

Efficacy of garlic extract and yeast against *Penicillium digitatum* causing post harvest fruit rot of citrus (*Citrus sinensis* L.)

■ R.P. SHINDE*, B.G. BARHATE AND N.A. MUSMADE

Department of Plant Pathology, Mahatma Phule Krishi Vidyaapeeth, Rahuri, AHMEDNAGAR (M.S.) INDIA

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ABSTRACT

Freshly prepared 1 per cent garlic extract and 5 per cent yeast solution were tested against *Penicillium digitatum* causing fruit rot (green mold) of sweet orange both under *in vitro* and *in vivo*. 1 per cent garlic extract gives 15.7 per cent control and 5 per cent yeast gives 15 per cent control against *Penicillium digitatum*. Artificially inoculated fruits treated with 1 per cent G. E. were observed in good condition upto 35 days and 5 per cent yeast treated citrus fruits upto 45 days. Use of garlic extract and yeast maintains the quality of citrus fruits, prolonged the shelf-life and delayed the rottenness presence on fruits. *Penicillium digitatum* could grow between temperature ranges of 20°C to 45°C, however, optimum temperature was $27 \pm 1^\circ\text{C}$ at which growth and sporulation of the fungus was maximum.

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*Corresponding author:

INTRODUCTION

Citrus (*Citrus sinensis* L.) belongs to family 'Rutaceae' an of hesperidium type of fruit. It occupies an area of 0.84 million ha and production being 7.46 million MT (Anonymous, 2011). Fruit rot caused by *Penicillium digitatum* is a serious problem in sweet orange causing economic loss to the crop by reducing fruit quality. Many fungicides such as thiophenate methyl, carbendazim etc. are being currently used to control these diseases but the chemicals have several adverse effects such as development of resistant strains and contamination of food etc. Taking in the consideration necessity of controlling post harvest diseases development of alternative strategies, which are ecologically safe and risk free to human being is

important. Biological control is one of the safest approach for controlling post harvest diseases. In view of post harvest diseases control in major fruit crops, it was thought to undertake use of garlic extract and yeast for study. Garlic (*Allium sativum*) when crushed, yields allicin, an antibiotic and antifungal compound (Phytocide). Yeast (single celled fungi) abundantly found in soil as well as plant phyllosphere also have antagonistic properties against some post harvest fungal plant pathogens (Droby and Hofstein, 1993).

MATERIAL AND METHODS

***In vitro* efficacy of garlic extracts and yeast post harvest pathogens :**

Garlic extracts prepared fresh cloves were

collected and washed. Then 100g of fresh sample was crushed in grinder in 100 ml sterile distilled water (1:1 w/v). Then extract was filtered through double layers of Muslim cloth. Thereafter extract solution was filtered through GG bacterial filter and solution thus, obtained was used as stock solution (Kota *et al.*, 2006). In order to study the effect of garlic extract under *in vitro* conditions, extract of 1 per cent concentration was prepared. The seven day old cultures of each isolate of pathogen having good growth were used separately for preparation of spore suspension in sterile water. Garlic extract was evaluated using paper disc method. 250 ml PDA media was prepared in 500 ml Borocil flasks autoclaved and cooled to 50°C spore suspension was added to each flask containing warm media and was quickly poured into sterile Petri-plates. The pathogen spore seeded plates were allowed to solidify. Sterilized paper disc (5mm dia) cut from Whatman filter sterilized aqueous garlic extract of 1per cent concentration. The discs loaded with aqueous extract were kept on to the surface of the Petri-plates containing spore seeded PDA medium. The paper disc loaded with sterile distilled water served as respective check. Each treatment was replicated thrice. The plates were incubated at 28±2°C for 7 days.

5 per cent yeast suspension (O.D 0.64) was prepared in a sterile test tube containing 5 ml sterile distilled water 48 hrs old yeast culture grown on yeast extract peptone agar medium with good shaking in order to get clear suspension and the procedure was carried out in laminar air flow. The optical density was adjusted to 0.64 OD with the help of spectrophotometer. The yeast suspension was centrifused at 10,000 rpm for 15 minutes. Same method *i.e.* paper disc method is used for evaluation of yeast strain against post harvest fungal pathogens and inhibition zone around the paper disc was measured with millimeter scale. Per cent inhibition was calculated by using formula $C-T/C \times 100$.

***In vivo* effects of garlic extract against post harvest pathogens :**

To study the antagonistic effect, experiments were laid out in *in vivo* conditions by wound inoculation methods. Small wounds (3mm wide x 5 mm deep) were made by pinching sterile paper pins on to healthy citrus fruits, washed in 1:1000 sodium hypo chloride for one minute followed by rinsing twice in sterile distilled water.

