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#### Raj Kumar S

Research Associate, Agriculture Research Station, Vizianagaram, Andhra Pradesh, India

#### Srinivas T

Professor (Plant Pathology), O/o Dean of Agriculture, Lam, Guntur, Andhra Pradesh, India

#### Prasanna Kumari V

Associate Professor, Department of Plant Pathology, Agricultural College, Bapatla, Andhra Pradesh, India

#### Sai Ram Kumar DV

Professor, Department of Entomology, Agricultural College, Bapatla, Andhra Pradesh, India

## Correspondence Raj Kumar S Research Associate, Agriculture Research Station, Vizianagaram, Andhra Pradesh, India

# In vitro studies on dry root rot in chickpea by using fungicides, natural farming products and organic amendments

#### Raj Kumar S, Srinivas T, Prasanna Kumari V and Sai Ram Kumar DV

#### Abstract

Dry root rot caused by Rhizoctonia bataticola (Taub.) Butler is emerging as a serious biotic constraint for chickpea production. It is the most important and widespread soil borne disease of chickpea. Among the several constraints affecting the productivity of chickpea, 10-35 percent loss in yields are due to dry root rot diseases. Among them, dry root rot caused by Rhizoctonia bataticola is becoming severe in most of the chickpea growing regions of Andhra Pradesh. So to find out the best management strategies for the disease through bio-control agents, fungicides, natural farming products and organic amendments, investigation was carried during rabi 2017-18 in the study area.

**Keywords:** Chickpea, *Rhizoctonia bataticola*, fungicides, natural farming products, organic amendments, *in vitro* studies

#### Introduction

Chickpea (*Cicer arietinum* L.) is one of the major grain legume pulse crops grown in India and other semi-arid regions of the world. In India, chickpea is cultivated in an area of about 10.22 M ha with a production of 9.8 M t and with the average productivity of 920 kg ha<sup>-1</sup> (Ministry of Agriculture and Farmers Welfare, 2016-17). In Andhra Pradesh, it is grown in an area of 6.30 lakh ha with an annual production of 9.12 lakh t and with a productivity of 1233 kg ha<sup>-1</sup> (Ministry of Agriculture and Farmers Welfare, 2016).

The chickpea crop was reported to be attacked by nearly 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma) from all over the world (Nene *et al.*, 1996) [9]. However, only a few have the potential in devastating the crop. Some of the serious diseases in chickpea are Dry root rot (*Rhizoctonia bataticola*), Wilt (*Fusarium oxysporum f. sp. ciceri*), Wet root rot (*Rhizoctonia solani*), Ascochyta blight (*Ascocthya rabiei*) and Collar rot (*Sclerotium rolfsii*). Of them, dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler, is a major hindrance for getting proper yields. The pathogen is soil borne, it infects the crop from seedling to maturity stages of the crop. Keeping this in view, an attempt was made to find out the suitable agents for the management against causal agent of dry root rot under *in vitro* conditions.

#### **Material and Methods**

The investigation was carried out in the laboratory of the Department of Plant Pathology, Agricultural College, Bapatla. The efficacy of different components were studied on the test fungus using Poison Food Technique. PDA plates with non poisoned medium inoculated with *R. bataticola* served as control. Radial growth of the *R. bataticola* was recorded in treatments and control as well as till the growth in control reached 9.0 cm. Percent inhibition of growth over control was calculated using the formula given by (Nene and Thapliyal, 1982) [7].

Percent inhibition = 
$$\frac{C - T}{C}$$
 X 100

I = percent inhibition

C = growth in control

T = growth in treatment

The components used for studying their efficacy in inhibiting the growth of R. bataticola were detailed below;

