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Research Article

Isolation, Identification and Pathogenicity of Leaf Spot Disease of Grape and their Control

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Abstract

Grapes (*Vitis vinifera*) belong to family *Vitaceae* which is one of the most significant fruit crop throughout the world and has attained the status of a dependable and profitable farming enterprise. Grapevine is one of the most widely grown crops in the world which is cultivated on every continent except Antarctica. In almost all the major grape growing countries, bulk of the grape produced is used for wine making with a small portion going for raisin and table purposes. Almost 50% of grapes are altering into wine. Grape is distress from many infections initiated by fungi, viruses, bacteria, and nematodes. Fungal diseases are most common on grapevines. Some common diseases of grape are downy mildew, powdery mildew, anthracnose, rust and leaf spot. Fungal diseases of trunk and root are not well understood because the disease is often not visible until it is late to manage. Example of such kind of disease is *Eutypa* dieback, a trunk disease which is caused by fungi *Eutypa lata* caused heavy losses worldwide. Different grape diseases samples were collected after the visual identification of leaves spots of the grapes from different areas. The diseases samples were transported aseptically to the molecular biology laboratory of plant pathology department for further analysis. Isolated specie of the fungi was treated with five different fungicides to check the effect upon. Out of five fungicides tested maximum mycelial progress inhibition (cm) was in case of Cabriotop (Metiram+Pyraclostrobin) followed by Dithene (Metalaxyl+Mancozeb) at 200 ppm while minimum mycelial growth inhibition was in case of Topsin-M (Thiophanate Methyl), Revus (Copper-Oxychloride) and Topas (Penconazole) at 50 ppm concentration. The aim of this investigation was to find the possible grapevine infection fungus and their ability in surviving in different concentration of fungicides.

Keywords: *Alternaria; Chemical Treatments*

Introduction

Grapes (*Vitis vinifera*) belong to family *Vitaceae* which is one of the most significant fruit crop throughout the world and has attained the status of a dependable and profitable farming enterprise [1]. The production of world wine is 27 million tons signifying about 36 million tons of grapes occupying more than 10 million hectares in different countries. The main producing countries of grape in world are Germany, Australia, Portugal, South Africa, Spain, France, Argentina, Italy, China, and United States [2]. Grapevine is one of the most widely grown crops in the world which is cultivated on every continent except Antarctica. In almost all the major grape growing countries, bulk of the grape produced is used for wine making with a small portion going for raisin and table purposes. Almost 50% of grapes are altering into wine [3]. Grape is important, profitable and most grown product of agriculture in Afghanistan. In a survey in 2009 showed, national productions of grapes were assessed about 58,000 tons which showed 12% of an increase since last ten years [4]. Grape-vine

grown best in all sorts of climates and soils, where the production of other deciduous fruits crops is limited. It can be grown well in cold and dry climates in valleys in high elevations. A soil having low water holding capacity sandy loam is the best for its growth [5]. However grape production is disposed to serious problems caused by insects, pest and diseases, which cause heavy losses up to 30%, decrease in the production [6]. Grape is distress from many infections initiated by fungi, viruses, bacteria, and nematodes. Fungal diseases are most common on grapevines [7]. Some common diseases of grape are downy mildew, powdery mildew, anthracnose, rust and leaf spot. Fungal diseases of trunk and root are not well understood because the disease is often not visible until it is late to manage. Example of such kind of disease is *Eutypa dieback*, a trunk disease which is caused by fungi *Eutypa lata* caused heavy losses worldwide [8]. *Alternaria* diseases are among the most common diseases of many grape plants throughout the world. They affect primarily the leaves, stems, flowers and fruits of annual plants, especially vegetables and ornamentals. Being saprophytes as well as plant pathogens, species of genus *Alternaria* are widely distributed in our environment. Members of this group are known to cause spots, rots and blights in plants [9]. Identification of *Alternaria* specie is very complex. Simmons [10] provided the detailed manual for *Alternaria* identification. *Alternaria spp.* are major plant pathogens, which cause at least 20% of agricultural spoilage; most severe losses may reach up to 80% of yield [11].

Methodology

Different grape diseases samples were collected after the visual identification of leaves spots of the grapes from different areas. The diseases samples were transported aseptically to the molecular biology laboratory of plant pathology department for further analysis. Isolated specie of the fungi was treated with five different fungicides to check the effect upon.

Purification and preservation of pathogen

Most frequently isolated fungal pathogen was purified and mass cultured on (PDA) potato dextrose agar media (Figure 1). Bit of mycological growth developed from the diseased foliage tissue was moved to (PDA) slants and incubated at 28° ± 2°C for 10 days.

