INT. J. BIOL. BIOTECH., 4 (1): 75-78, 2007.

FUNGICIDAL CONTROL OF POST HARVEST STEM-END ROTS OF KINNOW MANDARIN AND LEMON CAUSED BY *ATERNARIA CITRI* ELLIS AND PIERCE

M. B. Ilyas, M.U. Ghazanfar and T. Naveed

Department of Plant Pathology, University of Agriculture, Faisalabad,Pakistan

ABSTRACT

In *in vitro* evaluation Tilt followed by Rubigon was the most effective fungicide in inhibiting the mycelial growth of *Aternaria citri*, the cause of stem end rot of kinnow mandarin and lemon. Thiabendazole and Antracol were intermediate while Calixin, Polyram combi, Daconil and Calixin M, were the least effective fungicides. Nimrod was ineffective fungicide in inhibiting mycelial growth of the fungus. Daconil, Thiabendazole, Daconil+Rubigon (in combination of 1:1) were the most and equally effective in controlling stem end rot of kinnow mandarin with dip treatment while Tilt dip treatment was the most effective in controlling stem end rot of lemon fruits. Although there was an increased reduction in percent fruit rot with an increase in Tilt concentration, the lower concentrations of Tilt, which were ineffective for kinnow fruits, were quite effective for controlling stem end rot of lemon fruit

Keywords: Kinnow mandarin, lemon, stem end rot, *Aternaria citri*, fungicides.

INTRODUCTION

Kinnow mandarin (*Citrus reticulata* Blance) and lemon (*C. limon* Burn.) occupy a prominent position in the fruit industry and are extensively grown in many parts of Pakistan. These fruits assumed a special importance due to their being a very rich source of ascorbic acid (vitamin C.), sugars and mineral matters like calcium, phosphorus and iron. Beside this, they are a good source of foreign exchange. Kinnow mandarin and lemon are subjected to many post-harvest diseases during transit, storage and marketing, the most important being the fruit decay by fungal attacks. Fungi such as *Aternaria citri* Ellis and Pierce, *A. niger* Van Tiegh and *Penicillium digitatum* Sace which are mostly responsible for post-harvest fruit decay, are not capable of direct penetration through cuticle and epidermis of the fruits, but if they gain their entry through surface injury or natural openings, they cause devastating rots of mature fruit (Hussain, 1976., Farooqi, 1983). This paper reports on *in vitro* evaluation of various fungicides against mycelial growth of *A. citri* and *in vivo* post harvest control of stem end rot of kinnow mandarin and lemon caused by *A. citri* by fungicidal treatments.

In vitro sensitivity of *Aternaria citri* to various fungicides

The sensitivity of *A. citri* mycelium to each of the nine test fungicides at 50 ug/ml concentration (Table 1) was studied using modified Borum and Sinclair's technique (1968). A weighed quantity of each fungicide was amended to chickpea seed meal agar (CSMA) medium (chickpea seed meal 20 g, glucose 20g and agar agar 20g, dissolved in water to make 1000 ml volume) after autoclaving, to obtain required concentration. CSMA without fungicide served as control. Twenty-five ml. of the amended and non-amended medium was poured in each of the four 90 mm (diameter) petriplates. After solidification, 6 mm agar plug containing *A. citri* mycelium were cut from 7 days old CSMA culture plates using sterile cork-borer and placed in the centre of petriplate. The inoculated petriplates ere incubated at 25 $\pm 2^0$C. Radial mycelial growths (mm) of *A. citri* were recorded after 7 days of incubation and the data were analyzed statistically to see the difference among various treatments

Comparative efficacy of various fungicides in controlling post harvest decay of Kinnow mandarin and Lemon fruits by dip treatment

Out of the fungicides evaluated against mycelial growth of *A. citri*, four fungicides i.e. Tilt, Thiabendazole, Rubigon, Daconil and one combination of Daconil + Rubigon(1:1) were further evaluated for the control of citrus fruit decay by dip treatment. A weighted quantity of each of the fungicides was dissolved in appropriate quantity of water to make 750-ug/ml concentrations. Healthy sound and good textured fruits were selected for the experiment. Washed and surface dried fruits were injured at the stem end with the help of a sterilized needle. The injured fruits were dip treated with fungicide solution for 2 minutes and were inoculated with equal amount of mycelial spore inoculum of *A. citri* at the stem end. Non-treated, injury inoculated and non-treated non-inoculated fruits served as control. There were three replications for each treatment with ten fruits per replication. Fruits in all treatments were kept at room temperature for 10 days and data for percent fruit rotted were recorded in each treatment.

