in crucifers.

Singh *et al.*, (1980) reported that besides sulphur, neem contains a bitter yellowish substance which contains alkaloid, resins, glycosides, fatty acids and P-Amino benzoic acid which inhibit the growth and development of fungus.

The investigations indicated that Mehendi (*Lawsoniainermeris*) and Tulsi (*Ocimumsancatum*) leaf powders remained at par in their efficacies but inferior to Jatropa, Aak and *Eucalyptus* at 40th day when the experiment was terminated. Better plant stand was recorded in *Azadiracthaindica*. Followed by Tulsi (*Ocimumsancatum*) and Mehendi (*Lawsoniainermeris*). The result is in confirmation with the finding of Shahraj *et al.*, (2007) [9] who reported that all the plant parts of Neelgiri (*Eucalyptus globulus*) were effective in reducing the infection of *Rhizoctoniasolani*.

Sharma and Gupta (2003) while working with *Rhizoctoniasolani* on French bean reported the efficacy of *Ocimumsancatum* against the fungus when incorporated with the soil.

The efficacy of Karanj (*Pongamiapinnata*) was reported by Goswami *et al.*, (2007) on pigeonpea and Dubey (2002) on urd bean. Application of Karanj (*Pongamiapinnata*) may improve the soil conditions including nutritional status of soil supporting crop health (Dubey, 2002).

Rest of the leaf powders viz., Jatropa (*Jatrophacurcas*), Babul (*Acacia nilotica*), Ashok (*Polyanthialongifolia*) and Bougainvillea (*Bougainvillea sp.*) were recorded to be at par in their toxicity when used as soil amendment. Numbers of wilted plants were less than in the control pots.

The performance of these plant leaves may be due to presence of antifungal compounds which inhibited the growth of the pathogen but not so inhibitory against natural antagonistic micro biota specially fungal antagonists present in soil (Mahadevan 1982, Singh and Dwivedi, 1987 and Dubey and Patel, 2000). The active fractions of all the plant extracts showed fungicidal activity by way of inhibiting mycelial growth of *R. solani* (Shivpuri *et al.*, 1997).

Influence of leaf extracts as seed treatment on plant mortality

Minimum numbers of wilted plants were recorded with the seeds treated by Neem (*Azadiracthaindica*) leaf extract at 40th day of plant growth. Karanj (*Pongamiapinnata*) and Mehendi (*Lawsoniainermeris*) followed Neem (*Azadiracthaindica*) but their efficacy declined as the time lapsed. Rest of the treatments however inferior then Neem (*Azadiracthaindica*), Karanj (*Pongamiapinnata*) and Tulsi (*Ocimumsancatum*) but superior over control. Better results due to seed treatment with neem leaf extract were also recorded by Hashmi *et al.* (1992) while working with *F. oxysporum* on lentil and Khan and Husain (1988) with *R. solanion* cowpea.

Fungitoxicity of botanical products are considered to be the safer means of plant disease control, Tiwari *et al.*, (1998).

Influence of Bio control agents as soil amendment on plant mortality

In the present investigation *Trichodermaharzianum*, *T. viride*, *Basillissubtillus* and *Pseudomonas fluorescens* were tested *in vitro* and in pots conditions. *Trichodermaharzianum* was

found more effective as compared to other bio control agents and inhibited maximum fungal growth of *Rhizoctoniabataticola* followed by *Trichodermaviride*, while *P. fluorescens* was the least effective in growth inhibition of the *R. bataticola* under pot conditions, *T. harzianum* was found most effective in reducing pre and post emergence mortality, *T. viride* and *Basillussubtillus* were moderately effective in while *P. fluorescens* was least effective. These findings are in conformity of earlier finding of Deshmukh and Raut (1992), who reported that *T. harzianum* and *T. viride* were effective inhibiting the mycelial growth of *R. solani* and reducing the disease incidence in pot experiment Manczinger *et al.*, (2002) reported that *T. harzianum* and *T. viride* have a strong antagonistic properties against soil borne pathogens.

Influence of oil cake as soil amendment on plant mortality

Minimum numbers of wilted plants were recorded with the soil treated mustard (*Brassica junsea*) cake at 40th day of plant growth. Castor (*Ricinuscommunis*) and cotton (*Gossypium spp.*) were superior over rest of the treatment but (*Brassica junsea*), cotton (*Gossypium spp.*) and Castor (*Ricinuscommunis*) superior over control, better result due to soil treatment with Neem cake were also recorded by Mishra *et al.*, (2005) while working with *Rhizoctoniasolani* on mungbean and Khan *et al.*, (1988) with *R. solanion* cowpea.

Summary and conclusion

Summary

The present investigations deal with the evaluation of commonly available botanicals viz., Neem (*Azadiracthaindica*), Karanj (*Pongamiapinnata*), Aak (*Calotropisprocera*), Neelgiri (*Eucalyptus globulus*), Tulsi (*Ocimum sanctum*), Jatropa (*Jatrophacurcas*) Mehendi (*Lawsoniainermeris*) and Datura (*Daturasuaveolens*).

The pathogenicity of *Rhizoctoniabataticola* on chickpea was confirmed by growing the fungus on gram straw soaked in 0.5 dextrose and then mixing it with pot soil. The symptoms appeared within 13 days and the plants were killed within 16 days after sowing the test fungus was recovered from the roots of diseased plants.

Evaluation of plant leaf extracts was done under laboratory conditions using poisoned food technique. The plant leaves were also tested as soil amendment under pot condition where the leaf powder was mixed with the soil pre infested with *R. bataticola*. The plants grown on neem amended soil remained healthy upto 40 days where minimum mortality was recorded *E. globulus* and *P. pinnata* stood next in order of their efficacies in managing the root rot disease of chickpea.

The results obtained during the course of investigations that *in vitro* evaluation of plant leaves extracts neem was recorded to be superior in inhibiting the mycelial growth of *R. bataticola*. At all the concentrations under test *Eucalyptus* and *Jatropa* were also found superior in inhibiting the fungus under laboratory conditions but their efficacies declined as the time lapsed.

The experiment was also conducted to evaluate the plant leaves as seed treatment. Out of eight plant leaf extracts tested, neem again showed its superiority over rest of the treatments and control *E. globulus* was noted to be inferior than neem but superior over rest of the treatments. The leaf extract of Aak (*Calotropisprocera*) did not show any significant effect in managing the disease as seed treating agent.

In the present investigation *Trichodermaharzianum* was found more effective as compared to other bio control agents and

inhibited maximum fungal growth of *Rhizoctoniablatitcola* followed by *Trichoderma viride*, while *P. flurescens* was least effective in inhibition of the *R. bataticola* under pot conditions. The experiment was also conducted to evaluate the oil cakes as soil treatment mustard cake was most effective in inhibiting of the growth *R. bataticola*, Castor (*Ricinus communis*) and cotton (*Gossypium spp.*) were superior over rest of the treatments.

Conclusion

The wilt symptoms on chickpea appeared within 13 days and the plants were killed 16 days after sowing. The roots of the plants showed typical browning at the color region which advanced further and disintegrate the roots.

Neem (*Azadirachta indica*) leaf extracts was found superior under *in vitro* conditions in reducing the mycelial growth of *Rhizoctoniablatitcola*.

Minimum number of wilted plants were noted in pots where *A. indica* leaf powder was amended in soil.

Minimum numbers of wilted plants were recorded in soil treated with mustard oil cake which was found superior over rest of the treatments.

Trichoderma harzianum was found more effective as compared to other bio control agents and inhibited maximum fungal growth of *Rhizoctoniablatitcola*.

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