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Efficacy of Various Fungicides Against Grey mold of Pomegranate (*Punica granatum* L.)

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Abstract: The pomegranate (Punica granatum L.), is one of the ancient and highly praised favorite fruit belong family *Lythraceae* of *Myrtales* order which is mainly grown in tropical and subtropical regions of the world. Pomegranate is susceptible to many diseases and fruit production is the essential issue in the whole world. Due to the development of resistance towards fungicides in different pathogens, in the present study fungicides were evaluated for their potential against *Botrytis cinerea*. The samples showing typical symptoms were collected from selected markets of Faisalabad and brought into the Seed Pathology Laboratory for isolation, purification and identification of different pathogens associated with sample of pomegranate. Four fungicides including, tebuconazole, fludioxonil, propiconazole and carbendazim were evaluated at S, S/2 and S/4 concentrations after 5 and 7 days for inhibition of fungal growth under lab conditions. Among fungicides Fludioxonil gave highest reduction (78.44%) in fungal growth at standard (S) concentration after 7 days of inoculation under lab conditions. The experiment was conducted under Complete Randomized Design (CRD) and data was statistically analyzed using Least Significant Difference (LSD) test.
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Key words: pomegranate (Punica granatum L.), fungicides, Grey mold

Introduction

A wide range of distribution of pomegranate in various agro-climatic conditions of the world may be due to its adaptability and genetic diversity (Holland *et al*., 2009; Khan et al., 2021). Mediterranean climate possesses the optimal conditions for growth of pomegranates including high exposure to the not sever winters and lower temperature and it should not be lower than that of -12°C (Levin, 2006). These conditions lead to the development of best growth of pomegranate with aesthetic colour and juice content without splitting of the fruits. Today it is cultivated in the different climatic zones in whole world including tropical and subtropical areas. Pomegranate tissues of leaves, bark, flower and fruit contain phytochemicals that control blood pressure, act as antimicrobial and aging agents, act against chronic diseases like diabetes, male infertility and cancer (Holland *et al*., 2009). There has been impressive improvement of pomegranate genotypes with growing techniques and extension in pomegranate orchards, especially in the Western world because of public demand for pomegranate fruit and juice for good health. Mainly, pomegranate fruit is consumed as fresh fruit, but it is also used to prepare fresh juices, canned beverages, jellies and jams, and their consumption is increasing in the world (Vardin and Fenercioglu, 2003). Pomegranate is susceptible to many diseases and fruit production is the essential issue in the whole world (Bardas *et al*., 2009). Many fungal pathogens attacked on pomegranate, but the most important plant pathogenic fungi include gray mold (*Botrytis cineria*) rot, black heart (*Aspergillus niger*) rot, green mold (*Penicillium digitatum*) rot and Alternaria (*A. alternata*) rot and blue mold (*P. expansum*) rot (Adaskaveg, 2012).

B. cinerea forms appressoria during penetration, albeit not the highly organized appressoria that are typical for many plant pathogenic fungi. Several authors observed the swelling of hyphal tips of germ tubes and interpreted these as an appressorium-like structure (Cole et al., 1996). Recent microscopic and histochemical studies (Tenberge *et al*., 2002) and gene function analysis (Gourgues *et al*., 2003) indicate that these structures act as functional appressoria. The swelling of the hyphal tip may be the consequence of a rise in osmotic value in the hyphal tip, resulting in water absorption. In the absence of a rigid layer in the outer wall, swelling cannot result in an equally high turgor as in the appressoria of Magnaporthe grisea (de Jong *et al*., 1997). The extracellular matrix may contribute to the swelling by retaining water, as its major polysaccharide component (cinerean), is extremely hygroscopic.

Initial infection of postharvest disease due to grey mold takes place during blooming season through flower parts which leads towards dormant infection. At early stages of fruit formation, pathogen

enters through mechanical damage or wounds and openings created by insects. Under suitable conditions characteristic symptoms comprising of discoloration of complete fruit initiating from crown or blossom end and covers the fruit entirely. Several types of symptoms such as rotting, blackening, softening and bruising also appear on fruit surface at lateral stages. Depending on the growing season, it can destroy 30-50% of fruits in cold storage (Teksur *et al*., 2014).

Post-harvest rots of fresh commodities (fruits and vegetables) is mainly caused by fungal infections during storage and transport, which is responsible for causing huge economic losses during commercialization phase (Gatto *et al*., 2011). Due to these post-harvest infections shelf life of fruits is decreased which badly affect their market value. In addition, secondary metabolites produced by molds are harmful for human and animals (Zain, 2011). *Botrytis cinerea*, *Penicillium italicum*, *Penicillium digitatum* are most common postharvest pathogens causing grey mold, blue mold, green mold respectively in fruits and vegetables (Gatto *et al*., 2011).

