Pak. J. Bot., 39(3): 961-965, 2007.

CHEMOTHERAPEUTIC CONTROL OF POSTHARVEST DECAY OF KINNOW MANDARIN AND LEMON CAUSED BY *PENICILLIUM DIGITATUM* SACC.

M.B. ILYAS, T. NAVEED, *M. INAM-UL-HAQ, N. JAVED AND S.M. MUGHAL

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

Abstract

In an *In vitro* evaluation Daconil was found to be the most effective fungicide in inhibiting mycelial growth of *Penicillium digitatum* followed by Antracol, Rubigon, Calixin, Thiahendazole, Calixin M., Tilt and Nimrod. Though Tilt as dip treatment was the most effective in controlling post harvest decay of lemon fruit but it was comparatively less so in controlling decay of Kinnow fruit. Tilt, Thiabendazole and Daconil + Rubigon (1:1) were statistically equally effective in controlling decay of Kinnow fruits. There was an increased reduction in percent fruit decay with an increase in Tilt concentration. Tilt also caused reduction in lesion size of the decaying fruits. Lower concentration of Tilt which were ineffective for Kinnow fruit, were quite effective for controlling decay of lemon fruits.

Introduction

Kinnow mandarin (*Citrus reticulata* Blance) and Lemon *(Citrus limon* Burm) 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 *Alternaria citri* Ellis & Pierce, *Aspergillus niger* Von Tiegh and *Penicillium digitatum* Sacc, 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 fruits (Hussain, 1976). This paper reports *In vitro* evaluation of various fungicides against mycelial growth of *P. digitatum* and *In vivo* post-harvest control of decay of Kinnow mandarin and Lemon caused by *P. digitatum* by fungicidal treatments.

Material ad Methods

i. *In vitro* sensitivity of *Penicillium digitatum* to each of the test fungicides: The sensitivity of *Penicillium digitatum* 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 (seed meal agar 20gm, glucose 20g, agar 20g dissolved into H_2O to make volume one liter) 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 Petri plates. After solidification, 6 mm agar plugs containing *Penicillium digitatum* mycelium were cut from 7days old CSMA culture plates using sterile cork-borer and placed in the centre of Petri plate. The inoculated Petri plates were incubated at 25^{o}C. Radial mycelial growth (mm) of *P. digitatum* were recorded after seven days of incubation and data were analyzed statistically to see the differences among various treatments.

*Corresponding author email: dr_iuhaq@yahoo.com

Table 1. Radial growth (mm) of *P. digitatum* at 50 ug/ml concentration of each of the nine test fungicides.

Fungicides	Radial growth (mm)	Percent decrease over control
Antracol	23.00[d]	77.44
Calixin-M	26.25[cd]	70.83
Calixin	24.25[d]	73.05
Daconil	9.00[e]	90.00
Nimrod	29.25[bc]	67.50
Polyram Combi	30.25[b]	66.38
Rubigon	23.75[d]	73.61
Thiabendazole	24.50[d]	72.77
Tilt	29.25[bc]	67.50
Control	90.00[a]	0.00

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

ii. 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 *P. digitatum* four fungicides i.e., Tilt, Thiabendazole, Rubigon, Daconil and one combination of Daconil + Rubigon (1:1) which proved to be most effective in inhibiting mycelial growth were selected and further evaluated for the control of citrus fruit decay by dip treatment. A weighed quantity of each of the fungicides was dissolved in appropriate quantity of water to make 750 ug/ml concentration. Healthy sound and good textured fruits were selected for the experiment. Washed and surface dried fruits were injured with the help of a sterilized needle. The injured fruits were dip treated with fungicide solution for 2 minutes and inoculated with mycelial/ spore inoculum of *P. digitatum*.

Non-treated, injury inoculated and non-treated non-inoculated fruits served as control. There were three replications for each treatment with ten fruits/replication. Fruits in all treatments were kept at room temperature for 10 days and data for percent fruit decay were recorded in each treatment.

iii. Effect of dosage rates of tilt fungicide 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 decay was further evaluated at concentration of 200, 300, 400,500, 600, 700 ug/ml for Lemon decay and at concentration of 500,750, 1000, 1250, 1500 ug/ml for Kinnow mandarin by dip treatment procedure described above. Data on percent fruit decay were recorded at the expiry of 7 and 10 days for Lemon and Kinnow mandarin decay respectively. The data were analysed statistically to visualize the difference in the effect of different Tilt concentrations for the control of fruit decay.

