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RESEARCH ARTICLE

Effect of chemicals inducing systemic resistance and efficacy of bioagents and botanicals against pomegranate (*Punica granatum* L.) anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.)

■ A. PRASHANTHA[1]*, ARUN R. SATARADDI[2], S.V. PATIL[1], M.S. LOKESH[3], K. CHANDAN[1], AND S.B. GURUMURTHY[1]

[1]College of Horticulture [U.H.S.(B)], Sirsi, UTTARA KANNADA (KARNATAKA) INDIA
[2]College of Agriculture, University of Agricultural Sciences, DHARWAD (KARNATAKA) INDIA
[3]AICRP on Spices Horticulture Research Station [U.H.S.(B.)], Sirsi, UTTARA KANNADA (KARNATAKA) INDIA

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***Corresponding author:**
Email: pachu.prash@gmail.com

ABSTRACT

Pomegranate (*Punica granatum* L.) is a commercially an important fruit of both tropical and subtropical countries and belongs to the family Punicaceae. The fruits are susceptible to various biotic stress caused by fungi, bacteria and physiological disorders. Among the various fungal diseases, anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.) is one of the most serious diseases of pomegranate worldwide. Like many of pathogenic fungi it also remains latent during maturity of the fruit and expresses symptoms during storage. Further, conidia of the *C. gloeosporioides* which are abundant in the atmosphere of tropical plantation may lodge on the surface of the fruits as the fruits approaching maturity. Propagules of the pathogen cause lesions and decay of the fruit. Among the chemicals inducing systemic resistance *viz.*, salicylic acid and benzoic acid were found more effective in reducing the per cent disease index with high total soluble solid, low total titrable acidity and less physiological loss in weight after eight days of storage.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is a commercially an important fruit of both tropical and subtropical countries and belongs to the family Punicaceae. It is a symbol of health, fertility, eternal life and also being valued as medicinal plant to treat diabetes, cancer, hypertension, gastric inflammation, heart and kidney diseases and its bark, pericarp are used to control dysentery and diarrhoea. The rind of fruit contains tannins, which are used as dyeing material for cloth and leather. Pomegranate is gaining more importance in states of Maharashtra and Karnataka due to suitability of soil, hot and dry climate which are necessary for fruit development and ripening. The crop can also withstand low temperature in winter, drought and salt. In the world, India ranks third in position with respect to area and production and is mainly occupied in Karnataka, Maharashtra, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Gujarat, Rajasthan and Haryana. Pomegranate fruits are susceptible to various diseases caused

by fungi, bacteria and physiological disorders. Among the various fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is one of the most serious diseases of pomegranate worldwide. In India, anthracnose disease was first reported by McRae (1924). Conidia of the pathogenic fungus which are abundant in the atmosphere of tropical plantation may lodge on the surface of the fruits as the fruits approaching maturity. Propagules of these pathogens cause lesions and decay of the fruit. Hence, the present investigation was carried out to study the response of chemicals inducing systemic resistance, and efficacy of bioagents and botanicals against the anthracnose of pomegranate.

MATERIAL AND METHODS

The isolation of *C. gloeosporioides* from infected portion of anthracnose of pomegranate was carried out by following standard tissue isolation technique. The pure culture of fungus was obtained by single spore isolation technique, the pathogenecity was proved by pinprick method using detached fruit technique and growth phage.

***In vitro* evaluation of chemicals inducing systemic resistance against *C. gloeosporioides* :**

Chemicals inducing systemic resistance, such as salicylic acid, sodium nitrate, mannitol and benzoic acid were screened against the mycelial growth of *C. gloeosporioides* using poisoned food technique (Shravelle, 1961). The fungus, *C. gloeosporioides* was grown on Potato dextrose agar (PDA) medium for 13 days prior to setting up of experiment. The PDA medium was prepared and molten. The resistance inducing chemicals were added to the molten medium to obtain the desired concentration based on the molecular weight of the chemicals. The 20 ml of the medium was poured in each sterilized Petriplates. Suitable check was maintained without addition of resistance inducing chemicals. Mycelial disc of 5 mm was taken from the periphery of the 8 days old colony and placed in the center of the Petriplate and incubated at 27 ± 1° C for 13 days. Suitable numbers of replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was recorded. The per cent inhibition of growth of fungus was calculated by using the formula given by Vincent (1927).

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

where,
I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

List of chemicals inducing systemic resistance

Sr. No.	Name of the chemical	Chemical formula	Molecular weight (g)
1.	Salicylic acid	$C_6H_3(OH)(COOH).SO_3H.2H_2O$	254.22
2.	Sodium nitrate	$NaNO_3$	84.99
3.	Benzoic acid	C_6H_5COOH	122.12
4.	Mannitol	$C_6H_{14}O_6$	182.17

In vitro* evaluation of bioagents against *C. gloeosporioides:

Bioagents obtained indigenously and from the Project Directorate of Biological Control (PDBC), Hebbal, Bangalore and Tamil Nadu Agricultural University (TNAU), Coimbatore were tested for their efficacy under *in vitro* conditions using dual culture technique. The bio agents and the test fungus were inoculated side by side on a single Petridish containing solidified PDA medium. The 4 replications were maintained for each treatment with one control by maintaining only pathogen and bioagent separately. They were incubated for 13 days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. The per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1927).

List of bioagents used against *C. gloeosporioides* are mentioned below.

