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Research Article

Isolation, Identification and Pathogenicity of Anthracnose of Grapevine and its Possible Management

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Abstract

Grape (*Vitis vinifera*) is the most remunerative and economically important fruit crop and has a lot of uses. It is commonly attacked by a wide range of fungus species in which the most prominent is *Elsinoe ampelina* which cause anthracnose disease of grapevine. An *in vitro* trial was conducted in the Laboratory of Mycology, Department of Plant Pathology, Agricultural University Faisalabad, Pakistan, during 2016 - 2017 in order to properly manage Anthracnose disease of grape with systemic fungicides and plant extracts. Five systemic fungicides namely Diniconazole, Thiophanate-methyl, Myclobutanil, Difenoconazole and Hexaconazole were tested at 50 ppm, 100 ppm and 200 ppm in order to check their efficacy against percent growth inhibition of *Elsinoe ampelina*. Hexaconazole and Myclobutanil at 200 ppm inhibited 88.33% and 86.42% radial growth respectively and found to be significantly superior to Diniconazole and Thiophanate-methyl at 200 ppm. Difenoconazole was significantly inferior against *Elsinoe ampelina*. Different plant extracts of neem (*Azadirachta indica)*, onion (*Allium cepa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were evaluated at 3 different concentrations 10%, 20% and 30% against the growth inhibition of *Elsinoe ampelina*. Among all the plant extracts, the highest concentration of ginger (*Zingiber officinale*) was found to be significantly best in radial growth inhibition of *Elsinoe ampelina* followed by neem (*Azadirachta indica)*, garlic (*Allium sativum*) and onion (*Allium cepa*), each plant extracts were found to be significantly different from one another. Another field experiment was also conducted in which Diniconazole, Thiophanate methyl, Myclobutanil, Difenoconazole and Hexaconazole and plant extracts namely neem (*Azadirachta indica)*, onion (*Allium cepa*), ginger (*Zingiber officinale*) garlic (*Allium sativum*) and Lantana (*Lantana camara*) were further evaluated under greenhouse condition, among the five tested fungicides, Hexaconazole was found to be the most effective fungicide in controlling the disease incidence, for which the disease incidence was recorded very low (40.93%) followed by Myclobutanil and Diniconazole, while Difenoconazole was found to be less effective in controlling the disease incidence. Similarly among the five plant extracts, Lantana extract was found to be highly effective in controlling disease incidence, for which the disease incidence was recorded very low (38.04%) followed by ginger (*Zingiber officinale*) and neem (*Azadirachta indica),* while onion (*Allium cepa*) was found to be less effective in controlling disease incidence.

Keywords: *Pathogenicity; Identification; Management*

Introduction

Grapes (*Vitis vinifera*) is the most important horticultural and well remunerative fruit crop which is generally grown on about 57,800.00 hectares with annual production of 14,72,800.00 tons in India, among which 94% production area is generally fall in Nadu, Tamil, Karnataka, Andhra Pradesh and Maharashtra [5]. Grapevine belongs to "*Vitis*" genus and family vitaceae. Vitaceae family contains 14 living

