MANAGEMENT OF CROWN AND ROOT ROT CAUSED BY *Rhizoctonia solani* IN ACID LIME (*Citrus aurantifolia*) UNDER SCREEN HOUSE CONDITIONS IN KIRTIPUR, KATHMANDU

Susmita Sigdel[1,*], Srijana Bhandari[2], Kritika Adhikari[1], Gaurav Adhikari[1] and Hira Kaji Manandhar[3]

[1]Agriculture and Forestry University, Rampur, Chitwan Nepal
[2]Warm Temperate Horticulture Centre, Kirtipur, Kathmandu, Nepal
[3]Nepal Plant Disease Agro Associates, Balaju-Chakrapath, Kathmandu, Nepal
[*]Correspondence: susmitasigdel16@gmail.com

ABSTRACT

Crown and root rot disease was among the major problems in acid lime (*Citrus aurantifolia*) seedlings grown in the nursery of Warm Temperate Horticulture Centre, Kirtipur, Nepal. Samples of the diseased plants were investigated at the laboratory of Nepal Plant Disease and Agro Associates. *Rhizoctonia solani, Fusarium solani* and *Alternaria citri* were found associated with the disease samples. On pathogenicity testing *R solani* showed symptoms like those of the infected seedlings. Hence, a pot experiment was conducted in a completely randomized design with four replications to manage the disease. Acid lime seedlings grown in sterilized soil and inoculum of *R. solani* prepared in potato dextrose agar (PDA) were used for the experiment. The treatments included carbendazim 50WP @ 1000 ppm, *Trichoderma viride* @ 2 ml/l water, *Pseudomonas fluorescens* @ 2 ml/l water, A-arya 009 (essential oil and organic acids) @ 1 ml/l water, and control (water) which were applied by soil drenching before 15 days of pathogen inoculation. Carbendazim at 100 and 200 ppm was also evaluated against *R. solani* by poison food technique on PDA. In the pot culture, the effect of treatments was significant on the growth of root and shoot of seedlings and both disease incidence and severity. Carbendazim had significantly least disease incidence (43.75%) and least disease severity (18.75%) among the treatments 45 days after inoculation with the pathogen. Also, the fungicide at 100 ppm gave complete inhibition of *R. solani* in PDA culture. Hence, carbendazim could be suggested for the management of the citrus crown and root rot caused by *R. solani*. However, further study is required to find out appropriate concentration of the fungicide for effective management of disease.

Key words : Carbendazim, pathogenicity, reduction, *Rhizoctonia solani*

INTRODUCTION

Citrus stands as the most important fruit crop of Nepal in terms of area coverage, production, and export potential. Currently, citrus covers a total production area of 28,406 ha with a productivity of 9.57 t/ha and among citrus, acid lime shares 10% of the total production area of citrus (MoALD, 2019). However, the production of citrus fruits has been widely affected by various citrus diseases right from the nursery condition to fruit production causing excessive financial losses (NCDP, 2014).

The crown and root rot caused by *Rhizoctonia solani*, the anamorph of *Thanatephorus cucumeris*, is a destructive disease occurring in diverse conditions afflicting a diverse range of crops in varied

conditions with a capability to compete both above and below the soil surface (Baker, 2020; Butler & Bracker, 1970). It is mentioned among the aggressive fungus existing in the rhizosphere of the citrus seedling which flourishes well under high moisture regime making the seed-borne inoculum important for disease development (Mansour & El-Shimy, 2009; Keijer, 1996).

R. solani causes symptoms like rotting of rootlets, girdling around the crown, wilting of plant, and its eventual death thereby affecting many aspects of nursery seedling production, right from seed germination to finishing with high-quality trees (Ajayi-Oyetunde & Bradley, 2017; Butler & Bracker, 1970). But the management of the *R. solani* through cultural practices and chemical fungicides is not completely effective due to which the disease caused by *R. solani* remains a persistent problem (Baker, 2020; Butler & Bracker, 1970; Moni *et al.*, 2016). So far, the problem of *R. solani* in citrus seedlings has not been documented in Nepal with no studies on the management of crown and root rot problems caused by *R. solani* in citrus seedlings. Therefore, the aim of this study was to explore the pathogens associated with crown and root rot, the pathogenicity of the *R. solani* in a citrus sapling and assess disease management options in the screen house conditions.