It is difficult to manage *B. cinerea* infection because of its wide host range, diverse mode of action and its ability to survive in unfavorable conditions as sexual and asexual spores. So, for control of *B. cinerea* is based on application of synthetic fungicides which counts about 8 percent global fungicides market and annual expenditures of *B. cinerea* control exceeds from 1 billion in world (Dean *et al*., 2012). Various classes of fungicides including fludioxoul, tebuconazole, iprodione and boscalid have been mostly used for the management of grey mold. Improper use of fungicides and high genetic diversity of *B. cinerea* strains resulted in development of resistance to these chemicals (Khan et al., 2020). For example, benzimidazole fungicide were found most effective in late 1960s but after few years of application strains resistant to these fungicides were evolved which made these less effective. The application of synthetic fungicides remains the key component of disease control. Thus, it is dire need of time to use appropriate fungicides and evaluate the potential of currently used chemicals. In these four different chemical fungicides were evaluated against *B. cinerea*. Primarily synthetic fungicides are the key component of post-harvest disease management. However, it is need of time to develop alternative, effective, safe and economically suitable methods to manage post-harvest grey mold (Zafar et al., 2020).

Keeping in view all above factors present study is designed to use of essential oils against grey mold disease of pomegranate fruit. Study was carried out under following objectives

- To evaluate the different fungicides to control postharvest grey mould problem

MATERIALS AND METHODS

Survey and Sampling

A survey was carried out in different markets and shops of district Faisalabad, Pakistan in (December- February) 2019. Pomegranate fruits having the symptoms of rots and discoloration were collected randomly. Infected fruits were separated from healthy once and were kept in polythene bags. All the samples were labelled properly and were brought into the Seed Pathology Lab, Department of Plant Pathology, University of Agriculture Faisalabad for further studies. Percentage of disease incidence (PDI) was calculated by applying the following formula (Abdulsalam *et al*., 2015).

$$\text{Disease incidence}(\%) = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

Sterilization of glassware and preparation of media

All the glass wares comprising of Petri plates, conical flasks, test tubes etc that were used in laboratory experiments were initially washed with detergent powder by using tap water and were air dried. After drying these were wrapped in clean paper and sterilized in autoclaved.

Potato dextrose agar medium (PDA) was used for isolation and culturing the pathogen. The ingredients of PDA medium were as follows

Potato starch	20 g
Glucose	20 g
Agar	20 g
Water	1000 ml

Isolation of pathogen

Infected fruits showing the symptoms of rotting were selected for isolation of fungal pathogen. Small infected portions of pomegranate fruits were cut into small pieces (3-5 mm) and were sterilized with the help of sodium hypochlorite (NaOCl). Samples were then rinsed by using sterilized distilled water and placed on sterilized filter papers. Sterilized diseased samples were brought into laminar flow chamber for inoculation. Samples were than inoculated into petri plates containing PDA medium with the help of sterilized forceps. Pates were wrapped and placed in incubator at 24^0C which examined regularly for growth of fungus.

Purification and identification of fungus

Samples were purified by transferring the hyphal tips to separate plates containing PDA medium. Plates were then incubated at 25^0C and growth of fungus was observed regularly. Identification of fungi was made according to cultural and morphological characteristics using related literature (Gilman, 1957). Fungal cultures were stored by inoculating on agar slants and were maintained at 4^0C for further studies.

Evaluation of fungicides against *B. cinerea*

In vitro efficacy of four different fungicides was assessed against *B. cinerea*, isolated from pomegranate fruits. All these fungicides were collected from market of Faisalabad. Three concentrations (S, S/2 and S/4) of each fungicide were made in PDA medium and were evaluated against pathogenic fungi. Twenty ml of media was poured to each petri plate and was allowed to solidify. In control plates only water was added instead of fungicides in PDA medium. Twenty ml of media was added instead of fungicides in PDA medium. Then these plates was inoculated in the centre of plate with mycelial agar disc, radial growth of pathogen was calculated according to different morphological characteristic by using formula (Sunder *et al.,*1995).

Percent inhibition zone =X-Y

Statistical analysis

Data was analyzed statistically; ANOVA was used to check the significant result of data while for the mean value LSD (least significance differences) was used to contrast the difference.