and two fossil genera Over 90% of the world total grapes production comes from the cultivars of Vinifera [25]. There are approximately 10000 different *V. vinifera* varieties which are grown all over the world (Australian Wine and Brandy Corporation, 2010). Out of these, top 3 varieties among them have accounted for approximately 60% or more area of vineyard in the year of 2009: Chardonnay (18.51%), Shiraz (27.8%) and Cabernet sauvignon (17.1%) [1]. Production of grapes exceeds that of every other fruit in the World. In the world the total cultivation area of grapes is about 7.64 million hectares, which produce 58.45 metric tons production annually [2]. According to the uses the grapes are divided into five different classes: desk grapes, raisin grapes, canning grapes, sweet juice grapes and wine grapes. Approximately 78% of the world's total productions are pressed into wine, about 13% is dried and approximately 8% is freshly used [26]. The Growth and production of grapevine is however affected by biotic as well as abiotic factors. Pathogens, such as viruses, viroids, bacteria, protozoa, parasitic plants, nematode and fungi [6,10,11,15]. Anthracnose disease of grapevine, also called bird's eye rot or grapes black spot disease is more destructive disease of grapes caused by *E. ampelina* fungus affecting many areas of grapes production [13,16] causing more economical losses of grape crops in a frequent rainy area infecting aerial parts of the grapes, which induce different lesions on petioles, berries, rachises, peduncles, berries, leaves and shoots, and similarly cause early drop of berries and leaves [14,19]. Grapes anthracnose disease is particularly harmful to vine cultivars grown in rainy and humid areas [16]. During the spring season, Conidia produced and released by temperature raised above 2°C, meanwhile sclerotia hangs wet for minimum of 24 hrs [3,7,16]. The conidia of the *Elsinoe ampelina* can easily infect a young tissue at a temperature as ranging from 2 to 41°C, but the optimum and most suitable temperature is 3°C [22,23]. Surface moisture of the tissue is important for infection; however contradictory report is also present. Cost of different fungicides makes it more difficult to get benefit due to susceptible varieties in regions with warm and humid climates [16]. In growth medium, the optimum temperature for growth of *Elsinoe ampelina* is about 28°C, but at about 35°C not proper growth occur [24]. While the moisture period is about 3 - 4 hrs at 21°C and 7 - 10 hrs at 12°C (Brook, 1973). For proper development of disease the temperature ranges from 24 to 26°C [16].

Regular application of fungicides is essential to properly manage anthracnose disease of grapes in Florida, while in frequently raining there, it is difficult to effectively manage anthracnose disease in susceptible vine cultivars even by the using of fungicides each seven to ten days interval [14]. Thind SK., *et al*. [23] stated that in a controlled condition of humidity and temperature, minimum of 3, 4, 5 and 24 hours of leaf wetness is essential at the temperature of 25, 30, 20 and 15°C respectively. Suhag LS., *et al*. [19] *in-vitro* experiment showed that the cankers been successfully checked via using hinosan, brestan, dodine and ferrous sulphate while maneb, miltox, thiram, ziram and captan prevent the growth of mycelium of the grape anthracnose pathogen for the short time. Sastry MNL., *et al*. [20] showed the efficiency of Difolatan, Thiram and Bavistin inhibiting growth of mycelium. Pampanagouda B [18] reported benomyl, Thiophanate-methyl in the systemic fungicide and similarly mancozeb and Bordeaux mixture in non-systemic fungicides can halt growth of *Elsinoe*. Gupta JS., *et al*. [12] Reported phytinoids compound of neem, garlic and onion inhibiting spore germination of anthracnose pathogen.

Materials and Methods

The present investigations were undertaken in the Department of Plant Pathology, University Of Agriculture Faisalabad, during the year 2015 and 2016.

Survey and sample collection

Extensive roving and periodic survey was conducted during the year 2015 and 2016 in three districts of Kabul province, Shakar Dara, Mirbacha Kot and Qara Bagh, and two districts of Ghazni province, Gelan and Andhar. On the basis of visual observations, critical disease symptoms of anthracnose disease of grapes such as minute brown specks initially along the margins of the leaf lamina that gradually developed into dark brown lesions with white/gray center were observed during the survey. The samples then carefully isolated inside polythene bags in order to prevent dehydration. Carefully transferred the samples to the Mycology Lab, University of Agriculture Faisalabad.

Isolation and identification pathogen

The diseased samples were washed thoroughly under tape water and allowed to dry in shade under laboratory conditions. The infected portions along with some healthy part were cut into small pieces and surface sterilized in 1:1000 mercuric chloride solution for one minute. The excess traces of mercuric chloride on the surface of the leaf and shoot bits were removed by washing 2 - 3 times in sterile distilled water dried on sterilized blotting paper and transferred aseptically to Petri plates containing potato dextrose agar (PDA) medium. The inoculated Petri plates were then incubated at 28 ± 1°C and growth of the fungus was observed periodically. The pure colonies that developed from these infected leaves and stem bits were then transferred to the PDA slants aseptically. The grown cultures further processed to make slides for morphological microscopic examination based on hyphae, septation, color, width, spore and fruiting body.