MATERIALS AND METHODS

Location of the Experimental Site

The experimental was carried in the Warm Temperate Horticulture Centre (WTHC), Kirtipur, Kathmandu, Nepal from February 2021 to July 2021 with the geographical location of the site 27.6630ºN and 85.2774ºE and the elevation of 1520 m above sea level. Treatment details are shown in Table 1.

Pathogen Identification and Isolation

Plants showing symptoms of die-back, crown discolorations, yellowing, and necrosis in leaves were selected. Portions (shoot, crown, and root) of size around 2 cm were rinsed, and surface sterilized with 1% and 2% NaOCl for 1 minute, and further rinsed in sterile distilled water. While choosing the portion, only partially infected potions were chosen rather than fully dead ones to ensure the presence of the pathogens in the plant part. The cut pieces were transferred to sterilized petri-plates containing two layers of moist blotting papers and water agar separately. All the petri-plates were incubated at 25 ºC. for three days.

The pathogens were isolated and identified in Nepal Plant Disease and Agro Associates (NPDA), Balaju-Chakrapath, Kathmandu, Nepal. Three pathogens were isolated *Alternaria citri* (Fig. 1a), *R. solani* (Fig. 1b) and *Fusarium solani* (Fig. 1c). *R. solani* was identified based on hyphal branch that originates from dolipore septum with characteristic constriction in the branching point (Ajayi-Oyetunde & Bradley, 2017). The presence of pale colored conidiophore with branched septation with conidia arranged either singly or in chains confirmed the presence of *A. citri* (Bliss & Fawcett, 1944). *F. solani* was identified based on its hyaline slightly curved three septate conidia having pointed ends on both sides (Hafizi *et al.*, 2013).

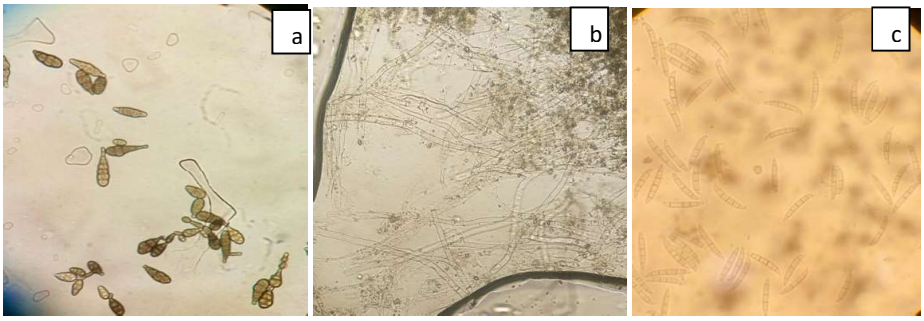

Fig. 1a. Microscopic view of *Alternaria citri*, **Fig. 1b.** Microscopic view of *Rhizoctonia solani*, and **Fig. 1c.** Microscopic view of *Fusarium solani.*

Identified pathogens were isolated and grown for 7 days in separate petri plates. Cultures were made several times to obtain the pure culture of the pathogen. Pure culture of the pathogen was maintained in several petri plates in potato dextrose agar for further use.

Seedling Preparation

The seed from the fruit of *Citrus aurantifolia* is extracted and then collected in a piece of muslin cloth. It was washed to make free from the pulp and the preliminary selection of the best seeds was based on their potential viability ascertained by placing seeds in water. The potent seeds were then treated with 1% NaOCl for one minute for surface sterilization and then washed thrice with distilled water. Then seeds were placed into filter paper for air drying for 24 hr. The soil was autoclaved at 121 ºC. for 15 min. Then one-third portion of vermicompost was mixed with a two-thirds portion of soil and the germination tray was filled with it. Thus fully sterilized and air-dried seeds were sown in the germination tray. The germination tray was then covered with a piece of black plastic and kept in a screen house maintained at an average temperature of 28 to 32 ºC. Watering to seeds was made once in three days.

