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SEEDLING ROOT ROT OF ROUGH LEMON (*CITRUS JAMBHIRI* LUSH) AND ITS CHEMICAL MANAGEMENT

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A B S T R A C T

Rough lemon (*Citrus jambhiri* Lush) is an important rootstock for citrus cultivation not only in Pakistan but all over the citrus producing areas around the globe. Seedling root rot disease of rough lemon cause devastating effect on the rough lemon nursery and lead towards negative impact on citrus production. In the present study, disease incidence data was recorded, fungal pathogen was isolated, purified and identified the "*Fusarium solani*". After phytopathogenicity test five fungicides were evaluated against pathogen under *in-vitro* conditions. Three fungicides thiophanate-methyl (Topsin M™), Ridomil gold™ and Score™ performed better. Topsin M was best in controlling mycelial growth at all concentrations. Afterwards, these three fungicides were evaluated in field conditions at their recommended doses All three fungicides significantly controlled the disease but Topsin M performed best in reducing the percentage infection under field conditions. It was concluded that Topsin M performed better under *in-vitro* and *in-vivo* both conditions and significantly reduced the percentage infection of seedling root rot disease in rough lemon nursery followed by Score and Ridomil gold. This study will be helpful to control the seedling root rot of rough lemon.

Keywords: Citrus jambhiri, *In-vitro, In-vivo, F. solani,* fungicides.

INTRODUCTION

Citrus has a distinctive position in the world among fruit crops. It belongs to the *Rutaceae* family. It is believed to be originated from southern slope of Himalayan region, the entire north eastern region of India and adjacent China (Gmitter *et al.,* 1990). Citrus is a good source of vitamin C. It contains many essential nutrients including carbohydrates, potassium, calcium, folate, niacin, thiamin, magnesium, phosphorus, vitamin B_6, riboflavin and pantothenic acid (Reiger, 2006). According to FAO (2011), 110 million tons of citrus fruit is produced annually in all over the world over an area of 7.5 million hectare.

Pakistan is at 13th position in producing citrus in the world. Citrus ranks first among all horticultural crops in Pakistan and it is cultivated over an area of 200 thousand hectare with an annual production of about 2.2 million tons (Anonymous, 2009). The propagation of citrus trees involves grafting the desired variety on to a specific rootstock. Budded trees are preferred over seedlings as they produce fruit sooner, true to type, have resistance to diseases, have higher quality of fruit, greater tolerance to cold, mature early, adapt better to soil conditions and can be dwarfed (Shailendra, 2005).

Rootstock is one of the major part of the citrus tree, have great effect on many aspects regarding yield, fruit quality, tree size, scion compatibility and tolerance to salts and diseases. It supports the upright growth and provide anchoress to tree in soil. Rootstocks are used in the various citrus producing areas of the world. Its selection is best adapted to the area in which it is used as rootstock (Syvertsen and Graham, 1985). Citrus rootstocks differ in their tolerance to cold injury, soil salinity and diseases. Rough lemon (*Citrus jambhiri*) rootstock is one of the rootstocks used for large fruit size, early maturity and to produce large healthy trees. Among all common rootstocks rough lemon shows

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quickest growth and is also easiest to propagate. It has the longest propagation season (Spiegel and Goldschmidt, 1996).

Average yield of citrus in Pakistan is about 12.78 tonnes per hectare while the potential yield of citrus is 18-20 tonnes per hectare in developed countries, so there is a big gap between its average and potential yield. This yield gap is attributed to a number of cultural and environmental factors as well as due to the attack of infectious diseases, caused by different pathogens on citrus or on its rootstocks. Therefore, in order to minimize the existing yield gap and to improve the quality of citrus, these problems need to be thoroughly investigated and properly addressed as rootstocks are the basic component for citrus growing.