vi. Effect of dosage rate of tilt fungicide on the extent (diameter) of fruit decay lesion: The Kinnow and Lemon fruits, which exhibited fruit decay/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

i. *In vitro* sensitivity of *P. digitatum* to various test fungicides: *P. digitatum* was found to be most sensitive to Daconil which, when amended into the growth medium, caused 90% inhibition in fungus growth over the non-amended control (Table 1). The fungus was less sensitive to Antracol, Rubigon,Calixin, Thiabendazole and Calixin M which caused 74.44, 73.61, 73.05, 72.77 and 70.83% inhibition respectively in mycelial growth over the control. However, there was no statistical difference between the effectiveness of Antracol, Rubigon, Calixin, Thiabendazole and Calixin-M. The least effective fungicides in inhibiting mycelial growth of fungus were Tilt, Nimord and Polyram Combi which exhibited same effectiveness statistically and caused 67.50, 67.50 and 66.38% reduction in mycelial growth of the fungus. There was also no statistical difference between the effectiveness of Calaxin-M,Tilt and difference between the effectiveness of Calaxin,-M, Tilt and Nimord in inhibiting mycelium growth of *P. digitatum*.

ii. Comparative efficacy of fungicidal dip treatments for post-harvest control of Kinnow Mandarin and Lemon decay caused by *P. digitatum*: The effectiveness of fungicidal dip treatment in controlling Kinnow and Lemon decay by *Penicillium digitatum* also varied greatly with the kind of fungicidal dip and the kind of fruit (Table 2). Although all fungicidal dip treatments significantly reduced decay of Kinnow and Lemon fruits over the untreated control, but the efficacy of dip treatments was more effective in controlling Kinnow decay over Lemon decay. Tilt, Thiabendazole and Daconil + Rubigon were statistically more but equally (among themselves) effective in controlling Kinnow decay and they caused 73.33, 66.66 and 63.33% decrease in Kinnow decay over the control. Tilt was also the most effective dip treatment in reducing decay of Lemon and it caused 96.67% decrease in Lemon fruit decay by *Penicillium digitatum.*

iii. Effect of dosage rate of tilt on the control of post harvest decay of citrus fruits: The effect of Tilt fungicide in controlling decay varied with the concentration of Tilt fungicide and there was an increased reduction in the fruit decay with an increase in Tilt concentration. The results concerning the effect of various dosage rates on the control of Kinnow and Lemon decay are as follows.

a. Kinnow mandarin: At all dosage rates evaluated, Tilt caused significant decrease in percent fruit rot by *Penicillium digitatum* (Table 3), thus dip treatment of Kinnow fruits in 500, 750, 1000, 1250 and 1500 ug/ml dosage decreased 16.67, 60, 76.67, 86.67 and 93.33% fruit rot respectively. However, there was no statistical difference between the effectiveness of Tilt at 1000 and 1250 ug/ml and between 1250 and 1500 ug/ml concentration in controlling Kinnow decay by *Penicillium digitatum.*

b. Lemon: In contrast to its higher dosage rates for Kinnow, Tilt was evaluated at lower dosage rates in case of Lemon. Tilt at 200 ug/ml dosage statistically did not reduce *Penicillium* decay of Lemon but at higher dosage rate of 300, 400, 500, 600, and 700 ug/ml it caused 16.67, 20, 43.33, 53.33 and 80% reduction in Lemon decay (Table 3). However, there was no statistical difference between the effect of Tilt at 300 and 400 ug/ml dosage rates and between 500 and 600 ug/ml dosage rates.

iv. Effect of dosage rate of tilt fungicide on the extent (diameter) of fruit of decay lesion: The size of decay lesion, on account of the activity of pathogen, depended on the dosage rate of dip treatment of Tilt and in general there was a progressive decrease in the diameter of the decay lesion with an increase in the dosage rate. Tilt at 500 ug/ml concentration did not reduce the lesion size of Kinnow by *Penicillium digitatum.* However, at dosage rate of 750, 1000, 1250 and 1500 ug/ml there was 34.92, 51.16, 70.14 and 86.17% reduction in lesion size over the control respectively (Table 4). Similarly there was 21.69, 40.11, 54.69, 71.61, 79.11 and 88.51% reduction in the lesion size of Lemon at dosage of 200, 300, 400, 500, 600 and 700 ug/ml respectively.

Table 2. Effect of various fungicidal dip treatment on the post–harvest decay of Kinnow mandarin and Lemon caused by *Penicillium digitatum.*

Fungicides	Kinnow mandarin		Lemon	
	Percent fruit rot	Percent decrease in fruit rot over control	Percent fruit rot	Percent decrease in fruit rot over control
Thiabendazole	33.33^{c*}	66.67	53.33^{c*}	46.67
Tilt	26.67^{c}	73.33	3.33^{d}	96.67
Rubigon	50.00^{b}	50.00	86.67^{b}	3.33
Daconil	50.00^{b}	50.00	76.67^{b}	23.33
Daconil + Rubigon	36.67^{c}	63.33	76.67^{b}	23.33
Control	100.00	0.00	100.00^{a}	0.00

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

Table 3. Effect of dosage rate of tilt on the control of post–harvest decay of Kinnow mandarin and Lemon caused by *Penicillium digitatum.*