Pathogenicity Test

The pathogenicity test was done with three replicates for three reported pathogens. The autoclaved soil was mixed with vermicompost in a 3:1 ratio. The mixture was filled in twelve poly-pots and acid lime seedlings at three-leaf stages developed in the germinated tray were transplanted and were left to grow up to 4 leaf stages. The trial included 12 plants in 4 replications; each replication included three pots having single plant in each. The pure culture of pathogen grown in 20 ml PDA in an incubator at 25 ºC for 7 days was used. The broth was prepared by mixing 40 ml PDA with 7 days old pathogen culture with 1 liter of distilled water. Then broth was filtered using muslin cloth. The procedure was same for preparing the inoculums of three pathogens. Four inoculums were prepared out of three pathogen-containing inoculums and one distilled water. At first distilled water was applied and then with the help of a spray bottle, the pathogen was inoculated in the plant rinsing its whole shoot portion. The test was carried out in the screen house at an average room temperature of 28 to 32 ºC. In alternate days the inoculums were prepared and sprayed in the same fashion until appearance of any disease symptoms. Regular observation and watering plants were performed to ensure the proper growth of the pathogen.

The trial included three pathogens, *A. citri*, *F. solani,* and *R. solani* were evaluated for about 45 days in the completely randomized design with three replicates. Among all the tested pathogens which were inoculated in the different plants, *R. solani* was shown to be pathogenic. Those plants inoculated with *R. solani* started wilting from about 30 days of inoculation. But under the evaluation of 45 days, other pathogens, *A. citri* and *F. solani* did not appear to have any pathological symptoms in the seedling of *C. aurantifolia*.

Nursery Trials

After pathogenicity trial, various treatments were assessed in the nursery condition. *Trichoderma viride*, *Pseudomonas fluorescens*, and A-arya 009 produced in Chitwan, Nepal and carbendazim produced in India were evaluated (Table 1). The soil was sterilized in an autoclave at 121 ºC for 15 minutes. The potting mixture was prepared by adding sterilized soil with vermicompost in the ratio of 1:3. Treatment was drenched in the sterilized soil with the help of a spray bottle and the soil was properly mixed. The mixture was then kept in a pot and 60 seedlings of the three-leaf stage at 35 DAS were transplanted in the poly-pots filled with autoclaved soil treated with different treatments.

The pure culture of pathogen grown in 20 ml PDA in an incubator at 25 ºC for 7 days was used. Similarly, 40 ml PDA with 7 days old pathogen culture was mixed with 1litre of distilled water to make the broth. Without filtration, the broth was drenched in each pot. The plants were inoculated only one time. After inoculation, regular watering was done. The pots were kept inside the screen house at the temperature of 28 to 32 ºC. First data was collected after 15 days of inoculation while second and third collections were done at an interval of 15 days. The data observation and data collection were continued until the plants in control plot died.

Table 1. Treatments details along with their concentration used against *Rhizoctonia solani* in nursery condition at Warm Temperate Horticulture Centre, Kritipur, Kathmandu, Nepal, 2021

Treatment	Active ingredient	FRAC Code	Formulation	Rate of application	Company
Bavistin	Carbendazim	1	50 WP	2 g/ litre of water	Crystal Crop Protection Ltd.
A-arya 009	Organic acids	NC	-	1 ml/ litre of water	Agricare Nepal Pvt. Ltd.
Guard	*Pseudomonas fluorescens*	BM 02	($1*10^9$ CFU/ml)	2 ml/ litre of sugar water	Agricare Nepal Pvt. Ltd.
Biocide Trivi	*Trichoderma viride*	BM 02	($1*10^9$ CFU/ml)	2 ml/ litre of water	Agricare Nepal Pvt. Ltd.
Distilled water	Control	-	-	1 ltr. of distilled water	-