Application of fungicides to control Grey mold of pomegranate

ANOVA indicated that all treatments (T), concentrations (C), days (D) and their interaction expressed significant results against *Botrytis cineria*. Maximum growth inhibition percentage was obtained by Fludioxonil (79.56%) followed by Propiconazole (72.8%), Tebuconazole (65.27%) and Carbendazim (56.27%). In interaction between treatments, concentration and days (T x C x D) maximum inhibition was shown by fludioxonil (86%) @ S, 81% @ S/2, 74.66 @ S/3 concentration) after 5 days and 84.66% @ S, 78% @ S/2 and 72.66 @ S/4) after seven days followed by Propiconazole which gave mycelial growth inhibition of 81 % @ S, 71 % @ S/2 and 64.66% @ S/4 concentration) after 5 days and (83.56% @ S, 69% @ S/2 and 67% @ S/3 concentration) after seven days followed by Tebuconazole which gave growth inhibition of 71.33% @ S, 65.33% @ S/2 and 58.66 @ S/3 concentration) after 5 days and (72.66% @ S, 67% @ S/2 amd 56.66% @ S/4 concentration) after seven days and Carbendazem which gave mycelial growth inhibition of (61% @ S, 55% @ S/2 and 48.33% @ S/4 concentration) after 5 days and (64% @ S, 57% @ S/2 aalnd 52.33% @ S/4 concentration) after seven days.

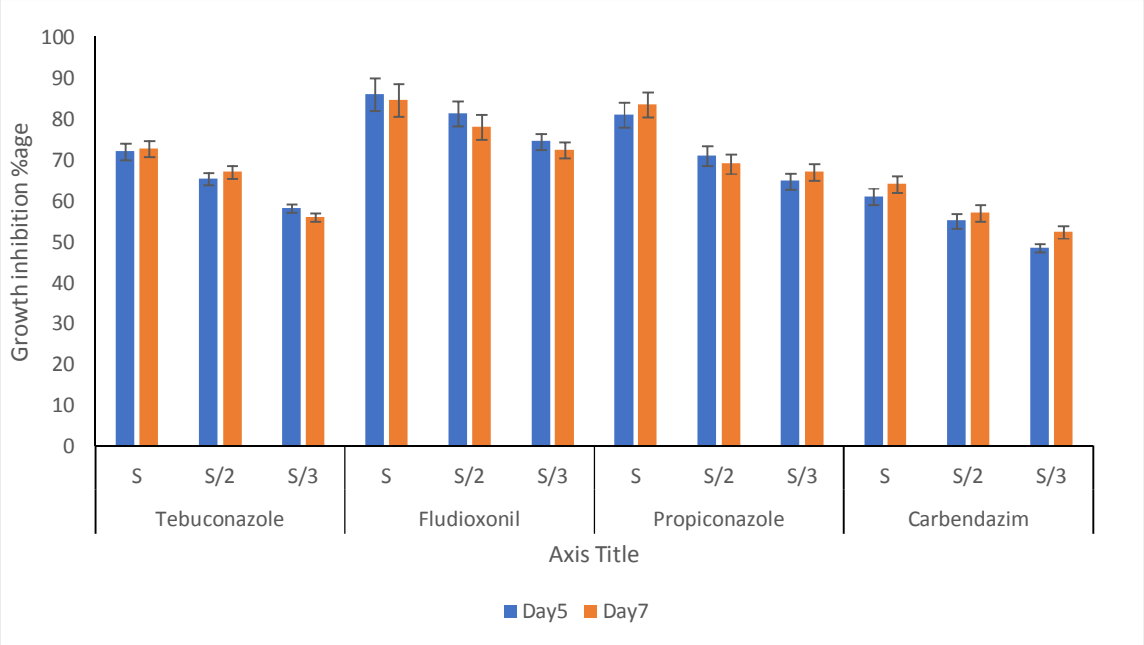

Fig 1.: Growth inhibition % age by application of fungicides at different concentrations

Table 1: ANOVA for Evaluation of different fungicides on growth inhibition % of *Botrytis cineria*

SOV	DF	SS	MS	F	P≥F
Rep	2	5.58	2.79		
Treatment (T)	3	5396.6	1798.87	1920.65	0.0000
Conc. (C)	2	2250.33	1125.17	1201.35	0.0000
Days (D)	1	5.01	5.01	5.35	0.0252
TxC	6	109.78	18.30	19.53	0.0000
TxD	3	62.71	20.9	22.32	0.0000
CxD	2	10.11	5.06	5.40	0.0078
TxCXD	6	28.67	4.78	5.10	0.0004
Error	46	43.08	0.94		
Total	71	7911.87			