Citrus is attacked by different factors both biotic and abiotic which effect its production. Biotic factors like fungus, bacteria, nematode and virus cause severe problems to the areas under citrus production (Timmer and Graham, 1992). Seedling root rot of rough lemon is a serious issue especially in those areas where other rootstocks are not grown that easily as rough lemon. It causes the rough lemon seedling to wilt and then ultimately die. Characteristic symptoms are like wilting, yellowing of leaves, followed by defoliation, die back of twigs from tip to downward and turns yellow to brown, size of root decreases as compared to healthy. Tap roots of seedlings are damaged and rotten while lateral roots almost die and ultimately root volume is reduced. Roots appeared dirty as compared to healthy roots because of having clinging soil particles and infected feeder roots remain stunted (Spina *et al.,* 2008). The seedlings have fewer fibrous roots with necrosis occur on roots (Vegas *et al.,* 1988).

Rough lemon is attacked by various fungal pathogens but *Fusarium* is of prime importance. Genus *Fusarium* occurs widely in nature as saprophytes in soils and decaying vegetables. Some species are plant parasites where specialized pathotypes cause stem rot, ear diseases, vascular wilts, fruit rot and damping off diseases (Booth, 1971). Root rot disease of citrus caused by *F. solani* is one of the most serious disease attacked mandarin trees especially that cultivated in new reclaimed lands in Egypt, it has been estimated to affect 11.6% of mandarin trees and caused 39.6% loss in fruit yield (Mohamedy, 1998) .The present study was designed to manage the disease in rough lemon. In this regard, pathogen was isolated and identified and afterwards different fungicides evaluated to control the seedling root rot.

MATERIALS AND METHODS

Locations of experimental units: All the *in-vitro* experiments were carried out in plant diagnostic laboratory department of plant pathology, University of Agriculture Faisalabad and *in-vivo* experiments were performed at green house of Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan.

Recording of disease incidence data: Seedling root rot disease was observed in nursery of rough lemon in green house of Institute of Horticultural Sciences, University of Agriculture Faisalabad. Area under rough lemon nursery was 120 m^2. This area was divided into 20 sub plots with dimension of 4×1.5m each. In order to make the estimation of disease severity in the field first of all disease incidence data was recorded. To collect disease incidence data from each sub plot 5 rows were selected randomly out of 17 rows. From each row total number of plants was counted and then plants representing the characteristic symptoms of seedling root rot were counted.

Collection of diseased samples: Samples were collected from nursery of rough lemon grown in a green house (Institute of Horticultural Sciences, University of Agriculture Faisalabad). Samples were collected from infected plants as well as from healthy plants to ascertain the association of different pathogens. Samples of soil, root, twigs and leaves were collected. From four to five places within plot sub samples were collected and then mixed together to form a composite sample. The samples were placed in paper bags and labelled with required data such as date, plot number, seedling condition etc. Then samples were brought to laboratory for further studies.

Isolation, purification and identification of pathogenic fungi: Isolation of fungi was done in a plant disease diagnostic laboratory (Department of Plant Pathology, University of Agriculture, Faisalabad). The collected specimens were used for the isolation of fungi by following the Ricker and Ricker (1936) procedure. After performing all the steps of procedure, petri plates were incubated at 27°C±2°C for 3-4 days and data was recorded. The fungi which colonized on these pieces were purified and identified on the basis of their morphological characters (Ellis, 1971). On the basis of the morphological characters the isolated fungus was identified *"Fusarium solani".* Because the isolated fungus

produces white cream mycelium, macro-conidia have three to four septa on average, are slightly curved, are rather wide and thick walled, and have a slightly blunted apical end. In addition, these are abundant, oval to kidney shaped, and formed in false heads on very long monophialides and chlamydospores are abundant (Ellis, 1971).

Plant material: A disease free nursery of Rough lemon was taken from the Citrus Nursery Sanitation Laboratory (Institute of Horticultural Sciences, University of Agriculture, Faisalabad) grown in sterilized soil and maintained in green house. A total of 25 plants were taken and kept in a net house (Department of Plant Pathology, University of Agriculture, Faisalabad).

