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In vitro evaluation of safer fungicides in management of *Penicillium digitaum* causing green mould of Kinnow

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Abstract
Green mould, caused by *Penicillium digitatum*, is one of most destructive post-harvest disease of citrus worldwide. *In vitro* study, we examined the antifungal activity of commercially available fungicides viz. difenoconazole 25 % EC, hexaconazole 5 % EC, tebuconazole 25 % EC, propiconazole 25 % EC, kresoxim-methyl 50 % EC, azoxystrobin 25 % EC against *Penicillium digitatum*. Among these six fungicides, difenoconazole was found to be most effective as well as significantly superior to all other treatments with 99.2 per cent average inhibition in mycelial growth followed by hexaconazole with 97.2 per cent inhibition at 100 ppm concentration.

Keywords: green mould, fungicides, Kinnow, *Penicillium digitaum*

Introduction
Citrus (*Citrus* spp.) is one of the most important fruit crop which includes lime, lemon, orange, tangerine, mandarins and Kinnow etc and these are commercially grown in 137 countries with tropical and sub-tropical agro-climatic conditions (Ismail and Zhang, 2004) [1]. Productivity of citrus in India is quite low (9.35 t/ha) in comparison to the other developed countries like Brazil, USA, China, Mexico and Spain which were having productivity of 30-40 t/ha (Anonymous, 2014) [2]. There are several reasons for the low productivity of citrus like lack of nutrition, poor management, handling practices and infestation of several pest and diseases. Among these damage caused by diseases is one of the dominant factors. Citrus is infected by many pathogens like fungi, bacteria and viruses which resulted in huge economic losses. Among fungal diseases, post-harvest diseases are also important and which resulted significant losses. Post-harvest fungal diseases reported to cause 30 to 50 per cent losses in citrus (Porat *et. al*., 2000; Embaby *et al*., 2013) [3, 4]. Many pathogens were reported to be associated with post-harvest fungal diseases in citrus which significantly reduce the yield as well as quality of citrus (Holmes and Eckert, 1999; Barkai-Golan, 2001; Plaza *et al*., 2003) [5-7]. Among post-harvest diseases of citrus, green mould (*Penicillium digitatum*) and blue mould (*P. italicum*) are most commonly observed in all citrus growing areas throughout the world (Holmes and Eckert, 1999; Palou *et al*., 2001; Skaria *et al*., 2003; Plaza *et al*., 2004) [5, 8, 9, 10]. Thus, the present studies were planned with the objective to find the in vitro efficacy of commercially used safer fungicide against *Penicillium digitatum*. So that possibilities of their use in the management of post-harvest disease of fruits could be explored.

Material and Methods
Isolation and Identification of the pathogen
Isolations of the pathogen with apparent symptoms of the green mould rot were made from diseased portion of Kinnow fruits. Small bits of 1 to 2 mm size were taken from juncture of diseased and healthy part of fruits with the help of sterilized and sharp blade. These bits were surface sterilized with mercuric chloride (0.1%) for 10 to 20 seconds and washed thrice with sterilized distilled water under aseptic conditions. The bits were then placed on the sterilized filter paper to remove the excess moisture and were subsequently transferred to sterilized Petri plates containing Malt Extract Agar (MEA) medium (malt extract 20 gm, peptone 1 gm, glucose 20 gm and agar-agar 20 gm in 1 litre distilled water). This medium has earlier been reported to support the maximum growth of *Penicillium* species (Timmer *et al*. 2000) [11]. Malt Extract Agar medium was added with *streptocycline* (30 mg/lt) while, pouring in Petri plates after sterilization, to restrict the growth of bacterial contaminants. The spores of test pathogen from the green area on infected fruits were also picked with precision with the help of

sterilized inoculating needle and were streaked on MEA medium. The inoculated Petri plates were incubated at 24±1 ºC in BOD incubator and examined daily for the mycelial growth. Pure culture was obtained by hyphal tip technique as well as single spore isolation (Sinclair and Dhingra, 1995) [12] and was further cultured on slants containing MEA. The morphological characters of the fungus were studied on host by inoculating the isolated test fungus on the Kinnow fruit with the help of pin prick method as well as in the culture grown on MEA medium. The causal organism was identified by studying its morphology and comparisons with standard and authentic description from literature on this fungus (Raper and Thom, 1949; John, 1979) [13, 14]. Size and other dimensions of mycelium, conidiophores and *phialides* were measured with the help of the software i.e. Magnus MIPS (Micro Image Projection System) installed and connected with Olympus microscope CX41 (Biocontrol laboratory, Department of Entomology), LEICA microscope (Virology laboratory, Department of Plant Pathology).