Methods deployed for pathogenicity identification

Detached leaf method

The healthy young (8 - 10 days) leaves of susceptible grape cultivar, Anabe-shahi were collected, surface sterilized with 0.1 per cent mercuric chloride solution followed by thorough washing in sterilized water. Spore suspensions from 15 days old fungal culture was prepared having approximately 2×10^4 conidia per ml in the suspension. The suspension was used for inoculating the healthy grape leaves. In another set instead of spore suspension only sterile water was sprayed which served as control. Inoculated leaves were kept in humid chamber. Observations were made at regular intervals for symptom development.

Detached twig method

Healthy grape vine twigs bearing leaves were identified from healthy grapevine cultivar Anab-e-shahi cut with sterilized pruning scissor; surface sterilized and collected in conical flask containing water. Twigs were inoculated with a spore suspension of 2×10^4 conidia/ml by ordinary baby sprayer, in ten different sterilized conical glass flasks and incubated in humid chamber. Un inoculated twigs sprayed with sterilized water were also maintained as control. Observations were made at regular intervals for symptom development.

Management trial

In vitro evaluation of fungicides against the mycelia growth of *Sphaceloma ampelinum* by applying poisoned food technique

Five different fungicides namely thiophanate methyl, hexaconazole, diniconazole, myclobutanil and difenoconazole were evaluated in *in vitro* to check their effect on percent growth inhibition of *Sphaceloma ampelinum* by applying poisoned food technique [17]. All fungicides were used at three different concentrations as 50 ppm, 100 ppm and 200 ppm. Fungicides suspensions of three different concentrations were prepared by adding requisite amount of each fungicide in warm PDA medium. About 15 ml of sterilized medium was poured in each 9 cm sterilized petri dish. After solidification, the plates were inoculated by placing 5 mm discs of 3 days old PDA cultures of *Sphaceloma ampelinum*. Three replicated plates were used for each concentration of every fungicide. Three replicated PDA plates received no fungicides served as control. The inoculated plates were incubated at 28°C and data on the radial colony diameter was recorded after 4 - 5 days of incubation when the growth of the control plates completely covered the plate. Diameter of the colonies on PDA with and without fungicide was measured from the bottom side of the Petri dishes. Inhibition of radial growth was computed based on colony diameter on control plate using the following formula as stated by [21]:

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

Where, C = Growth of control plate and T = Growth of fungicide treated plate.

In vitro evaluation of plant extracts against the radial colony growth of *Sphaceloma ampelinum* by applying poisoned food technique

Technique as described by [4] extracts of garlic, ginger, onion, lantana and neem were properly tested. Stalk solutions of these plants were formed by blending 100 gram of each plant material in 100 ml of sterilized water in a blender machine. PDA medium was amended with each individual plant extract at 0, 10, 20 and 30% (v/v). Requisite quantity of individual plant extract was added to the 100 ml conical flask with PDA medium to have concentrations of 0, 10, 20 and 30% (v/v). After thorough mixing with plant extracts the medium was autoclaved and approximately 15 ml of melted PDA mixed with extracts was poured into each 90 mm Petri dish. After solidification, the plates were inoculated by placing 5 mm discs of 3 days old PDA cultures of *Sphaceloma ampelinum.* Percent Growth Inhibition of *Sphaceloma ampelinum* fungi was computed with the help of a formula which had been described earlier [21].

In vivo evaluation of different systemic fungicides against anthracnose disease of grapevine by foliar spray

Different fungicides (systemic fungicides) had been evaluated in one season at three different concentrations (0.25, 0.5 and 1%) against the disease severity of grapes anthracnose caused by *Sphaceloma ampelinum.* The design that we had used for this experiment was Completely Randomized Block Design (RCBD) with 3 replications for each treatment on one year old grapes plants. The desired quantities of each fungicide was either pipette out or weighted with the help of micro-balance and properly dissolved in water in order to get desired concentrations of these chemicals. The systemic fungicides which were used against the anthracnose disease of grapes are given below.

Treatments	Fungi toxicant
T1	Hexaconazole
T2	Difenoconazole
T3	Thiophanate-methyl
T4	Diniconazole
T5	Myclobutanil
Control	Check (Water spray)

The observations had been recorded on disease severity/disease incidence by using 0 to 4 scale adopted by [8], similarly PDI (percent disease intensity) was recorded by using the Wheeler's formula. The obtained data was statistically analyzed.