Preparation of Inoculum of *Fusarium solani*: Isolates of *Fusarium solani* obtained from nursery of rough lemon rootstocks (*Citrus jambhiri* Lush) grown in Institute of Horticultural Sciences, UAF were used in pathogenicity test. *Fusarium* inoculum consisting conidial suspension, was obtained by growing the cultures into PDA broth from 7-days old culture on potato-dextrose agar (PDA). The flasks containing PDA broth were then inoculated with 14 day old *F. solani* culture using sterilized inoculating needle. Labelled flasks were placed in an incubator at 28±2°C and observed daily for mycelial growth. After 12 days entire surface area of each flask was covered with mycelial growth of *F. solani.* Afterwards, the inoculum of all the flasks was mixed and grinded in blender for 3-4 seconds to break mycelia into fragments. Then a suspension was obtained, containing spores and fragments of mycelium. The spores were counted by Haemocytometer, spore counting chamber.

Inoculation of *Fusarium solani*: Inoculation with freshly prepared spore suspension of *F. solani* was done by soil application. Depending on the size of pots 4-6 holes up to middle of the pots near the root system of the plants were made with the help of pointed wood. The rootstocks were inoculated with 45 ml of water suspension containing spore suspension of *F. solani* at rate of 1.2×10^7 conidia/ ml per pot. The suspension was distributed equally in each pot and covered with soil after inoculation to prevent drying. The pots were irrigated daily with tap water carefully to prevent loss of spore suspension through leaching or excessive drying.

Selection of Test Fungicides: The different fungicides (Table 1) were tested at different doses to select the most effective dose rate. Five fungicides were evaluated at five different concentrations *in vitro* and three fungicides out of five were evaluated at their recommended dose *in vivo.* The principle involved in this technique was to supply the nutrient medium with a toxic chemical and then allowing the test fungus to grow on the medium and evaluate the effect of such chemicals by measuring the diameter of fungus growth.

Table 1. List of fungicides used in the present study.

Sr.	Trade Name	Common Name	Formulation	Recommended Dose	Manufacturer
1	Topsin M	Thiophanate methyl	70%WP*	0.2g/100ml	Arysta LifeScience
2	Score	Difenoconazole	250EC***	0.2g/100ml	Syngenta
3	Ridomil Gold	Mancozeb 64%,	68%WP*	0.2g/100ml	Syngenta
4	Carbendazim	Metalaxyl 4% Carbendazim	75%WG**	0.2g/100ml	Syngenta
5	Antracol	Propineb	70%WG**	0.2g/100ml	Bayer Crop Science

WP= Wetable powder*

WG= Water Dispersible granules**

EC= Emulsify able concentrate***

Evaluation of different fungicides *in vitro* against *Fusarium solani:* The efficacy of different fungicides against the most frequently isolated fungal pathogen *F. solani* was studied by using poisoned food technique. Five different concentrations of each fungicide were made, 50, 100, 150, 200 and 250 ppm. To make ppm 1ml/mg of active ingredients in the chemical were weight and dissolved in 100 ml of water, it gave us stock solution. From this stock solution 50, 100, 150, 200 and 250 ppm were made by dissolving 0.5, 1.0, 1.5, 2.0 and 2.5 ml from stock solution in 100 ml of water respectively. Afterwards, 1 cm^3 of each fungicide concentration was added in the freshly prepared PDA (Potato Dextrose Agar) separately and allowed to cool to a pouring temperature of 40-45°C. 25 ml of these PDA amended with different fungicide at different rates was poured into 9cm diameter sterilized petri-dishes. Each plate including the control (without fungicide) on solidification was inoculated in the middle with 14 days old *F. solani* culture using sterilized inoculating needle.

Each concentration of each fungicides including control has five replications. Labelled petri dishes were placed in an incubator at 28±2°C and observed daily for mycelial growth. Radial mycelial growth was measured at five and ten day's interval after inoculation, by measuring the diameter along two perpendicular lines from the underside of the Petri dish (Mitra and Nandi, 1994).

