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Efficacy of garlic extract and yeast for the control of post harvest diseases of grape

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ABSTRACT

The present investigations were carried out with collection of diseased samples, isolation of the organisms responsible for post harvest spoilage, their pathogenicity, symptomatology, morphological characters and effect of temperature on growth and sporulation in order to suggest suitable control measures in respect of yeast as a bioagent and garlic extract as a botanical in controlling post harvest fungal diseases of grape. The causal organisms were isolated from infected grape berries and recorded association of three fungal pathogens *viz.*, *Aspergillus niger, Alternaria alternata* and *Penicillium digitatum*. The conidial size of *A. niger, A. alternata* and *P. digitatum* are measured as 4.4 µm, 42.47 x 16.37 µm and 4.33 – 4.5 µm in size (average), respectively. Use of 5 per cent yeast, (*Eremothecium cymbalariae*) was found effective against *Aspregillus niger* (both *in vitro* and *in vivo*) and 1 per cent garlic extract was found effective against *Alternaria alternata* (both *in vitro* and *in vivo*).Both yeast and garlic extract increases shelf-life and market quality of fruits, with increase in glossiness of fruits. Temperature 27 $\pm 1^0$C was found optimum for both growth and sporulation of all isolated fungal pathogens.

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INTRODUCTION

Grape (*Vitis vinifera* L.) belongs to family 'vitaceae' is one of the most remunerative horticultural crops in India. Grape berries are reported to be suffering from several post harvest diseases during transit and storage. Post harvest losses are reported upto 35 to 40 per cent with reduction in quality and quantity of marketable fruits. Chemical control is risky due to possibility of residual effect and awareness of people about health, and also results in considerable financial loss to the sellers as well as the consumers. Fungi are the important cause of storage rots, the fungal pathogens affecting the grapes after harvest includes *Aspergillus niger*, *Alternaria alternata*, *Penicillium digitatum* etc.

Among the fruit rots, rots caused by *Alternaria alternata* and *Aspergillus niger* are most common. Taking in the consideration necessity of controlling post

harvest diseases development of alternative strategies, which are ecofriendly 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 grapes, it was undertaken to undertake use of garlic extract and yeast for study. Garlic (*Allium sativum*) when crushed, yields allicin, an antibiotic and antifungal compound (Phytocide). Resultant sulphur compounds are responsible for the sharp or hot taste and antifungal properties of garlic remain for as little as half an hour after garlic is pressed or crushed.

The main antimicrobial effect of allicin is due to its chemical reaction with *thiol* group of various groups of an assortment of enzymes.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 *et al.*, 1993).

MATERIAL AND METHODS

Isolation and identification of pathogens associated with post harvest diseases of grape :

The micro organisms responsible for spoilage of fruits were isolated on PDA medium by tissue isolation method from diseased fruit collected from different markets at Rahuri and Ahmednagar.

Preparation of Garlic extract :

Fresh cloves were collected and washed. Then 100 g of fresh sample was crushed in grinder in 100 ml sterile distilled water (1:1w\v). The 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 vitro* effect of garlic extract against post harvest pathogens :**

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 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 50^0C spore suspension was added to each flask containing warm media and spore suspension was 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 1 per 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 ± 2^0C for 7 days.

***In vitro* effect of 5 per cent yeast suspension :**

5 per cent yeast suspension (0.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 centrifuged 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×100.

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

To study the antagonistic effect, experiments was 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 health fruits, grape bunches, 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 grape berries.

***In vivo* effect of yeast strain against post harvest pathogen :**

The wounded 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 fruits. Grape berries were observed for expression of symptoms.

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

The plates were inoculated at centre with different fungal post harvest plant pathogens disc of 5mm diameter and incubated at 0, 5, 20, 27, 35 and 45^{0}C. 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 4 .

Isolation and identification of pathogens associated with post harvest diseases of grape :

In the present studies, diseased grape samples were collected from market of Rahuri and also from grape orchards, Department of Horticulture, M.P.K.V., Rahuri, Taluka Rahuri, Dist. Ahmednagar (M.S.). Isolation of associated pathogens were studied in detail on PDA medium and it was revealed that the associated fungi were *Aspergillus niger, Alternaria alternata* and *Penicillium digitatum*. These fungi were responsible for spoilage of grapes after harvest either in storage or handling or during transport. The identification of fungi isolated was done at Department of Plant Pathology and Agril. Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Tq. Rahuri, dist. Ahmednagar (M.S.) on the basis of morphology and pathogenicity. The results obtained are in confirmation with the results obtained by Joshi and Vaidya (2007); Verma (2007) and Sangeetha and Rawal (2008) had pointed out association of fungal pathogens like *Penicillium, Aspergillus, Fusarium, Colletotrichum* and *Alternaria* are responsible for spoilage of fruits.