Fungicides								
S. No	Common Name	Trade Name	Active Ingredient		Concentration studied (ppm)		Source of Supply	
1	Captan	Captaf	50% WP		2000,25	00,3000	Rallis India Ltd., Mumbai	
2	Carbendazim	Zoom	50% WP		500, 100	00, 1500	United Phosphorus ltd., Gujarat	
3	Hexaconazole	Contaf	5% EC		1500,20	00,2500	Rallis India Ltd., Mumbai	
4	Validamycin	Valliant	3% L		500,100	00,1500	Mahindra and Mahindra ltd., Mumbai	
5	Pyraclostrobin	Headline	20% EC		1500,20	00,2500	BASF corporartion, USA	
6	Azoxystrobin	Amistar	23% SC		500,100	00,1500	Syngenta India Ltd., Pune, Maharashtra	
7	Tebuconazole	Folicur	25.9 %EC		500,100	00,1500	Bayer Crop Science Ltd., Mumbai	
8	Copper oxychloride	Blitox	50%WP		2500,3000,3500		Rallis India Ltd., Mumbai	
9	Propiconazole	Tilt	25% EC		500,1000,1500		Syngenta India Ltd., Pune, Maharashtra	
10	Mancozeb	Indofil M-45	75 %	WP 2500,3000,3500		00,3500	Indofil Industries Ltd., Mumbai	
Natural Farming Products				Concent	ration studied			
1	Panchagavya				10%, 20%			
2	Bheeja	Bheejamrutham			10%, 20%		Preparation details given below	
3	Bheejaraksha				10%, 20%			
Organic Amendments								
1	Neem cake			10%, 15%, 20% Sri Gopala		Sri Gopala	Krishna Oil Mills, Tamil Nadu, India.	
2	Castor cake			10%	10%, 15%, 20% Sri Gopala		Krishna Oil Mills, Tamil Nadu, India.	
3	Cotton cake			10%, 15%, 20% Sri Ragha		Sri Ragha	vendra Oil Mills, Chebrolu, Guntur.	

#### **Preparation of Panchagavya**

- Cow Milk 2litres
- Cow Curd 2 Litres
- Cow Urine 3 Litres
- Cow Ghee 500 grams
- Fresh cow dung 5 kg
- Sugarcane juice 3 kg
- Tender Coconut water 3 litres
- Banana ripe 12 numbers
- Toddy or grape juice 2 litres

Panchagavya was prepared in a wide mouth container made of mud, concrete or plastic. The container should not be made of any metal. The first step is to mix fresh cow dung and ghee in the conainer. Mix it twice a day for three days. On the fourth day, add the remaining ingredients to the container. Stir the mixture twice a day for the next 15 days. On the 19<sup>th</sup> day, the Panchagavya mixture will be ready for use.

### Preparation of Bheejamrutham Materials Required

- Cow Urine 5 Litres
- Cow Dung 5 Kgs
- Calcium (Sunnam) 50gms
- Water 20 Litres
- Container with 40-50 litres capacity.

Take twenty litres water in container and add urine / calcium in water. Tie the cow dung in a cloth and hang it half way into the container. Mix everything very well and let it sit over night (24 hrs). Make sure to stir the mixture clock wise every 8 hours and squeeze the dung each time you stir. In 24 hours solution is ready to use.

#### Preparation of Bheejaraksha Materials required

- Red sand 100grams
- Cow dung cake powder 100 grams
- Asofoetida (Inguva) 20 grams
- Turmeric 20 Grams
- Cow urine 10 ml

Mix red sand and cow dung cake powder and make them into fine powder. Then add 20 g of Asofoetida and turmeric and

mix it well by adding cow urine slowly by drop wise. Then shade dry. Now the bheejaraksha is ready.

## Preparation of aqueous extracts of natural farming products/ organic amendments/ fungicides

Desired concentrations (10% and 20%) of the natural farming product were prepared by adding 10g / 10ml of the product in 100ml water and mixed with the equal quantity of double strength PDA, sterilized and poured aseptically in sterilized Petri plates. The aqueous extracts of different organic materials *viz.*, neem cake, cotton cake, mustard cake were prepared by suspending 30grams of each organic material in 150ml sterilized distilled water in flask and left for 10days. The flasks were shaken on alternate day for thorough mixing and dissolution of the content. After 10 days, the flasks were thoroughly shaken and content was filtered through double layered muslin cloth and autoclaved at 1.2kg cm<sup>-2</sup> pressure for 20minutes. The autoclaved extracts were individually added in previously sterilized molten PDA medium in desired concentration.

Ten ml stock solution of 1,00,000 ppm concentration was prepared in sterilized distilled water. To obtain the desired concentration of fungicide in the medium, amount of stock solution to be added was calculated by using the following formula.

 $C_1V_I = C_2V_2$ 

Where,  $C_1$  = concentration of the stock solution (ppm)

 $V_1$  = volume of the stock solution to be added (ml)

 $C_2$  = desired concentration (ppm)

 $V_2$ = volume of PDA in which fungicide is to be amended (ml)

Required volume of the component was mixed with appropriate quantity of PDA in molten but cooled form. There after 20 ml of the poisoned medium was poured in to sterilized Petri plate (9.0 cm diameter) under aseptic conditions in Laminar air flow inoculation chamber and allowed to solidify. Each plate was inoculated in the centre with five mm diameter disc cut from the periphery of actively growing seven day old  $R.\ bataticola$  culture under aseptic conditions and incubated at  $28\pm1^{0}\mathrm{C}$  in a BOD incubator. Three replications were maintained for each treatment.