The spore suspension of pathogens was prepared from 7 days old culture on PDA media by flooding with sterile distilled water on the slants. Inoculation was made by dipping the wounded fruits in spore suspension of pathogenic fungi. After drying for 1 hour, fruits were sprayed with 1 per cent garlic extract solution and kept in moist chamber. Checks were also maintained by inoculating spore suspension of post harvest pathogens on citrus fruits.

The wounded citrus fruits were inoculated by dipping in spore suspension of pathogenic fungi. After drying for 1hr fruits were sprayed with yeast suspension having optical density 0.64 and kept in moist chamber *i.e.* desiccators. Check was also maintained by inoculating spore suspension of post harvest pathogens on healthy citrus fruits. The fruits were observed for expression of symptoms.

Effect of temperature on growth and sporulation of post harvest pathogens :

The study was conducted on potato dextrose Ager medium. Three replications were taken for each treatment. The plates were inoculated at centre with different fungal post harvest plant pathogens disc of 5 mm diameter and incubated at 0, 5, 20, 27, 35, 45°C and room temperature. Colony diameter was recorded by averaging of each plate of each culture observations on growth were recorded for each fungus.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads and Tables 1 to 3.

***In vitro* efficacy of garlic extract and yeast :**

The efficacy of 1 per cent garlic extract was evaluated against *Penicillium digitatum* post harvest pathogen associated with citrus fruits. According to observations recorded by inhibition zone method 1 per cent garlic extract gives 15.7 per cent control against *Penicillium digitatum*. The results obtained are in confirmation with results obtained by Choudhury and Rahin (2009). The efficacy of 5 per cent yeast solution was evaluated *in vitro* against *Penicillium digitatum* post harvest pathogen associated with citrus fruits. 5 per cent yeast gives 15 per cent control against *Penicillium digitatum*. The results obtained by using 5 per cent yeast

Table 1 : In vitro efficacy of garlic extract and yeast on *Penicillium digitatum*

Sr. No.	Biocontrol agents	Post harvest pathogen	<i>Penicillium digitatum</i>	
			Inhibition zone (mm)	% inhibition over control
1.	G. E. (1%)		14.3	15.7 %
2.	Yeast (5%)		13.0	15.0 %
3.	Control		00.0	00.0

G. E.= Garlic extract

Table 2 : In vivo efficacy of garlic extract and yeast on post harvest pathogens of citrus

Sr. No.	Treatments	<i>Penicillium digitatum</i>					
		7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	45 DAT
1.	G.E. (1%)	+	+	+	+	+	-
2.	Yeast (5%)	+	+	+	+	+	+
3.	Control	-	-	-	-	-	-

where, + = No infection - = Infection

Table 3 : Effect of temperature on growth and sporulation of *Penicillium digitatum*

Sr. No.	Temperature (°C)	<i>Penicillium digitatum</i>	
		MCD (mm)	Sporulation
1.	0	0.00	-
2.	5	0.00	-
3.	20	77.16	++
4.	27 ± 1	90.00	++++
5.	35	90.00	+++
6.	45	86.20	+++
	S. E. ±	0.106	
	C.D. (P=0.05)	0.327	
	C.V (%)	0.321	

* Average of three replications.

* MCD = Mean colony diameter in mm.

- = No sporulation, + = Poor sporulation, ++ = Moderate sporulation, +++ = Good sporulation,

++++ = Abundant sporulation

are in confirmation with results obtained by Zhang *et al.* (2010); Chanchaichaovivat *et al.* (2007); Capdeville *et al.* (2006) and Zhulong Chan and Shiping Tian (2005).

In vivo efficacy of garlic extract and yeast :

Fungus *Penicillium digitatum* was isolated from sweet orange and artificially inoculated fruits were treated with 1 per cent G. E. Treated fruits were observed in good condition upto 35 days. Use of garlic extract maintains the quality of citrus fruits, prolonged the shelf-life and delayed the rottness presence on fruits. The results obtained by Maria Amalia Brunini *et al.* (2012); Choudhury and Rahin (2009) and Yadav and Ojaha (1998). The artificially inoculated citrus fruits were treated with 5 per cent yeast. Treated citrus fruits remain healthy and in good condition upto 45 days after inoculation with *Penicillium digitatum*. The results obtained are in consonance with those reported earlier

by workers such as Zhang *et al.* (2010), efficacy of yeast antagonists individually or in combination with hot water and also by Chanchaichaovivat (2010) and Dan *et al.* (2003).

Effect of temperature on growth and sporulation of *P. digitatum* :

The temperature range of 0°C, 5°C, 20°C, 27 ± 1°C, 35°C and 45°C was taken for study of temperature effect on growth and sporulation. *Penicillium digitatum* could grow between temperature ranges of 20°C to 45°C, however, optimum temperature was 27 ± 1°C at which growth and sporulation of the fungus was maximum.

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