Certain aspects of management for Green mould rot (*Penicillium digitatum*) in Kachai lemon (*Citrus jambhiri* Lush.) in Ukhrul district, Manipur

S.NIMYAOLA, M.SHYAMKESHO SINGH AND N.IRABANTA SINGH

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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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Certain aspects of management for Green mould rot (*Penicillium digitatum*) in Kachai lemon (*Citrus jambhiri* Lush.) in Ukhrul district, Manipur

S. NIMYAOLA*, M. SHYAMKESHO SINGH AND N. IRABANTA SINGH[1]
Centre of Advanced Study in Life Sciences, Manipur University, Canchipur 795003, Imphal, Manipur

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Present investigation deals with development of suitable management practises for post-harvest disease of Kachai lemon caused by *Penicillium digitatum*. *In vitro* studies to assess the antifungal potentiality of 10 plant extracts were carried out. Among the phytoextracts, *Azaderachta indica* was found to have the highest inhibitory effect (69.27%, 71.22%, 72.62% and 73.46% at 5%, 10%, 15% and 20% concentration) on the mycelial growth of test fungus followed by *Zingiber officinale* and *Justicia adhatoda.* Thus, plant products could be successfully used as an effective alternative component of integrated pest management (IPM) due to their economic viability and eco-friendly nature.

Key words: Green mould rot, *Penicillium digitatum*, management, phytoextracts

INTRODUCTION

Citrus being a fruit crop of international importance is grown commercially in almost every country in different agro-climatic conditions . However, due to their higher water content and nutrient composition, citrus fruit is very susceptible to infection by microbial pathogens during the period between harvest and consumption .It is attacked by a number of fungal, bacterial and viral diseases. Among these, fungal diseases are of great importance since it causes huge economic losses up to 10 to 30 per cent . Out of various fungal diseases, Green mould rot caused by *Penicillium digitatum* (Pers.) Sacc. make severe post-harvest losses .*Penicillium digitatum* alone causes post harvest losses in major storage up to 26.2% in sweet oranges and 20.4% in acid lime (Reddy *et al*. 2008).

Thus losses due to this disease are ever increasing starting from fruit harvest stage till reaching to consumers through middle-men in the terminal markets resulting sharp acceleration of prices in the market by lowering the availability of fruits in the market. Keeping in view the destructive nature of the pathogen and extent of losses caused by this disease, the present study has been undertaken to develop an effective and economical disease management.

MATERIALS AND METHODS

Lemon fruits showing characteristics Green mould rot symptoms were collected during field survey for isolation of the associated pathogens. The bits of fruit surface from the margin of healthy and diseased area were taken, surface sterilized with 0.1% mercuric chloride for 30 seconds and repeatedly washed with sterilized distilled water. The bits were then placed on the sterilized filter paper to remove the excess moisture and were subsequently transfer to sterilize Petriplate containing PDA medium supplemented with streptomycin to avoid bacterial contamination. Spores of the test pathogen from the green area on infected fruits were also picked up gently with the help of sterilized inoculating needle and were streaked on PDA medium. The inoculated plates were incubated at 28 ± 1^0C in BOD incubator for 3-4 days and were examined for mycelial growth. Further, the pathogen was identified on the basis of morphological and cultural characters with the help of available standard literature.

* Corresponding author: nimyaos@gmail.com
[1]irabanta.singh@gmail.com

Etiology

Healthy fresh fruits of Kachai lemon were washed with tap water and then air dried. Surface sterilization was done by immersing to 70% ethanol for 1 minute and then inoculated with pure culture of test pathogen by pin prick method . The inoculated fruits were packed in plastic bag and incubated at ambient room temperature (25^{0}C) with >85%RH for 7-12 days. The non-inoculated fruits treated with sterile agar disc served as a control. Observation with regards to development of different stages of pathogen as well as disease was recorded. The causal pathogens were re-isolated from the infected culture fruit and compared with the original isolates.

In vitro evaluation of fungicides

The efficacy of four fungicides viz. Carbendazim 50% W.P, Thiophenate Methyl 70 % W.P, Captan 50% W.P, and Mancozeb 75% W.P were evaluated under laboratory condition at different concentrations viz., 50 ppm, 75 ppm and100 ppm respectively by using poisoned food technique. Each treatment was replicated thrice and the medium without fungicide served as control. The Petriplates were inoculated aseptically with colony bits (9mm) removed from 7 days old pure culture of test fungi and were incubated at 28 ± 1^{0}C in BOD incubators till the mycelial growth in the control reaches a maximum growth (120 hrs). The diameters of the colonies were measured after every twenty four hours and average values compared with control were taken as a measure of fungal toxicity. Growth inhibition (%) of test pathogen in treated plates was calculated by following the formula.