Evaluation of different fungicides *in vivo* against *Fusarium solani:* Five fungicides were evaluated *in vitro* against *F. solani,* out of these five fungicide chemicals three fungicides which performed better in laboratory experiment were selected for field trials against seedling rot of rough lemon. Nursery of rough lemon was in twenty sub plots, three fungicides were selected for field trial and one treatment of control with each fungicide. So, there were four treatment and 20 plots, each treatment was given five plots in random in this way that one fungicide/control is not applied on two consecutive plots. Solution of each chemical was made according to their recommended dose and then sprayed evenly on all seedlings in the plot. After applying one fungicide chemical, spray machine was thoroughly washed to remove all the remains of the previous fungicide. Spraying was done by keeping in mind all the precautions recommended. Three sprays of each fungicide at their recommended dose were applied after interval of 10-14 days and after each spray disease data was recorded.

Experimental designs and statistical analyses : Completely randomized design (CRD) was used for those experiments carried out in controlled laboratory conditions while in field experiments Randomized completely block design (RCBD) was applied. Results were statistically analyzed at 5% significance level using M-Stat version of statistics.

RESULTS

Recording of disease incidence data: Disease incidence data was recorded of each plot, total number of plants in each row was counted and also the number of infected plants showing characteristic symptoms of root rot was recorded. Average percentage infection in all 20 plots was 25.74%.

Evaluation of different fungicides *in vitro* against *Fusarium solani:* Five replications were made of each treatment. Effect of five fungicides Topsin M, Score, Ridomil Gold, Carbendazim and Antracol at five different concentrations of 50, 100, 150, 200 and 250 ppm was evaluated under *in vitro* conditions against *Fusarium solani.* All the fungicides under evaluation showed significant results against *F. solani.* The results of the present study suggested that as increased the concentration of the fungicide lead to the decreased chemical significance of the the mycelial growth of the fungus in the petri plate. None of the fungus reduced the mycelial growth to 100% at five different concentrations, data of colony growth was recorded every second day.

Results revealed that Topsin M was most effect at all five concentrations in significantly inhibiting the colony growth of *Fusarium solani* (Fig. 1).

At higher concentration of the Topsin-M minimum mycelial growth observed. Minimum colony diameter of 1.52 was at 250 ppm concentration. Score was next to Topsin M in controlling the colony growth of *F. solani*, it reduced the colony growth at all five different concentrations.

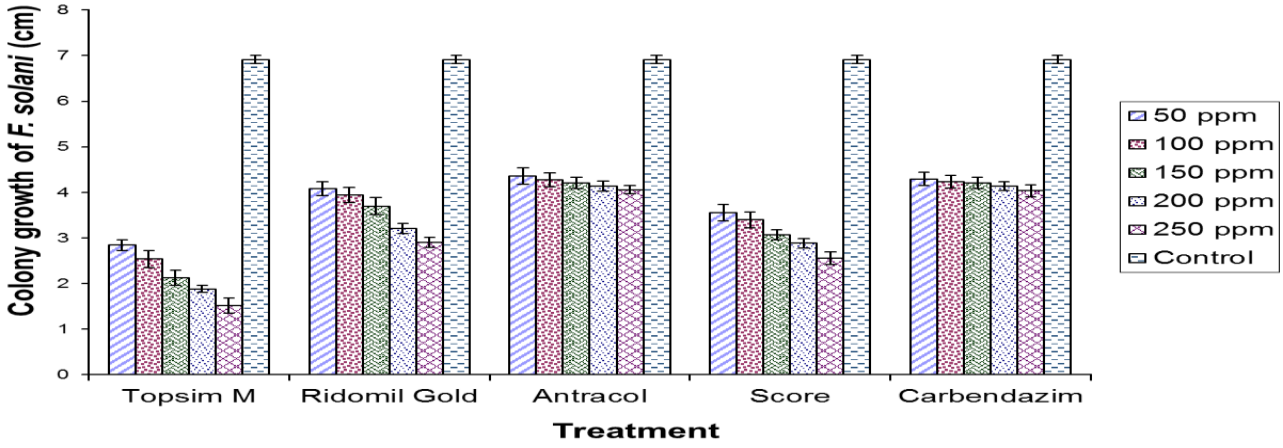

Figure 1. Representing the colony growth of *F. solani* against all five fungicides at five different PPM concentrations.