Pathogenicity test

To confirm pathogenicity grape plants (*Vitis vinifera*) were collected from nursery and grown in earthen pots of size (6" × 5") filled with treated soil. Plants were carefully washed with purified cleaned water by using humid cotton. Before inoculation the healthy plants with distilled water were carefully sprayed and were enclosed by bags of polythene for (24) hr. The inoculums suspension from ten days old culture was prepared in sterile distilled water. Spore suspension of *Alternaria* was done and spore density was set with the help of haemocytometer @ 1x 10^5 spores per ml of water sprayed on to the healthy grape plants.

Poisoned food technique

Five fungicides viz; Ditnene M 45, Revus, Cabriotop, Topas, and Topsin-M belonging to different chemical groups at three different concentrations were tested for their efficacy in vitro against *Alternaria* spp using poisoned food technique [12]. Three replications were retained for respectively concentration of individual experienced fungicides and plates were (incubated) at 28 ± 2 °C. Interpretations about mycelial development were recorded after 48 hrs. The PGI (per cent growth inhibition) of fungal pathogen over control was driven out by means of (formula) given by Arora and Dwivedi [13].

$$\text{Per cent growth inhibition (PGI)} = \frac{DC - DT}{DC} \times 100$$

Where:

PGI = Per cent growth inhibition.

DC = Average diameter of mycelial colony of control set (mm).

DT = Average diameter of mycelial colony of treated set (mm).

Trade name and formulation	Chemical name	Dose per liter
Dithene M 45 (72% WP)	Metalaxyl+Mancozeb	2.5 g
Revus (50% WP)	Copper-Oxychloride	2.5-3 g
Cabriotop (60% WDG)	Metiram+Pyraclostrobin	4 g
Topas (50% WP)	Penconazole	0.75 g
Topsin-M (70 % WP)	Thiophanate Methyl	2-2.5 g
Control		

Table 1: *Details of Treatments Employed in Experiment Along with Name of Fungicides.*

Statistical analysis

The composed data were evaluated statistically by means of the Fisher Analysis of Variance method. Treatment means were matched via (LSD) test at 5% probability (software M. Stat C) [14].

Results

In vitro evaluation of different fungicides against Alternaria spp by poisoned food technique

Out of five fungicides tested maximum mycelial progress inhibition (cm) was in case of Cabriotop (Metiram+Pyraclostrobin) followed by Dithene (Metalaxyl+Mancozeb) at 200 ppm while minimum mycelial growth inhibition was in case of Topsin-M (Thiophanate Methyl), Revus (Copper-Oxychloride) and Topas (Penconazole) at 50 ppm concentration.

S.o.V	DF	SS	MSS	F	P
Treatments (T)	5	404.706	80.9412	84108.2	0.000*
Days (D)	2	0.650	0.3252	337.96	0.000*
Conc. (C)	2	15.267	7.6336	7932.23	0.000*
T x D	10	0.488	0.0488	50.71	0.000*
T x C	10	14.154	1.4154	1470.82	0.000*
C x D	4	0.033	0.0082	8.55	0.000*
T x C x D	20	0.218	0.0109	11.35	0.000*
Error	108	0.104	0.0010		
Total	161	435.621			

Table 2: *ANOVA for the interaction of treatment, concentration and duration on*

mycelial growth of Alternaria spp.

**: Significant*

Grand Mean: 2.3206 CV: 1.34%.

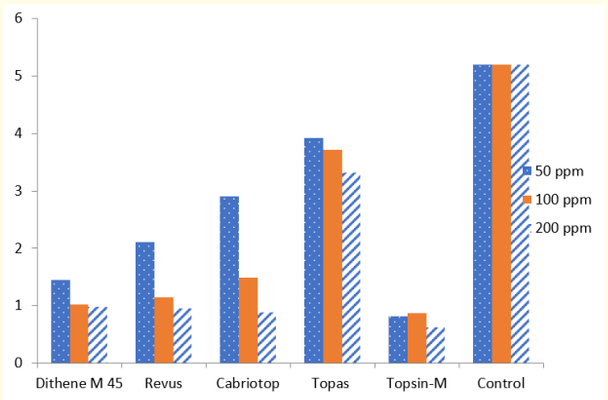

Figure 1: *Showing percentage inhibition of mycelial growth at different concentrations.*

Percentage growth inhibition of *Alternaria spp.* in the treated plates calculated with following formula:

$$\text{Per cent growth inhibition (PGI)} = \frac{DC - DT}{DC} \times 100$$

All fungicides applications expressively prevent the progression of (*Alternaria*) species under in-vitro conditions. It is proof from the above mentioned graphical representation (Figure 2) that development inhibition enhanced by increasing in the concentration of chemicals.