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

where,
PI= Per cent inhibition of mycelial growth
C= Radial growth of pathogen in control plates (cm)
T= Radial growth of pathogen in treatment plates (cm)

In vitro evaluation of phytoextracts effect on the growth of pathogen

Ten plants viz. *Centella asiatica, Tajeta petula, Allium tuberosum, Justicia adhatoda, Azaderachta indica, Ageratum conyzoide,Solanum anguivii, Zingiber officinale, Curcuma longa* and *Mentha spicata* locally known for their medicinal values for treatment of common human diseases were selected to determine the antifungal activity against *Penicillium digitatum*. Fresh plant parts of 25g from each plant were washed thoroughly 2-3 times with tap water and then again with sterilized distilled water. The surface sterilization was done finally with 90% ethanol. The plant materials were crushed in 100ml distilled water. The macerate was filtered through double layer cheese cloth and centrifuge at 3500 rpm for 20 minutes. The supernatant was filtered through Whatman no.1 filter paper. Extract (75%) thus obtained formed the standard plant extract solution. The plant extract so prepared were screened *in vitro* against *P.digitatum* using poisoned food technique (Nene and Thapliyal,1979) at four different concentrations viz. 5%, 10%, 15%, 20%. Require amount of stock solution of each plant extract was mixed thoroughly in molten PDA to get desired concentration just before pouring in sterilized petriplates. Each plate was inoculated with 9 mm disc of mycelia bit taken from 7 days old culture of *P.digitatum* and incubated at 28 ± 1 ^{0}C for 5 days.. Each treatment was replicated thrice and the medium without plant extract served as control. The per cent inhibition in the mycelia growth of the pathogen for each treatment was calculated.

Statiscal analysis

Data were statistically analysed following the method of variance. ANOVA was performed on the data and least significant difference (LSD) at 5 % level was calculated to determine significant differences between treatments.

RESULTS AND DISCUSSION

In vitro efficacy of fungicide

It is evident from the data that all the fungicides significantly inhibited the mycelia growth of the green mould rot pathogen in comparison to control. Carbendazim and Thiophenate methyl was found to be the most effective and significantly superior among all the treatment with cent per cent average mycelial inhibition which is followed by Mancozeb (97.76%) and Captan (96.36%).It was also observed that as the concentration of the fungicide increased, there was a corresponding increase in

percentage mycelial inhibition of the pathogen (Table 1, Fig. 1).

In vitro efficacy of plant extracts

Result from the data (Table 2) indicated that all tested plant extracts of *Centella asiatica, Tajeta petula, Allium tuberosum, Justicia adhatoda, Azadirachta indica, Ageratum conyzoide,Solanum anguivii, Zingiber officinale, Curcuma longa* and *Mentha spicata* showed a significant reduction in the linear mycelial growth of *P.digitatum* in comparison to control. This reduction was gradually increased by increasing the concentration of extracts in the growth medium. Extracts of *Azaderachta indica* was found to be most effective and significantly superior among all other phytoextracts with 69.27%, 71.22%, 72.62% and 73.46% mycelial inhibition at 5%,10%,15% and 20% concentration which is followed by *Zingiber officinale* (42.73%,60.47%, 68.85%,72.62%) and *Justicia adhatoda* (56.7%,60.89%, 64.24%, 69.27%) (Table 2, Fig.2).

Table 1: *In vitro* evaluation of effects of different fungicides on the linear mycelium growth of *Penicillium digitatum*

Fungicides	Radial growth diameter (mm) at 120 hrs		
	50 ppm	75 ppm	100 ppm
Carbendazim 50% W.P	0±0	0±0	0±0
Thiophenate Methyl 70% W.P	0±0	0±0	0±0
Captan 50% W.P	90.64± 0.12	92.59±0.09	96.36±0.23
Mancozeb 75% W.P	85.61± 0.38	97±0.14	97.76±0.15
Mean ± SE	0.11	0.17	0.095
C.D (p = 0.05)	0.23	0.34	0.19

Values are the mean of three replicates and ± S.E; Significant, p= 0.05

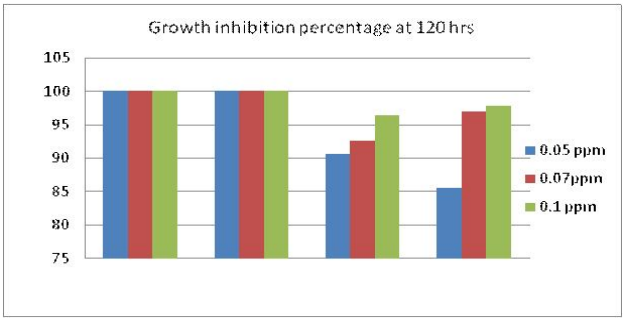

Fig.1: *In vitro* evaluation on the growth inhibition (%) of *Penicillium digitatum* by four commercial fungicides

