

# INTEGRATED MANAGEMENT OF JATROPHA ROOT ROT CAUSED BY *RHIZOCTONIA BATATICOLA*

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**KUMAR S, SHARMA S, PATHAK DV & BENIWAL J. 2011. Integrated management of jatropha root rot caused by *Rhizoctonia bataticola*.** Root rot of jatropha caused by *Rhizoctonia bataticola* is an important disease reported recently in Haryana, India. Management studies were initiated to reduce the disease using fungicides, plant extracts and biocontrol agents. Results revealed that Bavistin and Vitavax were effective in inhibiting the growth of *R. bataticola* and reducing the incidence of jatropha root rot. These fungicides resulted in 100% inhibition of mycelial growth at 50 ppm. Seed treatment with Bavistin and its soil drenching caused the least pre-emergence (16.7%) and post-emergence mortalities (10.1%). In *in vitro* studies, neem extract at 20% concentration provided 55.6% inhibition. *Trichoderma harzianum* showed the highest mycelial growth inhibition (58.9 %) against *R. bataticola*. Bavistin alone at 2 g kg<sup>-1</sup> seed was found to be the most effective treatment with 36.3% reduction in root rot. Integrated methods showed higher disease reduction compared with single method. Bavistin (2 g kg<sup>-1</sup> seed) + neem extract (20%) was the most effective treatment with 67.3% reduction followed by *T. harzianum* (15 g kg<sup>-1</sup> seed) + Bavistin (54.2%) and neem extract + *T. harzianum* (44.0%).

Keywords: Fungicide, plant extracts, biocontrol agents, *Trichoderma harzianum*, neem

**KUMAR S, SHARMA S, PATHAK DV & BENIWAL J. 2011. Pengurusan bersepadu reput akar jatropha yang disebabkan oleh *Rhizoctonia bataticola*.** Reput akar jatropha yang diakibatkan oleh *Rhizoctonia bataticola* merupakan penyakit penting yang dilaporkan baru-baru ini di Haryana, India. Kajian pengurusan dimulakan untuk mengurangkan penyakit ini dengan menggunakan fungisid, ekstrak tumbuhan serta agen kawalan biologi. Keputusan menunjukkan bahawa Bavistin and Vitavax sangat berkesan dalam menghalang pertumbuhan *R. bataticola* dan mengurangkan kejadian reput akar jatropha. Fungisid ini mengakibatkan 100% perencatan pertumbuhan miselium pada kepekatan 50 ppm. Bavistin yang digunakan untuk merawat biji benih serta membasahkan tanah mengakibatkan kematian pracambah (16.7%) dan kematian pascacambah (10.1%) yang paling rendah. Dalam eksperimen *in vitro*, ekstrak neem pada kepekatan 20% menyebabkan perencatan sebanyak 55.6%. *Trichoderma harzianum* menunjukkan perencatan pertumbuhan miselium yang paling tinggi terhadap *R. bataticola* iaitu sebanyak 58.9 %. Secara tunggal, Bavistin pada kepekatan 2 g kg<sup>-1</sup> biji benih didapati paling berkesan dengan pengurangan kejadian reput akar sebanyak 36.3%. Kaedah bersepadu menunjukkan pengurangan penyakit yang lebih tinggi berbanding kaedah tunggal. Rawatan Bavistin (2 g kg<sup>-1</sup> biji benih) + ekstrak neem (20%) paling berkesan dengan pengurangan sebanyak 67.3% diikuti dengan *T. harzianum* (15 g kg<sup>-1</sup> biji benih) + Bavistin (54.2%) serta ekstrak neem + *T. harzianum* (44.0%).

## INTRODUCTION

Physic nut (*Jatropha curcas*) globally known as jatropha belongs to the family Euphorbiaceae. It is a large shrub or small tropical tree widely distributed in arid and semiarid areas. Jatropha is the main commodity source for biodiesel in India. The tree has only a few pest and disease problems.

However, recently root rot caused by *Rhizoctonia bataticola* has been recorded as one of the most devastating diseases of jatropha (Sharma &

Kumar 2009). Fungicides are widely used as seed or soil treatment to combat various root diseases. However, use of fungicides causes environmental hazards and development of resistance in pathogen. In recent years, more emphasis has been given to the use of bioagents, plant growth promoting rhizobacteria (PGPRs) and plant extracts, either alone or in combination. Integrated management of root rot pathogens have been reported by several workers

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(Suriachandraselvan & Seethuraman 2002, Sharma & Gupta 2003, Sobti et al. 2005, Yadav et al. 2005). Unfortunately, no work has been done on this pathogen of jatropha. Therefore, the disease management strategies to reduce root rot in jatropha were investigated using plant extracts, biological agents and fungicide.