Effect of dosage rates of tilt on the control of decay of Kinnow mandarin and Lemon by dip treatment

Tilt fungicide which was found to be the most effective, in the dip treatment, for controlling post harvest rot of lemon fruits was further evaluated at concentration of 200, 300, 400, 500, 600, 700 µg/ml for lemon stem end rot and at concentration of 500, 750, 1000, 1250, 1500 µg/ml for kinnow mandarin stem end rot by dip treatment procedure described above. Data on percent fruit rotted were recorded at the expiry of 7 and 10 days for lemon and kinnow mandarin rot respectively. The data were analyzed statistically to visualize the difference in the effect of different Tilt concentrations for the control of fruit decay.

Effect of dosage rate of tilt fungicide on the extent (diameter) of fruit decay lesion

The kinnow and lemon fruits, which exhibited fruit end rot in the experiment on dosage rates of Tilt, were taken and the average diameter of the decay or rot lesion was measured for each dosage rate. The data were analyzed statistically to visualize the difference in lesion size between various dosage rates.

RESULTS

***In vitro* sensitivity of *Aternaria citri* to various fungicides amended in growth medium**

Aternaria citri was the most sensitive to Tilt fungicide which completely (100 percent) inhibited fungus growth (Table 1). Rubigon was next to Tilt in its effectiveness and it caused 92.5 percent inhibition in mycelial growth of Aternaria citri. The fungus exhibited intermediate sensitivity to Thiabendazole and Antracol, which caused 57.5 and 46.11 percent inhibition in mycelial growth respectively. The least effective fungicides in inhibiting mycelial growth were Calixin, Polyram combi, Daconil, Calixin M, which inhibited 28.33, 24.72, 24.72, and 23.61 percent growth respectively. However, there was no statistical difference between the effectiveness of Calixin, Polyram combi, Daconil and Calixin-M. Nimrod was found to be ineffective in inhibiting mycelial growth of the fungus.

Comparative efficacy of fungicidal dip treatments for the control of post harvest stem end tots of Kinnow mandarin and Lemon

The effectiveness of fungicidal dip treatments in controlling *A. citri* stem end rot varied greatly with the kind of treatment and the kind of citrus fruits (Table 2). Except for Tilt which was the most effective in controlling lemon rot (100 percent), all dip treatments were comparatively less effective in decreasing lemon rot but were more effective in decreasing kinnow rot. Thiabendazole, Tilt, Rubigon, Daconil, Daconil + Rubigon dip treatment reduced 60, 43.33, 26.27, 73.33 and 66.67 percent kinnow rot, respectively over the untreated control. There was no statistical difference between the effectiveness of Thiabendazole, Daconil and Daconil + Rubigon in reducing kinnow rot i.e. they exhibited same effectiveness. Tilt was the most effective in reducing lemon rot and it caused 100 percent decreased in lemon rot. Thabezdazol, Rubigon, Daconil and Daconil+Rubigon caused 33.33, 13.33, 23.33 and 19.67 percent decrease in lemon stem end rot. However, there was no statistical difference between the effectiveness of Thiabendazole and Daconil and between the effectiveness of Rubigon, Daconil and Daconil + Rubigon.

Table 1. Radical growth (mm) of *Aternaria citri* at 50 µg/ml concentration of each of the nine test fungicides amended in growth medium (CSMA).

Treatments	Radial growth (mm)	Percent decrease over control
Antracol	48.50[d]*	46.11*
Calixin-M	68.75 [b]	23.61
Calixin	64.50 [c]	28.33
Daconil	67.75 [bc]	24.72
Nimrod	87.50 [a]	2.77
Polyram combi	67.75 [bc]	24.72
Rubigon	6.75 [f]	92.50
Thiabendazole	38.25 [e]	57.50
Tilt	0.00 [g]	100.00
Control	90.00 [a]	0.00

* Figures with the same letters do not different at 5% level of significance.