Data Collection

In pot trials, growth parameters such as primary root length, number of secondary roots, number of leaf numbers, shoot length, root mass, shoot mass, and infected crown length were evaluated whereas as a part of pathological parameters, disease incidence and disease severity were assessed. Among vegetative parameters, number of leaves, plant height, primary root length, secondary root numbers, shoot mass, root mass and length of infected crown portion of individual sapling were taken using the destructive method. The total number of plants affected by disease after the inoculation was recorded. Twelve plants from each treatment were observed. Disease scoring of the affected plant was done

from the scale of 0-4 with 0 indicating no disease, 1 indicating slight bruises on the crown region without effect in root growth, 2 indicating slight bruises on the crown with decreased root growth, 3 indicating deeper bruise with decreased root growth, and 4 indicating the complete rot of crown and no root growth. Visually disease scoring was performed. The disease incidence percentage was calculated using the following formula (Rahman *et al.*, 2013).

$$\text{Percentage disease index} = \frac{\text{Sum of all the score}}{\text{Total number of plant considered x Maxium score}} \text{ x } 100\%$$

$$\text{Percentage Disease Control} = \frac{\text{Disease severity in control} - \text{Disease severity in treatment}}{\text{Disease severity in control}} \text{ x } 100\%$$

Laboratorial Confirmation

After attainment of result of the field trial, the best treatment was further evaluated under in vitro conditions. For this, carbendazim @ 100 ppm and carbendazim @ 200 ppm were evaluated in PDA plates using poison food technique.

Data Analysis Technique

All data were maintained in MS Excel (2013). The analysis of variance was done using R-Stat software (4.1.0) (R Studio Team, 2020). Mean comparison was done from the Fisher Test of Least Significance Difference (LSD) at 0.05 level of significance by using the Agricolae package.

RESULTS AND DISCUSSION

Effect on Vegetative Parameters

Table 2 shows the effect of different treatments (carbendazim, A-arya 009, *P. fluorescens*, *T. viride* and distilled water as control) in the vegetative growth of acid lime saplings at 45 days of *R. solani* inoculation. In comparison with untreated control (distilled water), all the tested chemicals fungicides significantly enhanced the vegetative growth of *R. solani* inoculated acid lime saplings. The treatments differed significantly ($p<0.05$) in enhancing the vegetative growth of the saplings. At the end of the experiment, carbendazim treated plants showed the better result in almost all with *T. viride* exceeding in terms of primary root length and secondary root numbers.

Effect on Disease Parameters

Table 3 shows the efficiency of various treatments on length of infected crown portion, percentage disease index, and disease incidence percentage. In all days of observation, all the tested treatments showed significant result at a $p<0.05$ level of significance. After 45 days of inoculation, carbendazim 50WP recorded the lowest length of infected crown portion (0.00), lowest percentage disease index (18.75%), and lowest disease incidence percentage (43.75%) after 45 days of disease inoculation until the 100% of the plants under untreated control were dead. Highest percentage disease reduction was observed in carbendazim (Fig. 2).

Table 2. Effect of treatments after 45 days on vegetative parameters of acid lime treated with various fungicides at Warm Temperate Horticulture Centre (WTHC), Kirtipur, Kathmandu, Nepal, 2021

Treatment (Fungicide)	Number of leaf	Plant height (cm)	Primary root length (cm)	Secondary root numbers	Shoot mass (g)	Root mass (g)
Carbendazim	16.00[a]	14.375[a]	21.50[a]	38.00[a]	1.371[a]	0.497[a]
A-arya 009	8.25[c]	8.800[d]	14.375[c]	23.00[bc]	0.576[bc]	0.183[cd]
Pseudomonas fluorescens	10.00[b]	9.750[c]	15.00[bc]	22.50[a]	0.656[bc]	0.303[bc]
Trichoderma viride	11.00[b]	10.875[b]	19.375[ab]	29.5[ab]	0.851[b]	0.367[ab]
Control	5.00[d]	6.250[e]	10.375[c]	15.25[c]	0.355[c]	0.135[d]
F-value	144.56	92.452	7.2924	9.163	14.207	8.735
F-test	***	***	**	***	***	***
SEm (±)	0.15	0.139	0.726	1.263	0.046	0.022
LSD	1.011	0.934	4.892	8.515	0.307	0.148
CV (%)	6.675	6.19	20.131	22.025	26.766	33.126
Grand Mean	10.05	10.010	16.125	25.65	0.761	0.297