With time increase in colony growth occurs. Minimum colony growth in case of Score was 2.54 colony diameters at 250 ppm concentration. It was moderately effective against *F. solani.* Ridomil Gold was next to Score in inhibition mycelial growth of fungus, *F. solani* Ridomil Gold also reduced the colony diameter at all different concentration in comparison to control. Diameter of *F. solani* colony growth was 2.90 at 250 ppm concentration of Ridomil Gold. Carbendazim and Antracol were found to be least effect against fungus, *F. solani* isolated from seedling affected with root rot in rough lemon. Minimum colony growth was 4.0 at 250 ppm concentration of Carbendazim and Antracol (Fig.1). They were effective when compared to control, colony growth was 6.9 in case of control.

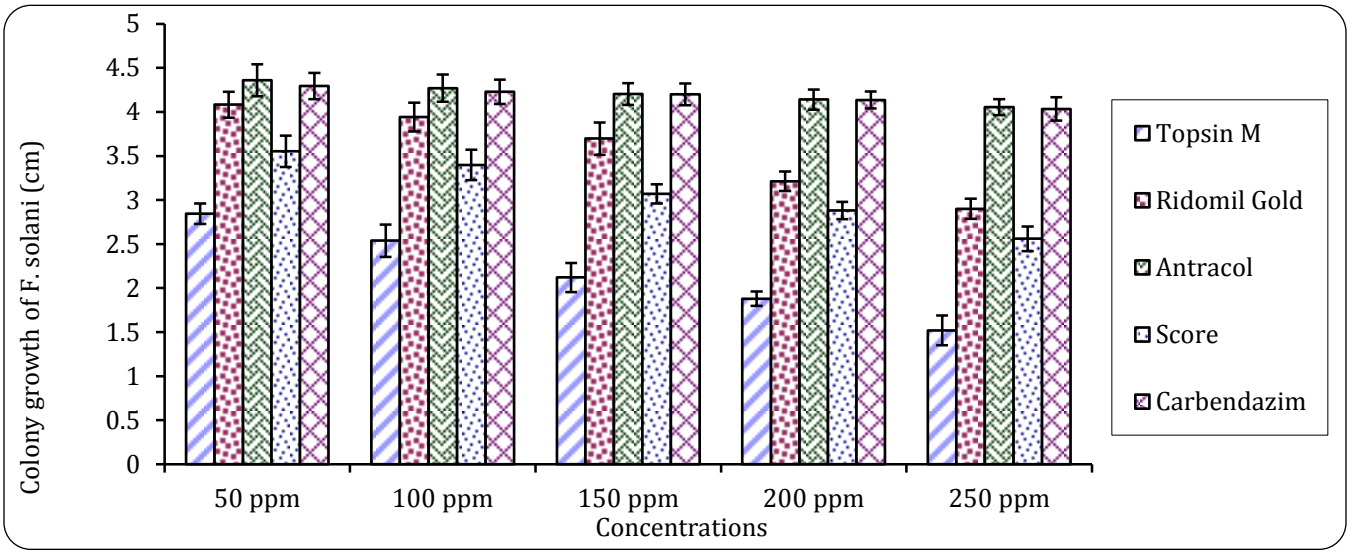

Figure 2. Efficacy of all five fungicides against different concentrations

Evaluation of different fungicides *in vivo* against seedling root rot of Rough lemon: Five fungicides were evaluated against *F. solani* under laboratory conditions out of these five fungicides three fungicides; Topsin M, Ridomil Gold and Score, performed best and were selected for field trial in controlling seedling root rot disease of rough lemon. Three sprays of each fungicide were done on their respective plots at recommended dose to evaluate their efficacy in controlling the disease. Spray was done after interval of 10-14 days and data was recorded after each spray. After first spray Topsin M reduced the percentage infection from 25% to 14%, Ridomil reduced from 26% to 22% and score reduced from 23% to16%. In the same way Topsin M was best in results after 2nd spray (Fig. 3 and 4). After third spray percentage infection was only 3%, before spray in these plots percentage of disease was 25%. Score was second best at 6.5% and Ridomil Gold was at third almost 9% infections.