***In vitro* evaluation of chemical fungicides**

Six fungicides, namely difenoconazole 25 % EC (Score), hexaconazole 5 % EC (Contaf), tebuconazole 250 % EC (Folicur), propiconazole 25 % EC (Tilt), Kresoxim methyl 50 % EC (Ergon), azoxystrobin 25% EC (Amistar) were evaluated under laboratory condition at (50,100,250 and 500 ppm) by poisoned food technique (Falck, 1907) [15]. Double strength MEA medium was prepared by doubling the amount of constituents except distilled water and the medium was sterilized at 1.05 kg/cm^2 pressure for 20 minutes. Simultaneously, equal volume of solution of each of the fungicide was prepared in sterilized distilled water at double concentration so as to get desired concentration of fungicides after mixing the fungicide solutions in the double strength medium. Fungicide solutions were added separately to equal quantities of double strength MEA medium aseptically before pouring in Petri plates. These plates were then inoculated with 4 mm mycelial bit of the seven days old culture of *P. digitatum.* A control treatment was also kept in which only plain sterilized water was added to double strength medium. Each treatment was replicated thrice and the inoculated plates were incubated at 25±1 ºC in BOD incubator. The colony diameter of test pathogen in each treatment was recorded till the control plates were full with diametric growth of the mycelium of the test pathogen. The per cent inhibition in the mycelial growth of test pathogen for each treatment was calculated according to formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I - Per cent inhibition of mycelial growth
C - Linear mycelial growth in control (mm)
T - Linear mycelial growth in treatment (mm)

Result and Discussion

The efficacy of six fungicides viz. difenoconazole 25% EC (Score), hexaconazole 5% EC (Contaf), tebuconazole 25% EC (Folicur), propiconazole 25% EC (Tilt), kresoxim-methyl 50 % EC (Ergon), azoxystrobin 25% EC (Amistar) was tested under *in vitro* conditions against the green mould pathogen (*P. digitatum*) at 50, 100, 250 and 500 ppm concentrations by poisoned food technique (Falck, 1907) [15]. It is evident from the data presented in Table 1. that all the treatments significantly inhibited the mycelial growth of green mould pathogen in comparison to control. Difenoconazole was found most effective and significantly superior among all the treatments with 99.2 per cent average inhibition in mycelial growth of the green mould pathogen followed by hexaconazole with 97.2 per cent inhibition. Propiconazole and azoxystrobin reduced the growth of the fungus by 94.1 and 67.3 per cent, respectively. However, kresoxim-methyl was found least effective among all the treatments with 60.2 per cent average inhibition in mycelial growth of the pathogen. It was also noticed that as the concentration of the fungicides increased, there was corresponding increase in per cent mycelial inhibition of the pathogen (Fig.1**)**. Effectiveness of difenoconazole, azoxystrobin, hexaconazole, tebuconazole, propiconazole and kresoxim-methyl against *P. digitaum* and other post-harvest pathogens has been reported by different workers under in vitro conditions. Difenoconazole has been reported to be highly effective in controlling the green mould rot pathogen (Cunningham, 2005*;* Taverner, 2010) [16, 17]**.** Abdelmalek and Salaheldin (2016) [18] observed 94.5 per cent mycelial growth inhibition against green mould pathogen (*P. digitatum*) at 150 ppm concentration of difenoconazole. The triazoles are very specific in their mode of action because they inhibit the biosynthesis of sterol, a critical component for the integrity of fungal cell membranes (Fishel, 2005) [19]. New generation and safe fungicides from strobilurin group like azoxystrobin have been reported to be effective for the control of fruit rotting caused by *Penicillium* spp. (McKay *et al*., 2007) [20]. Kanetis *et al*. (2008) [21] also reported higher effectiveness of azoxystrobin and difenoconazole against *Penicillium* spp. under *in vitro* conditions. McKay *et al*., (2012) [22] also observed efficacy of propiconazole against mycelial growth of *P. digitatum*. Difenoconazole was found to be the most effective with 99.5 per cent average mycelial inhibition followed by hexaconazole against green mould pathogen (Chand, 2013) [23].