Note: DADI, Days after Disease Inoculation, SEm ±, Standard Error of Mean; CV, Coefficient of Variation; LSD, Least Significant Difference. Means in the column with the same letter (s) in superscript indicate no significant difference between treatments at 0.05 level of significance; '***' Significant at 0.001 level of significance; '**' Significant at 0.01 level of significance; '*' Significant at 0.05 level of significance.

Table 3. Effect of treatments on length of infected crown, percentage disease index and disease incidence percentage of the collar and root rot in acid lime after 45 days of disease inoculation at Warm Temperate Horticulture Centre, Kritipur, Kathmandu, Nepal, 2021

Treatment (Fungicide)	Length of infected crown	Disease Index	Disease Incidence
Carbendazim	0.000[b]	18.75[d]	43.75[c]
A-arya 009	0.125[b]	75.00[ab]	68.75[b]
Pseudomonas fluorescens	0.200[b]	56.25[bc]	62.50[b]
Trichoderma viride	0.050[b]	50.00[c]	62.50[b]
Control	1.350[a]	87.50[a]	100.00[a]
F-value	35.219	10.211	11.464
F-test	***	***	***
SEm (±)	0.043	3.679	2.70
LSD	0.288	24.803	18.20
CV %	55.376	28.62	17.89
Grand Mean	0.345	57.5	67.50

Note: DAI, Days after Inoculation, SEm±, Standard Error of Mean; CV, Coefficient of Variation; LSD, Least Significant Difference. Means in the column with the same letter (s) in superscript indicate no significant difference between treatments at 0.05 level of significance; '***' Significant at 0.001 level of significance; '**' Significant at 0.01 level of significance; '*' Significant at 0.05 level of significance.

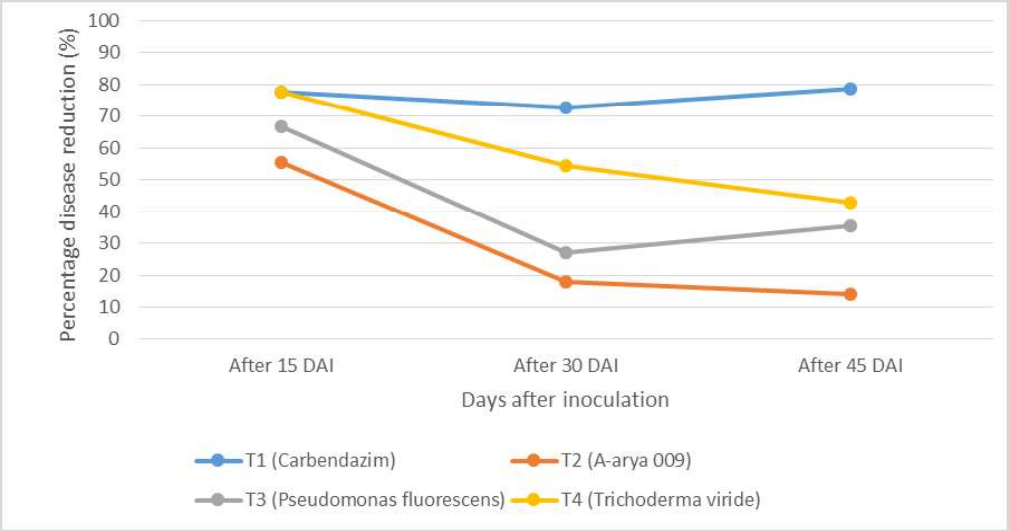

Fig. 2. Percentage Disease reduction by various treatments in acid lime treated at Warm Temperate Horticulture Centre, Kritipur, Kathmandu, Nepal in 2021.