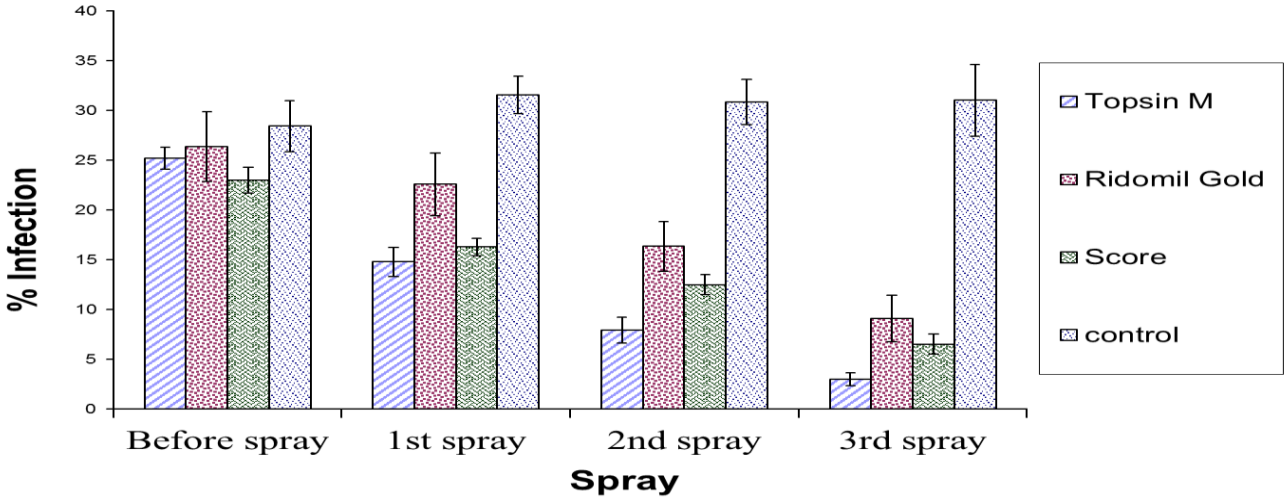

Figure 3. Percentage of infection with respect to interval of spray times

Statistically after first spray Score performed better than Ridomil Gold, after second spray Ridomil Gold and Score were sharing one letter in common but score has one lower letter than Ridomil. After third spray they were showing same results statistically as after second spray. Topsin M performed better than these two fungicide chemicals after each spray, but showed best results after 3rd spray.

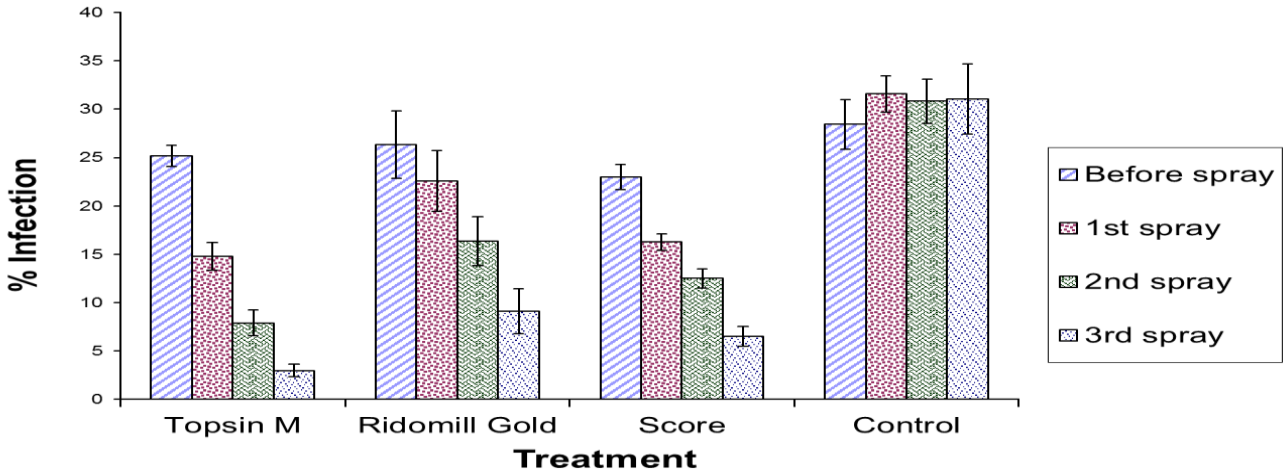

Figure 4. Representing the different fungicides performance with respect to different intervals of sprays.