#### **Results and Discussion**

Among the three natural faming products, beejamrutham is found to be highly effective and inhibited the growth of the mycelium at both 100 and 200 ppm concentrations. Beejarkasha did not showed any affect and Panchagavya at 200ppm concentration recorded 25.50 percent inhibition on the mycelium growth of the test fungus (Table 1 and Plate 1). Castor cake and neem cake did not show any effect on the growth of the *R. bataticola* and at par with control. Cotton cake at 200 ppm inhibited growth of the mycelium to an extent of 88.22 percent followed by 150ppm concentration (63.66%) and were significantly different from each other (Table 2 and Plate 2).

The efficacy of natural products and organic amendments on the growth of the mycelium is follows;

Control=panchagavya=beejarksha=neem cake= castor cake<cotton cake<br/>deejamrutham

Ashlesha and Paul (2004) [2] found that cow urine was most inhibitory to all the soil pathogens alone under *in vitro* conditions and in combination with other organic inputs under *in vivo*. Devakumar *et al.* (2014) [13] revealed that, Beejamrutha would give best result if it is used on the day of preparation. Dhingani *et al.* (2013) [4] found that neem cake was found to be high effective in reducing the growth of *M. phaseolina* causing dry root rot disease of chickpea followed by farm yard manure and whereas mustard and castor cake were poorer in reducing the growth of *M. phaseolina*.

Table 1: Effect of natural farming products on the growth of R. bataticola

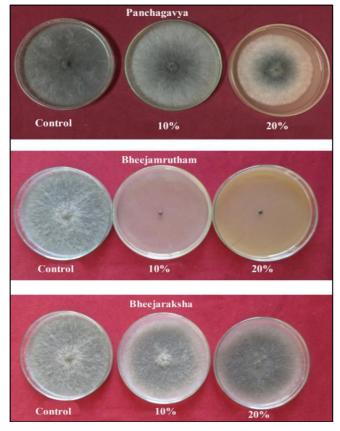
Product	Growth of m	ycelium (cm)	% inhibition over control			
Product	100ррт	200ppm	100ppm	200ppm		
Panchagavya	9.00 (3.08)	6.70 (2.68)	0.00	25.50		
Bheejamrutham	0.00 (0.71)	0.00 (0.71)	100.00	100.00		
Bheejaraksha	9.00 (3.08)	9.00 (3.08)	0.00	0.00		
C.D(P≤0.05)	0.008					
C.V% 0.24						

<sup>\*</sup>Figures in the parenthesis are square root transformed values

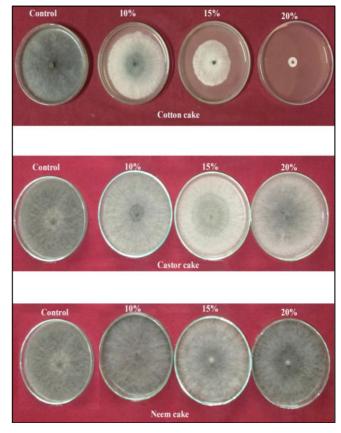
**Table 2:** Effect of organic amendments on the growth of *R. bataticola* 

Product	Grow	th of myceliun	% inhibition over control			
Froduct	100ppm 150ppm		200ppm	100ppm	150ppm	200ppm
Cotton cake	7.73 (2.87)	3.27 (1.94)	1.06 (1.25)	14.22	63.66	88.22
Castor cake	9.00 (3.08)	9.00 (3.08)	9.00 (3.08)	0.00	0.00	0.00
Neem cake	9.00 (3.08)	9.00 (3.08)	9.00 (3.08)	0.00	0.00	0.00
C.D(P≤0.05)		0.15				
C.V%	1.19					

<sup>\*</sup>Figures in the parenthesis are square root transformed values.



**Plate 1:** *In vitro* efficacy of natural farming products on the mycelial growth of *R. bataticola* by poisoned food technique.