Effect of dosage rates of tilt on the control of stem end rot of Kinnow mandarin and Lemon by dip treatment

Stem end rot by *A. citri* was also significantly reduced at all dosage rates of Tilt evaluated and there was a significant decrease in stem end rot with an increase in the Tilt concentration (Table 3). Thus at 500, 750, 1000, 1250 and 1500 µg/ml dosage, Tilt reduced 20, 30, 33.33, 76.67 and 96.97 percent stem end rot of kinnow fruits. However, there was no statistically difference between the effectiveness of Tilt at 500 and 750 µg/ml dosage and between 750 and 1000 µg/ml dosage.

Tilt at all dosage rates evaluated significantly reduced *Alternaria* stem end rot of lemon (Table 3). Thus at 200, 300, 400, 500, 600and 700 ug/ml dosage rates, Tilt caused 28.56, 39.27, 60.71, 71.42, 82.13 and 85.72 percent reduction in stem end rot of lemon. However, there was no statistical difference between the effect of Tilt at 200 and 300 µg/ml, between 400 and 500 µg/ml and between 500, 600 and 700 µg/ml dosage rates.

Table 2. Effect of various fungicidal dip treatment on the post harvest rot of Kinnow mandarin and lemon caused by *Aternaria citri*

Treatments	Kinnow mandarin		Lemon	
	Percent fruit rot	Percent decrease in fruit rot over control	Percent fruit rot	Percent decrease in fruit rot over control
Thiabendazole	40.00*[d]	60.00	66.67*[c]	33.33
Tilt	56.67[c]	43.33	0.00[d]	100.00
Rubigon	73.33[b]	2 6.6 7	86.67[b]	13.33
Daconil	26.67[d]	73.33	76.67[bc]	23.33
Daconil + Rubigon	33.33[d]	66.67	80.33[b]	19.67
Control	100.00[a]	0.00	100.00[a]	0.00

* Figures with the same letters do not differ at 5% level of significance

Table 3 Effect of dosage rate of tilt on the control of post-harvest rot of Kinnow mandarin and Lemon caused by *Aternaria Citri*

Treatments	Kinnow mandarin		Dosage rate of Tilt	Lemon	
	Percent fruit rot	Percent decrease in fruit rot over control		Percent fruit rot	Percent decrease in fruit rot over control
500 ug/ml	80.00[b*]	20.0	200ug/ml	66.67*[b]	28.56
750 ug/ml	70.00[bc]	30.0	300ug/ml	56.67[b]	39.27
1000 ug/ml	66.67[c]	33.33	400ug/ml	36.67[c]	60.71
1250 ug/ml	23.33[d]	76.67	500ug/ml	26.67[cd]	71.42
1500 ug/ml	3.33[e]	96.67	600ug/ml	16.67[d]	82.13
Control	100.00[a]	0.00	700ug/ml	13.33[d]	85.72
-	-	-	-	93.33[a]	-

* Figures with the same letters do not differ at 5% level of significance

Effect of dosage rate of tilt fungicide on the extent (diameter) of stem end rot lesion in Kinnow mandarin and Lemon.

Tilt at 500, 750, 1000, 1250 and 1500 µg/ml concentration reduced 18.13, 25, 33.45, 58.34 and 85.54 percent lesion size of the kinnow fruit (Table 4). However, there was no different between the effectiveness of 500 and 750 µg/ml concentration in reducing lesion size of kinnow fruits. Tilt dip at 200, 300, 400, 500, 600 and 700 µg/ml concentration, similarly caused 32.59, 50.14, 58.89, 67.52, 75.91 and 91.51 percent reduction in lesion size of lemon fruits

Table 4. Effect of tilt dosage rate on the extent of decay Lesion (average diameter) of Kinnow Mandarin and Lemon by *Alternaria citri.*

Dose rate of Tilt	Kinow Mandrine		Dose rate of Tilt	Lemon	
	Diameter of lesions (mm)	% Decrease in decay lesion over control		Diameter of lesions (mm)	% Decrease in decay lesion over control
500ug/ml	49.67[b*]	18.13	200ug/ml	16.4[b]	32.59
750ug/ml	45.50[b]	25.00	300ug/ml	12.13[c]	50.14
1000ug/ml	46.37	33.45	400ug/ml	10.00[d]	58.89
1250ug/ml	25.27[d]	58.34	500ug/ml	7.90[e]	67.52
1500ug/ml	8.77[e]	85.54	600ug/ml	5.86[f]	75.91
Control	60.67[a]	0.00	700ug/ml	2.06[g]	91.53

* Figures with the same letters do not differ at 5% level of significance

DISCUSSION

Measures for controlling post harvest citrus stem end rot by *A. citri* are based upon prevention of infection, eradication of incipient infections, retarding the progress of pathogen in infected fruit and finally imparting

resistance in fruit tissue against the spread and multiplication of the pathogen. Since fungicidal application to citrus fruit surface can fulfill these purpose (Echert, 1975), a lot of nine fungicides was evaluated against vegetative growth of *A. citri* as well as against the citrus stem end rot disease by this fungus.