	Kinnow mandarin			Lemon	
	Percent fruit rot	Percent decrease in fruit decay over control	Dosage of tilt (ug/ml)	Percent fruit rot	Percent decrease in fruit decay over control
500	83.33^{b*}	16.67	200	96.67^{ab*}	3.33
750	40.00^{c}	60.00	300	83.33^{bc}	16.67
1000	23.33^{d}	76.67	400	80.00^{c}	20.00
1250	13.33^{de}	86.67	500	56.67^{d}	43.33
1500	6.67^{e}	93.33	600	46.67^{d}	53.33
Control	100.00^{a}	0.00	700	20.00^{e}	80.00
			Control	100.00^{a}	0.00

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

Table 4. Effect of tilt dosage rate on the extent of the decay lesion (average diameter) of Kinnow mandarin and Lemon by *Penicillium digitatum.*

	Kinnow mandarin			Lemon	
	Diameter of decay lesions (mm)	Percent decrease in decay lesion over control	Dosage of tilt (ug/ml)	Diameter of decay lesions (mm)	Percent decrease in decay lesion over control
500	66.50^{a*}	10.46	200	21.10^{b*}	21.69
750	48.33^{b}	34.92	300	19.96^{c}	40.11
1000	36.67^{c}	51.16	400	15.10^{d}	54.69
1250	22.17^{d}	70.14	500	9.46^{e}	71.61
1500	10.27e	86.17	600	6.96^{f}	79.11
Control	74.27^{a}	0.00	700	3.86^{g}	88.41
–	–	–	Control	33.33^{a}	–

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

Discussion

Measures for controlling post harvest citrus decay by fungi depend upon prevention of infection, eradication of incipient infections retarding the progress of pathogens in infected fruits and imparting resistance in fruit tissue against the spread and multiplication of the pathogens. Since fungicidal application to citrus fruit surface can fulfill these purposes (Eckert, 1975), nine fungicides were evaluated against mycelial growth of *Penicillium digitatum* as well as citrus decay caused by this fungus. Thus

Daconil was found to be most effective fungicide in inhibiting the mycelial growth of *P. digitatum* followed by Antracol, Rubigon, Calixin, Thiabendazole, Calixine M., Tilt, Nimord and Polyram combi. However, there was no statistical difference among the effectiveness of Antracol, Rubigon, Calixin, Thiabendazole and Calixine M. Similarly there was no statistical difference among the effectiveness of Calixine M., Tilt, and Nimord in inhibiting mycelial growth. The differential sensitivity of *P. digitatum* to various fungicides may be due to differences in the rate of uptake of these fungicides and their detoxification by the fungus. The differential sensitivity may also be attributed to structural changes in fungicides in certain physiological or metabolic processes of the fungus (Vyas, 1984).

The effectiveness of fungicidal dip treatments in controlling Kinnow and Lemon decay by *P. digitatum* varied greatly with the kind of fungicide dip and the kind of fruit treated. However, the efficacy of dip treatments was more in controlling Kinnow decay over that of Lemon decay. Tilt, Thiabendazole and Daconil + Rubigon were the most and statistically equally effective in controlling Kinnow decay while Tilt was the only most effective in reducing decay of Lemon. Thiabendazole has already been reported to control post harvest *Penicillium* decay (Eckert, 1979, Ramana, e*t al.*, 1979; Morales *et al.*, 1981; Brown, 1984; Gutter, 1985). We report Tilt to be better and effective in controlling citrus fruit decay by *Penicillium* sp.

The effect of dosage rates of Tilt in controlling Kinnow and Lemon decay varied greatly and there was an increased reduction in percent fruit-decay with an increase in Tilt concentration. Similarly there was also an increased reduction in lesion size of the fruit which exhibited rottening. It was interesting that lower concentrations of Tilt which were not effective for Kinnow fruit, were quite effective for controlling decay 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 this aspect needs to be confirmed during further studies.

References

Broum, 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(5): 415-418.

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

Eckert, J.W., M.J. Kolezen, M.L. Rohm and J.K. Eckord. 1979. Influence of benomyl and methyl 2-benzimidazolecarbamate on development of *Penicillium digitatum* in the pericarp of orange fruits. *Phytopath*., 69(9): 934-939.

Gulter, Y. 1985 Combined treatment with Thiabendazole and 2-aminobutane for control of citrus fruit decay. *Crop Protection*, 4(3): 346-350.

Morals, M.A.R., A.M. Alvarz and A.L. Sanchez. 1981. Physiochemical control of green mold on Lemon. *Tecnica*, 41(4): 187-191.

Ramana, K.V.R., G.R. Seity, N.V.N. Moorthy, S. Saroja and A.M. Manjunadaswamy. 1979. Efffect of ethephen, benomyl, thiabendazole and wax on colour and shelf life of Kinnow mandarins. *Tropical Sci*., 21(4): 265-272.

Vyas, S.C. 1984. *Systemic fungicides*. Teta Mc Graw Hill Publ. Co. Ltd., New Delhi. P-360.

(Received for publication 26 October 2006)