Sr. No.	Bioagents	Source
1.	*Trichoderma viride* (I)	Department of Plant Pathology, College of Agriculture, Raichur
2.	*T. harzianum* (I)	Department of Plant Pathology, College of Agriculture, Raichur
3.	*B. subtilis* (PDBC)	PDBC, Bangalore, India
4.	*Pseudomonas fluorescens* (Pfr-1)	TNAU, Coimbatore, India

In vitro* evaluation of botanicals against *C. gloeosporioides:

The present investigation was carried out to evaluate the extracts of different plant species to know the possible presence of fungitoxicant properties against predominant pathogen of pomegranate anthracnose (*C. gloeosporioides*) using poisoned food technique (Shravelle, 1961). Fresh healthy plant parts of 50 g (leaves/root/bulb) collected from field were washed with distilled water and air dried and crushed in 50 ml of sterile water. The crushed product was tied in muslin cloth and collected the filtrate. The prepared solution gave 100 per cent, which was further diluted to required concentrations of 5.0, 7.5 and 10.0 per cent. The extracts were tested against *C. gloeosporioides* on the media using poisoned food technique under *in vitro* condition as described earlier for evaluation of fungicides against *C. gloeosporioides*.

List of plant extracts used for study *in vitro* condition.

Sr. No.	Plants (Common name)	Botanical name	Plant part used
1.	Neem	*Azadirachta indica*	Leaf extract
2.	*Datura*	*Datura stramonium*	Leaf extract
3.	Eucalyptus	*Eucalyptus* sp.	Leaf extract
4.	Garlic	*Allium sativum*	Bulb extract
5.	Tulsi	*Ocimum sanctum*	Leaf extract
6.	Onion	*Allium cepa*	Bulb extract
7.	Coat buttons	*Tridax procumbens*	Leaf extract

Effect of chemicals in inducing systemic resistance and evaluation of bioagents and botanicals against pomegranate anthracnose on detached fruit technique :

Two best treatments were screened *in vitro* evaluation of induced systemic resistance chemicals, plant extract and bioagents which were selected with respect to mycelial inhibition for to study on effect of chemicals in inducing systemic resistance and efficacy of bioagents and botanicals against pomegranate anthracnose on detached fruit technique. The efficacy of different treatments were cultural filtrates of *Pseudomonas fluorescens*, *Trichoderma viride* and leaf extracts (10 %) of eucalyptus and garlic. Resistance inducing chemicals of salicylic acid and benzoic acid (1 %) were studied under this experiment. The fruits of pomegranate were soaked in above mentioned treatments for 4 hrs followed by 30 minute shade drying and the fruits were inoculated with spore suspension (1 x 10^6 spores/ ml) of *C. gloeosporioides*. The fruits treated with distilled water along with fungus inoculation and distilled water alone, were maintained for comparison. The fruits were kept in humid chambers for incubation up to eight days with appropriate number of replication in each treatment (6 fruits/replication). After eight days, per cent diseases index was calculated using 0 to 5 scale by the formula (Wheeler, 1969), physiological loss, total soluble solids and total titrable acidity were also recorded.

RESULTS AND DISCUSSION

The management of disease through host plant resistance has been an optimum choice in all crop improvement programmes. The present investigation was undertaken by utilizing induced systemic resistance in the host by treating the fruit with systemic resistance inducing chemicals and efficacy of bioagents and botanicals using detached fruit technique. Among them the fruit treated with salicylic acid and benzoic acid recorded less PDI (40.20 and 46.67), higher TSS and less physiological loss as compared to other treatments at 8 days after storage. It was followed by fruit treatment with *P. fluorescens* which recorded less PDI at 8 days after storage with minimum physiological loss in weight as compared to control. It may be due to the development of antifungal compounds in the host plant. It is attributed in induction of phenols, which reduces the development / incidence of anthracnose in pomegranate. Mosa (2002) studied various biotic and abiotic resistance inducing agents in rice against blast disease. Among the inducing agents tested, abiotic agents like salicylic acid and BTH (benzo 1, 2, 3) thiabendazole-7 carbothioic acid s-methyl ester) were found to be effective. Among biotic agents, *Pseudomonas fluorescens* strain Pf5 seed treatment resulted in reduction of disease severity by the induction of systemic resistance. Zainuri *et al.* (2001) studied the pre or post-harvest dip with two-host defense promoting compounds (salicylic acid and potassium phosphonate) for suppression of anthracnose of mango without affecting their firmness and colour of the fruit.

Table 1 : Effect of chemicals inducing systemic resistance, botanicals and bioagents against pomegranate anthracnose by using detached fruit technique.

Sr. No.	Treatments	Concentration	Per cent diseases index		TSS (%)	Physiological loss weight (g)
			4 days	8 days		
1.	Control	–	41.12 (39.90)**	76.67 (61.12)	15.06	42.33
2.	Distilled water + fungus	–	43.56 (41.28)	86.67 (68.59)	14.46	63.33
3.	Salicylic acid	1%	20.89 (27.15)	40.20 (39.35)	17.10	13.00
4.	Benzoic acid	1%	23.56 (29.04)	46.67 (43.09)	16.56	19.66
5.	*P. fluorescens*	1%	30.45 (33.47)	53.45 (46.97)	15.90	29.66
6.	*T. viridae*	2×10^8 spores/ml	33.45 (35.34)	70.12 (56.89)	15.06	23.00
7.	Eucalyptus extract	10%	35.60 (36.63)	73.45 (58.99)	15.43	27.00
8.	Garlic extract	10%	26.56 (31.04)	50.12 (45.09)	15.30	30.00
S.Em±			0.267	0.068	0.239	0.706
C.D.at 1%			1.126	0.287	1.007	2.971

**Arcsine transformed values

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