Laboratorial Confirmation

In in-vitro evaluation, both the concentration of carbendazim @ 100 and @ 200 ppm showed 100% inhibition of mycelial growth of the pathogen. No growth was seen in both the petri plates (Fig. 3).

Fig. 3. Growth of *Rhizoctonia solani* in carbendazim 100 ppm and 200 ppm in the laboratory of Warm Temperate Horticulture Centre, Kirtipur, Kathmandu, Nepal in 2021.

R. solani is one of the devastating soils borne plant pathogens that causes disease of economically important. Once this pathogen gets established in soil, it becomes extremely difficult to eradicate from the crop field (Bolton *et al.*, 2010). The continuous infection of this fungus can lead to eventual

death of plant causing rot in crown and root region (Zaman *et al.*, 2015). Citrus industry is one of the major fruit industries in Nepal contributing total of 22.37% of total food production, so the management of such diseases in citrus seedling is crucial. Though modern fungicides are urgently needed to manage this disease, but at the same time management practices that aims at biocontrol activity and reduce the risk of crown rot in citrus seedling is needed. So, in this study an endeavour was made to find if the biological control agents like *Trichoderma* spp. and *Pseudomonas* spp. could provide the better management of *R. solani*.

The disease in acid lime in this study was best controlled by carbendazim as supported by the findings of Hans *et al.* (1981). Similarly, Leng *et al.* (2014) observed up to 91 % in-vitro inhibition of the mycelial growth of *R solani* by carbendazim. After carbendazim, *T. viride* and *P. fluorescence* showed better efficacy in managing *R. solani* in this study. The better efficacy in management of *R. solani* by *Trichoderma* spp. was also observed by Rehman *et al.* (2012), who found that the seedbed treatment with *Trichoderma* spp. in combination with farmyard manure offered better performance against the fungus. Similarly, the experiment of Kotasthane *et al.* (2015) exhibited the highest in-vitro antagonism of *Trichoderma* strain against *R. solani* and also stated the reason to be higher production of inorganic phosphate, indole acetic acid and siderphores. In this present experiment, *P. fluorescens* showed at par results with *T. viride* in terms of disease incidence and disease severity. The efficacy of *P. fluorescens* in suppressing *R. solani* was also observed by Mavrodi *et al.* (2012) and Bautista *et al.* (2007). The strain of *P. fluorescens* is capable of detoxifying oxalic acid, a toxin produced by *R. solani* with a plasmid of *Pseudomonas* being involved in oxalic acid detoxification (Nagarajkumar *et al.*, 2004). In the light microscopy analysis of treatment *R. solani* with *Pseudomonas* strain as done by Elkahoui *et al.* (2015) revealed the induced thickening of the cell-wall, vesiculation of protoplasm and blockage of fungal hyphae branching the *Pseudomonas* strain. *T. viride* and *P. fluorescens* recorded an increase in the plant seedling vigor with an increase in the vegetative growth in the acid lime seedlings. This result is supported by the findings of Rini and Sulochana (2008) and Shanmugaiah *et al.* (2009), who also reported high plant vigor in *Trichoderma* and *Pseudomonas* treated tomato and cotton respectively. The present study showed that *Trichoderma* and *Pseudomonas* spp. could be included in the integrated pest management of the *R. solani*. But for the urgent control, chemical fungicide like carbendazim might be the best treatment.

CONCLUSIONS

R. solani found to be pathogenic which caused crown and root rot in acid lime (*C. aurantifolia*) in nursery conditions. The application of carbendazim @ 1000 ppm was found the most effective for the promotion of sapling growth by restricting the disease. Along with it, *T. viride* and *P. fluorescence* showed the promising result in case of primary root length and secondary root number. Therefore, nurseries producing citrus seedlings are suggested to treat their soil with carbendazim at regular intervals to prevent the proliferation of the soil-borne pathogen. However, these fungicides should be further tested under field condition for further verification to optimize the control efficacy.

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