## MATERIALS AND METHODS

### *In vitro* studies

#### *Fungicide*

Stock solutions (500 ppm) were prepared for six fungicides, namely, Bavistin (active ingredient carbedazim 50 WP), Vitavax (carboxin 75 WP) Blitox (copper oxychloride 50 WP), Captan (captan 75 WP), Thiram (thiride 75 WP) and Kitazin (Kitazin 48 EC) using double distilled sterilised water. The solution was further diluted with distilled sterilised water to give double strength concentrations, i.e. 100, 200, 300, 400 and 500 ppm. Double strength potato dextrose agar (PDA) medium (40 g dextrose and 40 g agar per litre) was prepared and sterilised in an autoclave for 20 min at 1.1 kg cm<sup>-2</sup> pressure. Seventy-five ml of medium and an equal amount of fungicide solution of each concentration were poured into a sterilised conical flask and mixed together under aseptic condition. Finally the mixture gave 50, 100, 150, 200 and 250 ppm concentrations. The medium containing the required dose of fungicide was dispensed into five sterilised Petri plates and kept for solidification. Each Petri plate was then inoculated with a 5-mm mycelial disc in the centre. A separate check without fungicide was also maintained for each treatment. The inhibition of *R. bataticola* at different doses of treatment was determined on the basis of decrease in area of mycelial growth with respect to the control.

#### *Plant extracts*

For the evaluation of antifungal activities of different plant extracts, fresh leaves of neem (*Azadirachta indica*), jamun (*Syzygium cumini*), clerodendron (*Clerodendron enermii*), safeda (*Eucalyptus globulus*), aak (*Calotropis procera*), marwah (*Mirabilis jalapa*) and ashwagandha (*Withania somnifera*) were collected and washed twice or thrice with sterilised water. Leaves of

each plant species were crushed in distilled sterile water at 1:1 ratio and filtered through a piece of muslin cloth. The homogenate was then centrifuged at 5000 rpm for 10 min to produce a clear supernatant. This formed the standard plant extract solution (100%). To obtain sterilised extracts, the supernatant was passed through a Millipore filter (0.40 µm) using a filter adaptor attached to a glass syringe (20 ml). These extracts were immediately used for experiments. The desired concentrations of 5, 10 and 20% were prepared by adding appropriate amounts of standard solution of plant extract to 15 ml sterilised medium. The extract solution was then poured into sterilised Petri plates. Each treatment was replicated three times. Petri plates containing water instead of plant extract served as control. Each plate was inoculated with a 5-mm diameter mycelial disc taken from five-day-old culture raised on PDA. The inoculated plates were incubated at 28 ± 2 °C in a BOD incubator for five days. Observations of colony diameter were recorded after six days and per cent inhibition was calculated as in the previous experiment.

#### *Biocontrol agents*

Antagonistic effect of different bacterial/fungal culture, namely, *Pseudomonas fluorescens*, *P. maltophilia*, *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* were evaluated *in vitro* against *R. bataticola* using the dual culture technique. Discs measuring 5-mm diameter from five-day-old culture of the pathogen and antagonists were placed equidistantly to the pathogen on sterilised Petri plates containing PDA medium. Plates were incubated at 28 ± 2 °C in a BOD incubator and each treatment was replicated three times. Mycelial growth was recorded after six days and per cent inhibition was measured.

#### *In vivo* studies

To evaluate the effectiveness of the plant extract, biocontrol agent and fungicide in the field, neem extract, *T. harzianum* and Bavistin, which showed the highest inhibition, were used for the following experiments. For antagonistic treatment, the culture was grown on PDA medium. Seeds were surface sterilised with 2% sodium hypochlorite solution and then washed two to three times with sterilised distilled water. Cold water extract of

fresh neem leaves was used for seed treatment with plant extract. Neem leaves were washed in sterilised water and homogenised with sterile distilled water in 1:1 ratio in a pestle and mortar. The homogenate was filtered through a piece of muslin cloth and centrifuged at 5000 rpm for 10 min. The extract was then heated to 40 °C for 10 min to avoid contamination (Jaganathan & Narasimhan 1988). The seeds were then soaked in extract for half an hour and air dried under the shade.