Table. 2. *In-vitro* evaluation of different fungicides against*Botrytis cineria*

Sr#	Treatments	Growth inhibition (%)
T_1	Tebuconazole	65.27 c
T_2	Fludioxonil	79.55 a
T_3	Propiconazole	72.73 b
T_4	Carbendazim	56.27 d
	LSD	0.6493

Table 3: **Interaction of means of growth inhibition produced by different treatments and concentration (TxC) against *Botrytis cineria***

Sr.No.	Treatments	Growth inhibition (%)		
		S	S/2	S/3
1	Tebuconazole	72 e	66.16 g	57.65 i
2	Fludioxonil	85.4 f	79.76 c	73.65 d
3	Propiconazole	82.3 b	70 f	65.83 g
4	Carbendazim	62.5 f	56 j	50.45 k
5	LSD	1.124		

Table 4: Interaction of means of growth inhibition (%) by different treatments and days

Sr.No.	Treatments	Growth inhibition (%)	
		Day 5	Day 7
1	Tebuconazole	65.11 e	65.54 e
2	Fludioxonil	80.53 a	78.44 b
3	Propiconazole	72.22 d	73.26 c
4	Carbendazim	54.78 g	57.75 f
5	LSD	0.9183	

(TxD) against *Botrytis cineria*

Table 5: **Interaction of means of growth inhibition (%) by different concentration and days (CxD) against**

Sr.No.	Concentration	Growth inhibition (%) at different days	
		Day 5	Day 7
1	S	74.83 b	76.25 a
2	S/2	68.16 c	67.75 c
3	S/4	61.57 d	62.16 d
	LSD	0.7953	

Table 6: Interaction of means of growth inhibition (%) produced by different treatments, concentrations and days (T x C x D) against *Botrytis cineria*

Treatment	Growth inhibition (%)					
	After 5 days			After 7 days		
	S	S/2	S/4	S	S/2	S/4
Tebuconazole	71.33 fg	65.33 j	58.66 l	72.66 f	67 i	56.66 m
Fludioxonil	86 a	81.34 c	74.66 e	84.66 ab	78 d	72.66 f
Propiconazole	81 c	71 g	64.66 j	83.56 b	69 h	67 i
Carbendazim	61 k	55 n	48.33 p	64 j	57 m	52.33 o
LSD	1.5906					

Discussion

Pomegranate (*Punica granatum* L.*)* is important fruit of *Punicaceae* family. It thrives in different climate and soil conditions and can tolerate drought and salt stress. Pomegranate fruits are consumed fresh or used for the production of wine. Pomegranate production has been increasing worldwide in response to increase popularity due to its health benefits (Basu and Penugond, 2009).

Pomegranate is susceptible to many diseases and fruit production is the essential issue in the whole world (Bardas *et al.,* 2009). Many fungal pathogens attacked on pomegranate, but the most important plant pathogenic fungi include gray mold (*Botrytis cineria*) rot, black heart (*Aspergillus niger*) rot, green mold (*Penicillium digitatum*) rot and Alternaria (*A. alternata*) rot and blue mold (*P. expansum*) rot (Hebert and Clayton, 1963; Sharma and Jain, 1978; Snowdon, 1990; Adaskaveg, 2012). The most important pathogen that cause fruit decay worldwide is *Botrytis cineria* Pers (Tedford *et al.,* 2005).

Results indicated by Sharma and Tripathi, 2006 described the maximum growth inhibition oils due to their inhibitory effect on germ tube growth, spore germination as well as mycelia growth against 10 post-harvest disease spreading agents. Xing *et al*., 2012 evaluated the *in vitro* and *in vivo* antifungal activities of clove oil against *Aspergillus flavus*, *Penicillium citrinum* and *Rhizopus nigricans*. The minimum inhibitory concentrations of clove oil against *A. flavus*, *P. citrinum* and *R. nigricans* were 25, 25 and 50 µL/mL respectively. These results indicated that clove oil has a good potential to be a natural antifungal agent for fruit applications. Whereas in this study sage oil was most effective in controlling the pathogen.

Postharvest fungal disease infection on pomegranate fruit in markets can be minimized by use of fertilizer a common practice with quick results. Fungicide use to manage postharvest pathogens although gives best results, but the pathogen population may develop resistance against fungicides. Chemical use with good practice against postharvest fungal pathogens causing fruit rot can be applied for protective uses (Kumar *et al*., 2017).

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