DISCUSSION

Seedling root rot of rough lemon is a serious issue for citrus industry and is becoming serious day by day not only in Pakistan but all over the world where rough lemon is used as rootstock. It is caused by *F. solani* which inhabits in rhizosphere. Infected seedlings on early stage of infection show flaccid leaves and symptoms of water deficiency. Seedlings become wilted and color of the seedling becomes yellowish brown. After few days leaves shows symptoms of chlorosis and start dying from tips and turn (Kore and Mane, 1992; Praksasm *et al.,* 1992; Verma *et al.,* 1999).

It had been reported that *F. solani* to be present in roots of different cultivated crops such as beans (Silbernagel and Mills, 1990) and agricultural crops (Latiffah *et al.,* 2000), The spread of most of *Fusarium* species depends upon the climatic factors (Bajwa and Javed, 2007). *Fusarium solani* has reported to be widely distributed in numerous native soils such as in sub-tropical, semi-arid and grassland and desert soil soils (Zaccardelli *et al.,* 2008). *Fusarium* species have found to be present in all the four soil types i.e. sandy loam, silt clay loam, silt loam and silt clay soils (Latiffah *et al.,* 2000). Occurrence of *F. solani* in orchards may be due to the intercropping in the orchards.

Our results strongly disagree with the work of the different researchers who concluded that *F. solani* is non-pathogenic to citrus with inability to develop symptoms during pathogenesis (Ghaffar, 1992). The *Fusarium* spp., are predominantly common soil fungi, present in almost all parts of the world as a colonizer of root surfaces or a weak invader of the root cortex of many plants and cause wilts and root rots (Armstrong, 1975).

In vitro comparative effect of different fungicides on mycelial growth of *F. solani* was studied after five days and ten days interval to check the fungi toxic effect of fungicides. It was observed that an increase in the inhibition of mycelial growth of the fungus occur with an increase in the concentration of fungicide. Although none of the fungicide gave 100% inhibition at its three concentrations. The results revealed that the Topsin M was the most effective at all the concentrations. It inhibits the colony growth maximum at its all concentrations. The cheapest and environmentally safe method to control the most of the diseases is the cultivation of resistant varieties but resistance breaking occurs in citrus rootstocks. This suggests the use of fungicides against the disease to minimize the losses.

The effectiveness of fungicides being tested in inhibiting the mycelial growth of *F. solani* varied in great deal and in general a significant increase in the inhibition of mycelial growth occurs with an increase in fungicide concentration. According to our studies, the most effective fungicides inhibiting the mycelial growth of *F. semitectum* was Topsin M. Other fungicides like Ridomil

Gold, Score, Carbendazim and Antracol also proved to be effective at all the five concentrations but at fewer rates compared to Topsin M. *In vitro* effectiveness of Topsin M at all the concentrations has also been reported (Mehrotra and Garg, 1977; Utikar *et al.,* 1978).

In vivo evaluation of three different fungicides Topsin M, Score and Ridomil Gold was also carried out against seedling root rot of rough lemon in controlling the percentage infection of disease. From the experiment it was concluded that Topsin M performed better as compared to Ridomil Gold and Score. 100% infection under field conditions was not controlled but all the fungicides reduce the percentage infection significantly as compared to control. In plots of Topsin M where before fungicide application infection was 25%, it was just 3% after spraying the three splits of chemical. After each spray disease percentage was reduced. Results of fungicide application are also confirmed from literature as many other scientists also worked on fungicide application against rough lemon or citrus (Singh, 1988; Bajwa and Javaid, 2007; Rehman *et al*., 2011).

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