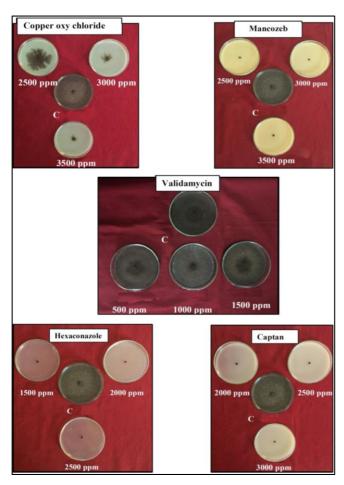


**Plate 2:** *In vitro* efficacy of organic amendments on the mycelial growth of *R. bataticola* by poisoned food technique

The data on efficacy of fungicides on mycelial growth of *R. bataticola* was presented in the Table 3 and in Plate 3 and Plate 4. The efficacy of ten fungicides on the growth of *R. bataticola* revealed that at all the concentrations carbendazim, pyraclostrobin, captan, hexaconazole, tebuconazole, mancozeb and propiconazole were found to be highly effective in completely inhibiting the growth of the fungus (100%).

Validamycin did not show any effect on the growth of the test fungus and at par with the control where as azoxystrobin inhibited the growth of *R. bataticola* to an extent of 31.88 percent at 1500 ppm concentration, 30.55 and 27.33 percent inhibition at 1000 and 500 ppm concentrations respectively and they were significantly different from each other. Copper oxychloride also found to be effective in inhibiting the growth of the fungus to an extent of 87.44 percent at 3500 ppm concentration.

Khan and Gangopadhyay (2008) [1] tested the efficacy of fungicides like carbendazim, carboxin, captan, chlorothalonil on mycelial growth of M. phaseolina and reported that carbendazim and captan were highly inhibitory to the pathogen. Veena et al. (2014) [11] found that fungicides copper oxy chloride, captan, hexaconazole and tebuconazole were found to be highly effective in inhibiting mycelial growth of bataticola, the highly virulent pathogen at all concentrations tested. Ravichandran and Hedge (2017) [10] evaluated five contact fungicides and four systemic fungicides against R. bataticola causing dry root rot disease of chickpea, among the contact fungicides chlorothalonil and mancozeb at 0.2% were effective in completely inhibiting mycelia growth and among systemic fungicides, carbendazim, difenoconazole and tebuconazole were best with cent percent inhibition of mycelial growth at all the concentrations tested.



**Plate 3:** *In vitro* efficacy of different fungicides on mycelial growth of *R. bataticola* using poisoned food technique.



**Plate 4:** *In vitro* efficacy of different fungicides on mycelial growth of *R. bataticola* using poisoned food technique.

**Table 3:** *In vitro* efficacy of fungicides on the growth of *R. bataticola* 

	Concentration	% inhibition over		
Fungicide	(ppm)	Mycelial growth (cm)	control	
Carbendazim	500	0.00 (0.71) *	100.00	
(50%WP)	1000	0.00 (0.71)	100.00	
	1500	0.00 (0.71)	100.00	
Pyraclostrobin	1500	0.00 (0.71)	100.00	
(20%EC)	2000	0.00 (0.71)	100.00	
( ,	2500	0.00 (0.71)	100.00	
Azoxystrobin	500	6.54 (2.55)	27.33	
(23%EC)	1000	6.25 (2.57)	30.55	
(237020)	1500	6.13 (2.59)	31.88	
Captan	2000	0.00 (0.71)	100.00	
(50% WP)	2500	0.00 (0.71)	100.00	
(3070 111)	3000	0.00 (0.71)	100.00	
Validamycin	500	9.00 (3.08)	0.00	
(3%L)	1000	9.00 (3.08)	0.00	
(3%L)	1500	9.00 (3.08)	0.00	
Hexaconazole	1500	0.00 (0.71)	100.00	
(5%EC)	2000	0.00 (0.71)	100.00	
(370EC)	2500	0.00 (0.71)	100.00	
Tebuconazole	500	0.00 (0.71)	100.00	
(25.9%EC)	1000	0.00 (0.71)	100.00	
(23.9%EC)	1500	0.00 (0.71)	100.00	
Copper oxy	2500	6.95 (2.77)	22.88	
chloride	3000	2.45 (1.73)	72.77	
(50% WP)	3500	1.13 (1.30)	87.44	
Mancozeb	2500	0.00 (0.71)	100.00	
(75%WP)	3000	0.00 (0.71)	100.00	
(73% WP)	3500	0.00 (0.71)	100.00	
D	500	0.00 (0.71)	100.00	
Propiconazole	1000	0.00 (0.71)	100.00	
(25%EC)	1500	0.00 (0.71)	100.00	
Control	-	9.00 (3.08)	-	
S.Em±	-	0.01	-	
C.D.	-	0.04	-	
C.V%	-	2.15	-	

<sup>\*</sup> Mean of four replications

<sup>\*</sup>Figures in the parenthesis are square root transformed values.

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