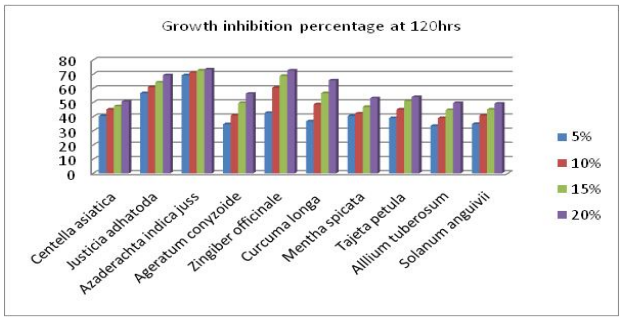

Fig.2: *In vitro* evaluation on the growth inhibition (%) of *Penicillium digitatum* by ten phytoextracts

Table 2: *In vitro* evaluation on effect of different phytoextracts on the linear mycelial growth of *Penicillium digitatum*

Phytoextracts	Radial growth diameter (mm) at 120 hrs				
	Control	5%	10%	15%	20%
Centella asiatica	71.6	40.5±0.20	45.11±0.15	47.48±0.05	50.69±0.25
Justicia adhatoda		56.7±0.1	60.89±0.17	64.24±0.15	69.27±0.1
Azaderachta indica		69.27±0.1	71.22±0.11	72.62±0.05	73.46±0.1
Ageratum conyzoide		34.77±0.15	40.92±0.05	49.72±0.1	56.28±0.05
Zingiber officinale		42.73±0.36	60.47±0.15	68.85±0.25	72.62±0.20
Curcuma longa		36.73±0.15	48.74±0.25	56.70±0.15	65.64±0.05
Mentha spicata		40.5±0.20	42.31±0.20	46.92±0.41	53.07±0.30
Tajeta petula		39.1±0.20	45.11±0.44	51.11±0.24	53.91±0.20
Allium tuberosum		33.51±0.19	39.1±0.278	44.69±0.12	49.72±0.3
Solanum anguivii		34.91±0.17	40.92±0.09	45.11±0.09	49.3±0.11
Mean ± SE		0.34	0.38	0.30	0.30
C.D (p=0.05)		0.16	0.19	0.15	0.15

Values are the mean of three replicates and ± S.E; Significant, ≤ 0.05

The result of the current research was to study the effect of fungicides and plant extracts on the mycelium growth of *P.digitatum* that is pathogen

for the post- harvest disease of citrus. Evaluation of four fungicides under *in vitro* condition against the test pathogen indicated that Carbendazim and Thiophenate methyl were most effective and significantly superior over all the treatment showing cent per cent inhibition on mycelial growth of *P.digitatum* followed by Mancozeb (97.76 %) and Captan (96.36) the least effective. These results are in agreement with the finding of previous workers who have also reported triazole and benzimidazole (carbendazim, thiophanate M, thiabendazole, benomyl) to be highly effective in controlling green mould rot. The higher effectiveness of azoxystrobin, carbendazim and difenoconazole against *Penicillium* spp. under *in vitro* conditions was also reported by Kanetis *et al*. (2008).

Evaluation of different botanical extracts under *in vitro* conditions indicated that *Azaderachta indica* was found to be most effective with 73.46 % average inhibition of pathogen at 20% concentration followed by *Zingiber officinale* (72.62%) *Justicia adhatoda* (69.27) *Curcuma longa* (65.64%) *Ageratum conyzoide* (56.28%) *Tajeta petula* (53.91%) *Mentha spicata* (53.07%) *Centella asiatica* (50.69%) *Allium tuberosum* (49.72%), *Solanum anguivii* (49.3%) per cent inhibition. Plant extracts have been shown to possess great potential as an alternative to synthetic fungicides . The extracts of neem, aonla and garlic have been reported effective against *Penicillium* sp. (Mossini *et al.* 2009). The leaf extracts of *Ocimum sanctum* and *Azadirachta indica* were effective against post-harvest fruit rot pathogens in grapes, citrus and guava . Investigation on the mechanisms of disease suppression by plant products have suggested that the active principle present in plant extracts may either act on the pathogen directly or induce systemic resistance in host plants resulting in a reduction of the disease development. The present study revealed the application of fungicide was more effective than the application of phytoextracts. However, the disease control by bio control agents reduced problems such as toxic to non target species, lower risk of fungicide resistance and lower environmental negative impact.

For the sustainable management of green mould rot caused by *Penicillium digitatum* in citrus, an integrated approach with minimum application of fungicide as well as use of fungicide with different mode of action is very important for the lower cost of control, lower risk of fungicide resistance and lower environmental negative impact. Further, plant extracts can integrate very well with IPM approaches for the effective and sustained management which in turn paves way for enhancing lemon production in an ecologically sustainable manner.

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