Treatments	Ist week.			2nd week.			3rd week		
	C1	C2	C3	C1	C2	C3	C1	C2	C3
T1	1.3400 p	0.8867 vwx	0.6733 b	49.333 nopq	1.0500 t	0.9800 u	1.5400 m	1.1233 rs	1.3000 p
T2	1.8333 l	1.0500 t	0.7967 za	2.2000 k	1.1533 r	0.9800 u	2.3000 j	1.2333 q	1.0833 st
T3	2.8733 i	1.4567 o	0.8467 xyz	2.8733 i	1.4733 no	0.8633 xy	2.9467 h	1.5233 mn	0.9300 uv
T4	3.8733 c	3.6533 e	3.2633 g	3.9233 bc	3.7200 d	3.3267 f	3.9600 b	3.7567 d	3.3467 f
T5	0.7533 a	0.8400 xyz	0.5800 d	0.8133 yz	0.8600 xy	0.6200 cd	0.8733 wx	0.9200 vw	0.6700 bc
T6	5.2000 a	5.2000 a	5.2000 a	5.2000 a	5.2000 a	5.2000 a	5.2000 a	5.2000 a	5.2000 a
LSD	0.0502								

Table 3: *Effect of interaction of Treatment, Concentration and duration on the mycelial growth (cm) of Alternaria spp.*

S.o.V	DF	SS	MSS	F	P
Replication	2	42.4	21.2		
Conc. (C)	2	226.8	113.4	6.60	0.0028
Treatments (T)	2	22751.0	11375.5	622.29	0.0000
Week (W)	2	2616.9	1308.5	76.18	0.0000
C x T	4	273.6	68.4	3.98	0.0068
C x W	4	200.3	50.1	2.92	0.0299
T x W	4	3396.1	849.0	49.43	0.0000
C x T x W	8	226.5	28.3	1.65	0.1338
Error	52	893.2	17.2		
Total	80	30626.7			

Table 4: *ANOVA for the interaction of treatments and weeks on disease severity of leaf spot of grape plant*

**: Significant*

Grand Mean: 57.951 CV: 7.15.

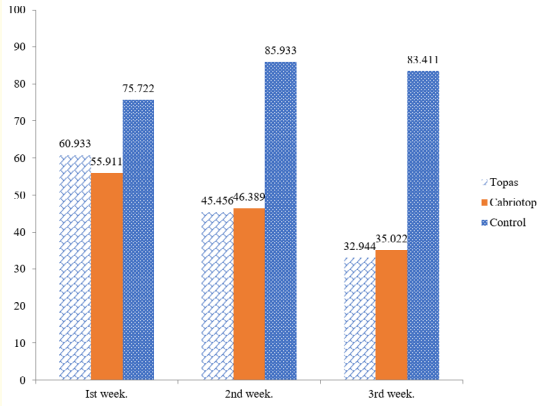

Figure 2: *Showing percentage disease severity of leaf spot of grape plant (vitis vinifera) after 1-3 weeks of inoculation.*

The results showed that use of fungicides had expressively condensed the development of leaf spot infection in grape plants infested with inoculum of *Alternaria* species after changed intervals of inoculation than unprocessed (control plants).

Conclusion

The isolated fungus from the infected leaves were treated *in-vitro* and *in vivo* both with different fungicides. Cabriotop was effective at all three concentrations, while Dithene M 45 (Metalaxyl+Mancozeb), Carbendazim, Topas and Topsin M were moderately effective at low concentration but highly effective at higher concentrations. The results of in-vitro were different when the healthy plants were sprayed *in vivo*, cabriotop showed minimum disease severity 55.91, 46.38 and 35.02 as compared to control 75.72, 85.93 and 83.41% after 1st, 2nd and 3rd week of inoculation correspondingly. In the same way Topas displayed disease severity of 60.93, 45.45 and 32.94%. The consequences confirmed that application of fungicides can protect grape plant against *Alternaria* leaf spot disease. Infections or plants diseases are organized and managed through many diverse approaches, included as application of cultural practices. Cultivation of resistant cultivars or variety, usage of many chemicals pesticides and fungicides and by biological control managers. Even though all these approaches of management apply have specific significance, however not any of them is totally effective when applied alone for management of infection.

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