To test the fungicide, dry seed treatment was carried out using Bavistin at 2 g kg<sup>-1</sup> seeds. Seeds used for this treatment were coated with the slurry of mycelial mat of antagonist. For this, the required quantity of mycelial mat (15 g kg<sup>-1</sup> seed) was put into a container and sufficient quantity of water was added to produce a thin paste, but not too watery. Carboxy methyl cellulose (0.1%) was added to the paste for better adherence of antagonist to seeds. The seeds were mixed thoroughly with the paste for even distribution of the antagonist. The seeds were then dried in shade before sowing.

In the co-treatment using Bavistin + *T. harzianum*, seeds were at first treated with Bavistin (2 g kg<sup>-1</sup> seeds) followed by *T. harzianum* (15 g kg<sup>-1</sup> seeds). A six-hour interval was kept in between two subsequent treatments. During this period seeds were placed under shade (Sobti et al. 2005). Similarly, for combination seed treatment with neem extract and *T. harzianum*, seeds were treated with neem extract (20%) by dipping for half an hour and then with *T. harzianum* at 15 g kg<sup>-1</sup> seeds. Co-seed treatment with neem extract and Bavistin was carried out as with neem extract and *T. harzianum*, i.e. seeds were first treated with neem extract followed by Bavistin.

Fifteen treated seeds were sown in earthen pots (40 × 40 cm) filled with 5 kg sterilised soil. Oat meal sand inoculum (20 g kg<sup>-1</sup> soil) was mixed with the top soil before sowing. The control was maintained by sowing untreated seed. Three replications of each treatment were maintained including control. Pots were watered on alternate days. The disease incidences in terms of pre-emergence and post-emergence mortalities were recorded 7 and 30 days after sowing respectively. Disease reduction was calculated using the formula:

$$\text{Disease reduction (\%)} = \frac{D_c - D_t}{D_c} \times 100$$

where  $D_c$  is disease (mortality) in control pots and  $D_t$  is disease (mortality) in treatment pots. Treatments in this experiment were: neem extract (20 %) as seed soaking (for half an hour); *T. harzianum* (15 g kg<sup>-1</sup> seeds) as seed coating with mycelial slurry; Bavistin seed treatment at 2 g kg<sup>-1</sup> seeds; neem extract (20 %) + *T. harzianum* (15 g kg<sup>-1</sup> seeds); neem extract (20 %) + Bavistin (2 g kg<sup>-1</sup> seeds); Bavistin (2g kg<sup>-1</sup> seeds) + *T. harzianum* (15 g kg<sup>-1</sup> seeds) and untreated control.

### Integrated chemical control

*Rhizoctonia bataticola* was raised on oat meal sand medium for five days at 28 ± 2 °C. This inoculum (20 g kg<sup>-1</sup> soil) was mixed in a pot filled with 5 kg sterilised soil and allowed to stabilise for five days before sowing. For seed treatment experiments, seeds were treated with formulated wettable powder of fungicides (Bavistin and Vitavax) at 2 g kg<sup>-1</sup> seeds. For soil drenching treatment, the required solution was prepared by adding 2 g Bavistin per liter of distilled water. For combination of seed treatment and soil drenching, seeds were treated first and sown in pots. After sowing, 200 ml of Bavistin solution were drenched in each pot. Soil drenching was also maintained for control, which did not have any fungicidal treatment including seed treatment. Three replications of each treatment and control were kept at the same time.

Fifteen seeds of jatropha were sown in earthen pots (40 × 40 cm) and irrigated regularly. As in the *in vivo* studies, observations of disease incidence were recorded as pre-emergence (7 days after sowing) and post-emergence (30 days after sowing) mortalities. Per cent disease control was calculated using the formula above. Treatments in the experiments were: Bavistin at 2 g kg<sup>-1</sup> seeds as seed treatment; Vitavax at 2 g kg<sup>-1</sup> seeds as seed treatment; Bavistin at 0.2% as soil drenching; Vitavax at 0.2% as soil drenching; Bavistin (seed treatment) + Bavistin (soil drenching); Vitavax (seed treatment) + Vitavax (soil drenching) and control.

In all experiments, treatments were replicated thrice and the statistical analysis of the data was done using completely randomised design on the basis of angular transformed values (Panse & Sukhatme 1985).

## RESULTS AND DISCUSSION

### *In vitro* studies

#### *Fungicide*

The basic approach before recommending chemical control against a particular disease is to screen the fungicides against pathogen under laboratory conditions. Increase in concentrations of fungicides caused a decrease in mycelial growth of the fungus thereby resulting in increased inhibition (Table 1). From the various fungicides tested, Bavistin and Vitavax were found to be more effective, showing 100% inhibition of mycelial growth at 50 ppm followed by Kitazin which showed 100% inhibition at

100 ppm. Thiram, Blitox and Captan were the least effective. These results are similar to earlier findings by Rana and Tripathi (1983), Prajapati et al. (2002), and Dubey and Kumar (2003).