Table 1: *In vitro* efficacy of fungicides against green mould pathogen (*P. digitatum*)

Fungicide	Per cent inhibition in mycelial growth at different concentrations (ppm)				
	50	100	250	500	Mean
Difenoconazole	97.07 (80.15)	100.00 (89.39)	100.00 (89.39)	100.00 (89.39)	99.26 (87.08)^a
Tebuconazole	80.00 (63.42)	92.57 (74.22)	100.00 (89.39)	100.00 (89.39)	93.14 (79.10)^b
Propiconazole	83.30 (65.86)	93.30 (75.00)	100.00 (89.39)	100.00 (89.39)	94.15 (79.91)^b
Kresoxim-methyl	48.53 (44.14)	52.20 (46.24)	62.57 (52.26)	73.13 (58.78)	59.11 (50.36)^d
Azoxystrobin	57.80 (49.47)	62.57 (52.26)	67.80 (55.41)	81.10 (64.25)	67.32 (55.35)^c
Hexaconazole	91.10 (72.61)	97.80 (81.44)	100.00 (89.39)	100.00 (89.39)	97.22 (83.21)^a
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	65.40 (53.66)	71.20 (59.79)	75.76 (66.46)	79.17 (68.66)	

* Figures in parentheses are arc sine transformed values
**Figures denoted by same letter do not differ significantly

$CD_{(0.05)}$		
Fungicide	:	0.51
Concentration	:	0.67
Fungicide x Concentration	:	1.35

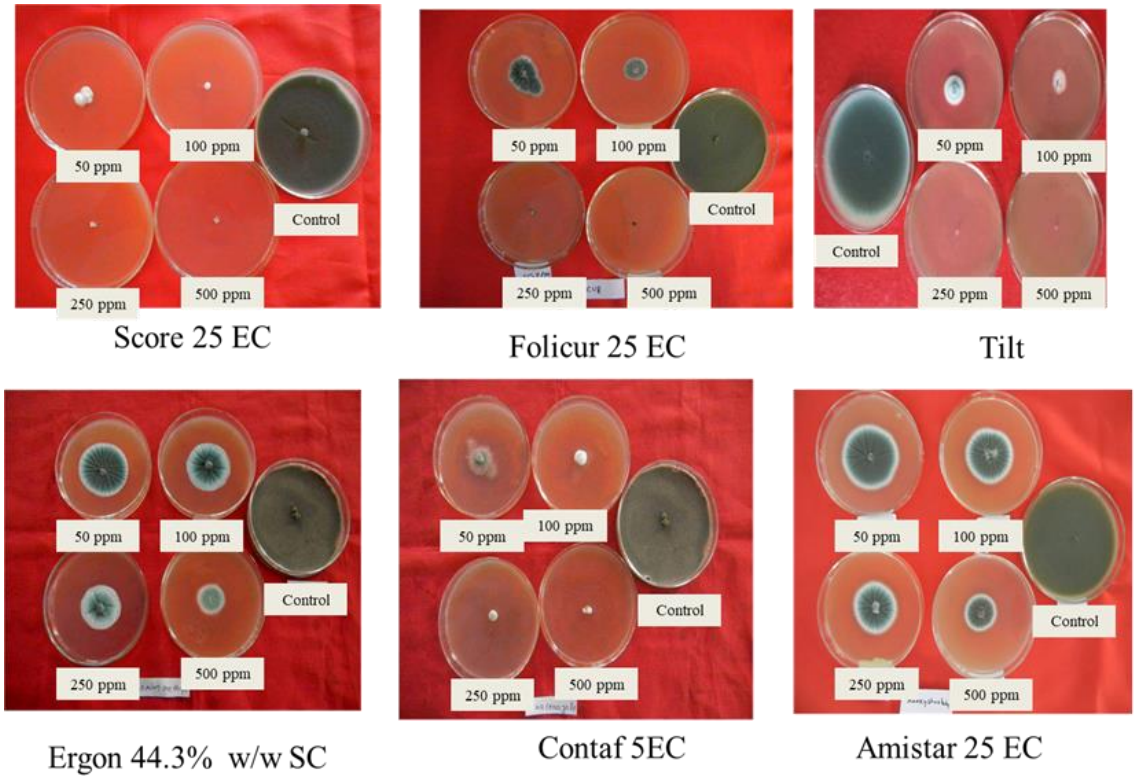

Fig 1: *In vitro* evaluation of fungicides against green mould rot pathogen (*P. digitatum*)

Conclusion

Among different commercially available fungicides evaluated under in vitro at various concentrations, difenoconazole, hexaconazole and propiconazole exhibited its highest ability even in low concentrations against the mycelial growth of green mould pathogen in comparison to control. Therefore, aforesaid evaluated fungicides can be used as potential source of less expensive and more efficient source of different crop protection management strategies for numerous post-harvest diseases.

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