***In vitro* efficacy of 1 per cent garlic extract :**

The efficacy of 1 per cent garlic extract was evaluated against three post harvest pathogens associated with Grape. According to observations recorded by inhibition zone method 1 per cent garlic extract gives 6.7 per cent control against *Aspergillus niger*, 27 per cent control against *Alternaria alternata*, 15.7 per cent control against *Penicillium digitatum*. In present investigation good control was obtained against *Alternaria alternata*. The results obtained are in confirmation with results obtained by Maria Amalia Brunini *et al.* (2011); Mukherjee *et al*. (2011) and Raja

Table 1 : *In vitro* efficacy of garlic extract and yeast on *Aspergillus niger*

Sr. No.	Post harvest pathogen / Biocontrol agents	*Aspergillus niger*	
		Inhibition zone (mm)	% inhibition over control
1.	G. E. (1%)	22.0	25.0 %
2.	Yeast (5%)	36.0	46.0 %
3.	Control	00.0	00.0

Table 2 : *In vitro* efficacy of garlic extract and yeast on *Alternaria alternata*

Sr. No.	Post harvest pathogen / Biocontrol agents	*Alternaria alternata*	
		Inhibition zone (mm)	% inhibition over control
1.	G.E. (1%)	24.3	27.0 %
2.	Yeast (5%)	32.6	36.3 %
3.	Control	00.0	00.0

Table 3 : *In vitro* efficacy of garlic extract and yeast on *Penicillium digitatum*

Sr. No.	Post harvest pathogen / Biocontrol agents	*Penicillium digitatum*	
		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 4 : *In vivo* efficacy of garlic extract and yeast on post harvest pathogens of grapes

Sr. No.	Treatments	*Aspergillus niger*					*Alternaria alternata*					*Penicillium digitatum*				
		2 DAT	4 DAT	8 DAT	10 DAT	12 DAT	2 DAT	4 DAT	8 DAT	10 DAT	12 DAT	2 DAT	4 DAT	8 DAT	10 DAT	12 DAT
1.	G.E. (1%)	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-
2.	Yeast (5%)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

where, + = No infection - = Infection DAT = days after treatment

(2010).

***In vitro* efficacy of 5 per cent yeast suspension :**

The efficacy of 5 per cent yeast suspension was tested *in vitro* against three post harvest pathogens associated with grape. According to observations recorded by inhibition zone method, 5 per cent yeast gives 5.6 per cent control or growth inhibition against *Aspergillus niger*, 36.3 per cent against *Alternaria alternata*, 15 per cent control against *Penicillium digitatum*. It is seen from above results that the good control with 36.3 per cent inhibition was obtained against *Alternaria alternata.* The results obtained by using 5 per cent yeast are in confirmation with results obtained by Senthil *et al*. (2011); Zhang *et al*. (2010) and Chanchaichaovivat *et al*. (2007).

***In vivo* efficacy of 1 per cent garlic extract against isolated post harvest pathogens of grape :**

The three fungal pathogens were isolated from grape berries are *Aspergillus niger, Alternaria alternata* and *Penicillium digitatum*. Grape berries artificially inoculated with these pathogens are treated with 1 per cent garlic extract and observations were taken.

Treated grape bunches are observed in good condition upto 8, 10 and 8 days against *Aspergillus niger, Alternaria alternata* and *Penicillium digitatum*, respectively. Use of garlic extract maintains the quality of fruits, prolonged the shelf-life and delayed the rottness presence on fruits. The result obtained by Maria Amalia Bruniri *et al*. (2012) and Mukherjee *et al*. (2011).

***In vivo* efficacy of 5 per cent yeast solution :**

The artificially inoculated fruits of grape were treated with 5 per cent yeast. Treated grape bunches were observed in good condition upto 12 days against *Aspergillus niger*, 12 days against *Alternaria alternata* and 12 days against *Penicillium digitatum*. The results obtained are in consonance with those reported earlier by workers such as Senthil *et al.* (2011). studied efficacy of biocontrol agents against post harvest pathogens. Optimum temperature was 27 ± 1^0C at which growth and sporulation of the isolated fungi were maximum.

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