#### *Plant extracts*

Several higher plants and their constituents have shown success in controlling plant diseases while proving to be harmless and non-phytotoxic (Dubey 1991, Shinde & Patel 2004, Kiran et al. 2006). In the present findings, of the seven extracts evaluated, neem extract proved to be more toxic to the mycelial growth of *R. bataticola* with inhibition between 38.8 and 55.6% followed by marwa (36.2 to 53.6%) and aak (34.0 to 52.0%) (Table 2). The efficacy of the three best

**Table 1** Effects of different concentrations of fungicides on the inhibition of the radial growth of *Rhizoctonia bataticola* in jatropha

Fungicide	Growth inhibition (%)					Mean
	Concentration (ppm)					
	50	100	150	200	250	
Bavistin	100.0	100.0	100.0	100.0	100.0	100.0
Vitavax	100.0	100.0	100.0	100.0	100.0	100.0
Blitox	42.2	78.5	91.0	100.0	100.0	82.3
Captan	49.2	70.7	87.5	100.0	100.0	81.5
Thiram	39.4	62.4	76.3	89.0	100.0	73.4
Kitazin	81.9	100.0	100.0	100.0	100.0	96.4
Mean	68.78	85.27	92.47	98.17	100.0	-
CD at 5%					Fungicide	0.51
					Concentration	0.32
					Fungicide × concentration	1.25

**Table 2** Effects of plant extracts on the inhibition of radial growth of *R. bataticola* in jatropha

Plant extract	Growth inhibition (%)			Mean	
	Concentration (%)				
	5	10	20		
Neem ( <i>Azadirachta indica</i> )	38.8	50.00	55.6	48.1	
Jamun ( <i>Syzygium cumini</i> )	9.1	24.6	34.3	22.7	
Clerodendron ( <i>Clerodendron enermii</i> )	7.8	14.8	31.7	18.1	
Safeda ( <i>Eucalyptus tereticornis</i> )	29.0	35.0	49.8	37.9	
Calotropis (aak) ( <i>Calotropis procera</i> )	34.0	40.8	52.0	42.3	
Marwa ( <i>Mirabilis jalapa</i> )	36.2	48.1	53.6	46.0	
Ashwagandha ( <i>Withania sominifera</i> )	19.2	31.6	44.0	31.3	
Mean	24.9	35.0	45.9		
CD at 5%				Extract	0.890
				Concentration	0.72
				Extract × concentration	1.238

extracts, i.e. neem, marwa and aak, may be due to the presence of antifungal constituents in the form of phenolics, resins and gummy and non-volatile substances of unknown nature. The effectiveness of neem as well as *Allium cepa* and *A. sativum* against *R. bataticola* has been reported by Sindhan and Jaglan (1988).

Neem flower extract, nimbidine and neem oil, all at 10% concentration, were found to be highly inhibitory to hyphae and sclerotial formation of *Macrophomina phaseolina* (Desai & Srikant 2002). Azadirachtin at 25% concentration inhibited the radial growth of *M. phaseolina*, which causes charcoal rot in soybean by 84.2% (Dubey & Kumar 2003). The efficacy of the other leaf extracts, namely, *Acacia arabica*, *A. cepa*, *A. sativum* has also been reported to be able to stop mycelial growth of *M. phaseolina* completely even at 5% (Dubey & Dwivedi 1991).

#### Biocontrol agents

The role of antagonists in suppressing the growth of soil-borne pathogens has been well documented (Garret 1980). The biocontrol potential of *Trichoderma* spp. against *Rhizoctonia* spp. in different plant species has been reported by Kehri and Chandra (1991) and Singh et al. (1998). The present study also 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* (Table 3). The inhibition by the different antagonists

ranged from 38.9 to 58.9%. These observations are similar to the findings of Pathak et al. (2003) who reported maximum inhibition of *R. bataticola* by *T. harzianum*. The supremacy of *T. viride* over *P. fluorescens* and *Gliocladium virens* in reducing the colony diameter and sclerotial production of *S. rolfsii* has also been reported (Kuldeep et al. 2005).