The sensitivity of *A. citri* mycelium to each of the nine test fungicides varied greatly. It was found to the most sensitive to Rubigon and Tilt fungicide, intermediate to Thiabendazole and Antracol and least sensitive to Calixin, Daconil, Polyram combi and Calixin M. However, *A. citri* mycelium was insensitive to Nimrod fungicide. Such a differential sensitivity of *A. citri* to various fungicides may be due to their different modes of action, their differential rates of up take by the fungus,, their variable detoxification by the fungus. Differential sensitivity may also be attributed to structural alterations in the fungicides in certain physiological or metabolic processes of the fungus, particularly those involved in nucelie acid synthesis and respiration. Physical or metabolic transformation of a fungicide to more or less toxic product by the fungus may add or reduce the effect of the fungicide on the growth the fungus (Vyas, 1984).

The effectiveness of fungicidal dip treatment in controlling kinnow and lemon stem end rot varied greatly with the kind of fungicide used and the kind of fruit to which dip treatment was applied. Tilt completely i.e. 100 percent controlled or reduced the stem end rot of lemon but it was not so (less) effective (almost 57 percent) in reducing the stem end rot of kinnow mandarin. In general, dip treatments of Thiabendazole, Daconil, Daconil + Rubigon and Rubigon exhibited greater effectiveness in controlling stem end rot of kinnow mandarin but were comparative less effective in controlling stem end rots of lemon. The effectiveness of Thiabendazole + Daconil were statistically equal in controlling stem end of kinnow as well as that of lemon. The control of Alternaria rot with Guazatine has been reported by Singh (1980), with 2, 4-D has been reported by Chalutz *et al*., (1983) and with Thiabendazol by Brown (1984). We report Tilt to be better and effective in controlling stem end of kinnow mandarin and lemon.

The effect of dosage rates of Tilt in controlling stem end rot of kinnow and lemon varied greatly and there was an increased reduction in percent stem end rot with an increase in Tilt concentration. Similarly, there was an increased reduction in lesion size of the fruits which exhibited rotting. It was interesting that lower concentration of Tilt which were not effective for kinnow fruits, were quite effective for controlling stem end rots of lemon fruits. The exact reason for this is not known, probably there may be some metabolites in the dermis of lemon fruits which increased the efficacy of lower dosage rates of Tilt dip on lemon fruits.

REFERENCES

Borum, D.F. and J.B. Sinclair (1968). Evidence for systemic protection against *Rhizoctonia solani* with vitavax in cotton seedlings. *Phytopath*., 58: 976-980.

Brown, G.E. (1984). Efficacy of citrus post harvest fungicides applied in water and resin solution, water wax. *Plant Disease*, 68: 415-418.

Chalutz, E., A. Giser, N. Kaplan, J. Waks, Y. Guller, S. Nadel, E. Lomanice and B. Weiss (1983). Semi commercial trials to control *Alternaria* in grape fruits. *Alon Hanotea*, 37: 365-366.

Eckert, J.W. (1975). Post harvest diseases of fresh fruits and vegetables. Ecology and control. *Symposium post harvest biology and handling of fruits and vegetables*. The AVI Publishing Co & Inc. Connecticut, USA, P-193.

Farooqi, W.A. (1983). *Biochemical and physiological basis of shelf life extension of citrus fruits by refrigeration and controlled atmosphere storage. NIAB Faisalabad, Pakistan*. Technical Report (PL-480Project): 103.

Hussain, K. (1976). *Fungi causing rot of citrus fruits in the market and storage*. M.Sc. Thesis. Deptt. of Plant Pathology Univ. of Agri., Faisalabad.

Singh, V. (1980). Control of Alternaria rots in citrus fruit. Abst. *Pl. Path*., 9: 12-13.

Vyas, S.C. (1984) *Systemic fungicides*. Tata McGraw-Hill Publishing Co. Lted. New Dehli: 360.

(*Accepted for publication November 2006*)