#### In vivo studies

In the present study, integration of different treatments, namely, plant extracts, bioagents and fungicides provided an additive effect over single practice. The disease reduction varied from 17.0 to 36.2% in single treatment compared with 44.0 to 67.3% in combination treatment. Bavistin alone could reduce the disease up to 36.2% but with *T. harzianum* the reduction was 44.0%. However, the most effective combination treatment was Bavistin plus neem extract which resulted in 67.3% disease reduction (Table 4). These findings agree with the observations of a study to control *R. bataticola* in soybean using *T. viride* or *T. harzianum* and carbendazim (Vyas 1994). Similarly, *T. harzianum* also controlled soil-borne plant pathogens when introduced in combination with carbendazim (Mehta et al. 1993). *Trichoderma viride* in combination with carbendazim (2 g kg<sup>-1</sup> seeds) reduced post-emergence mortality of blackgram caused by *R. bataticola* compared with when both were applied separately (Suriachandraselvan & Seethuraman 2002).

**Table 3** Effects of plant growth promoting rhizobacteria and bioagents against *R. bataticola* using dual culture technique

PGPRs/bioagent	Mycelium growth (mm)		Inhibition (%)
	Pathogen	Antagonist	
<i>Bacillus subtilis</i>	45	55	38.89
<i>Pseudomonas fluorescens</i>	46	54	48.89
<i>Pseudomonas maltophilia</i>	48	52	46.67
<i>Trichoderma viride</i>	41	59	54.45
<i>T. harzianum</i>	37	63	58.89
Control	90	-	0.0
CD at 5 %	0.72	1.15	

PGPRs = Plant growth promoting rhizobacteria

*Integrated chemical control*

In the present findings, integration of methods, namely, seed treatment and soil drenching showed a synergetic effect against root rot of jatropha. Seed treatment and soil drenching using Bavistin provided the highest disease control (61.9%) compared with individual methods (29.0 and 44.2% respectively) (Table 5). A similar trend

was also observed with Vitavax. These results are in agreement with the observations made by Vyas (1994) and Sharma et al. (2005).

It is thus concluded that root rot of jatropha can be controlled by treating seeds with Bavistin (2 g kg<sup>-1</sup> seed) + neem extract (20%). Seed treatment with Bavistin (2 g kg<sup>-1</sup> seed) + soil drenching (2 g l<sup>-1</sup> water) is another option to minimise the disease.

**Table 4** Effects of seed treatment with fungicide, plant extract, bioagent and their integration with each other on the incidence of root rot in jatropha

Treatment	Germination (%)	Pre-emergence mortality (%)	Post-emergence mortality (%)	Disease reduction (%)
Neem extract	62.22* (52.07)	37.78 (37.89)	28.51 (32.19)	17.03
<i>T. harzianum</i>	64.45 (53.39)	35.55 (36.57)	24.07 (29.29)	24.76
Bavistin	68.89 (56.10)	31.11 (33.86)	19.39 (26.11)	36.27
Bavistin + <i>T. harzianum</i>	71.11 (57.49)	28.89 (32.47)	15.45 (22.96)	44.04
Neem extract + <i>T. harzianum</i>	75.55 (60.39)	24.45 (29.57)	11.86 (19.85)	54.18
Neem extract + Bavistin	82.22 (65.13)	17.78 (24.84)	8.12 (16.54)	67.31
Untreated control	51.11 (45.62)	48.89 (44.35)	30.35 (33.34)	-
CD at 5 %	2.26	1.36	2.06	

Figures in parentheses are angular transformed values; \* = average of three replications

**Table 5** Effects of fungicidal seed treatment, soil drenching and their integration on the incidence of root rot in jatropha

Treatment	Germination (%)	Pre-emergence mortality (%)	Post-emergence mortality (%)	Disease control (%)
Bavistin (ST)	70.83 (57.31)	29.17 (32.65)	20.70 (26.93)	29.0
Vitavax (ST)	66.50 (54.73)	33.50 (35.23)	24.84 (29.83)	16.9
Bavistin (SD)	77.09 (61.42)	22.91 (28.54)	16.24 (23.75)	44.2
Vitavax (SD)	72.92 (58.65)	27.08 (31.32)	19.95 (26.44)	33.0
Bavistin (ST + SD)	83.33 (66.09)	16.67 (23.99)	10.07 (18.22)	61.9
Vitavax (ST + SD)	79.17 (62.87)	20.83 (27.10)	13.25 (23.42)	51.5
Control (inoculated)	58.33 (49.79)	41.67 (40.18)	28.52 (32.19)	-
CD at 5%	1.44	1.44	1.61	

Figures in parentheses are angular transformed values; ST = Seed treatment; SD = soil drenching

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