In vivo evaluation of different plant extracts against anthracnose disease of grapevine by foliar spray

Five different types of plant extracts namely garlic, ginger, onion, lantana, camara and neem evaluated at three different concentrations (10, 20 and 40%) to check efficacy of fungicides against anthracnose disease. The experimental design was Randomized Complete Block Design (RCBD) in which each treatment replicated three times. Each fungicide was sprayed for three weeks with one week interval and with three different concentrations. Data on the disease intensity and incidence had been recorded for all five fungicides over 3 weeks. The observations had been recorded on disease severity/disease incidence by using 0 to 4 scale adopted by Chatta [8], similarly PDI (percent disease intensity) was recorded by using the Wheeler's formula [27]. The obtained data was statistically analyzed.

Disease incidence

Disease incidence (%) = Number of diseased unit/Total number observed × 100.

Disease intensity

The disease intensity was recorded by visual observations using 0 - 4 scale in table below [8]:

PDI = Sum total of numerical rating/Total units observed × Maximum numerical value × 100

Category	Numerical Value	Description
I	0	Healthy grapes foliage or grapes leaf spots in traces
II	1	Upto 10 percent leaf area covered with Anthracnose disease lesions
III	2	10.1-25 percent leaf area covered with slight twig infection i.e. 1 - 3 cankers per twig
IV	3	25.1-50 percent leaf area covered with heavy twig infection i.e. 4 - 10 cankers per twig
V	4	Above 50 percent leaf area covered with very heavy twig infection i.e. above 10 cankers per twig and heavy berry infection

Results

In-vitro evaluation of different fungicides by applying poisoned food technique against the mycelial growth of *Sphaceloma ampelinum*

Five fungicides viz Diniconazole, Thiophanate-methyl, Myclobutanil, Difenoconazole and Hexaconazole at three different concentrations as 50 ppm, 100 ppm and 200 ppm were evaluated. However, when the fungus growth at various concentrations of fungicides at the incubation period of 5 days at 25°C compared based on all means of all concentrations of fungicides (Table 3). Hexaconazole, Diniconazole, Thiophanate-methyl, Myclobutanil and Difenoconazole caused 82.38%, 71.42%, 60.23%, 78.33% and 15.47% reduction in the growth of fungal mycelium respectively.

Analysis of variance shows a significant interaction between concentration and mycelial growth of *Sphaceloma ampelinum* (Table 3), there was no any statistical difference between the effectiveness of Diniconazole at 200 ppm and Hexaconazole at 100 ppm (Table 3).

Source of variance	DF	SS	MS	F	P
Concentration	2	5.25	2.62	8345.48	0.0000**
T	5	96.57	19.31	61351.1	0.0000**
C*T	10	2.95	0.29	938.82	0.0000**
Error	36	0.01	0.0003		
Total	53	104.79			

Table 1: *ANOVA of Systemic fungicides used under lab condition.*
***Highly significant at P ≤ 0.05%.*

S. No	Treatment	Mean Mycelial Growth (cm)	Percentage Fungal inhibition over control
1	Myclobutanil	0.91 e	78.33
2	Diniconazole	1.20 d	71.42
3	Thiophanate-methyl	1.67 c	60.23
4	Difenoconazole	3.55 b	15.47
5	Hexaconazole	0.74 f	82.38
6	Control	4.20 a	

Table 2: *LSD All-Pair-wise comparisons test of mean mycelial growth (cm) for treatment.*
Mean values in this column having similar letters do not differ significantly as determined by LSD test (P ≤ 0.05).

Treatments	Mean colony growth (cm) at various concentration		
	50 ppm	100 ppm	200 ppm
Myclobutanil	1.30 h	0.87 k	0.57 m
Diniconazole	1.76 f	1.07 i	0.77 l
Thiophanate-methyl	2.66 e	1.49 g	0.86 k
Difenoconazole	3.80 b	3.62 c	3.23 d
Hexaconazole	0.96 j	0.78 l	0.49 n
Control	4.20 a	4.20 a	4.20 a

Table 3: *LSD All-Pair-wise comparisons test of mean mycelial growth (cm) for concentration* treatment. Mean values in this column having similar letters do not differ significantly as determined by LSD test (P ≤ 0.05).*

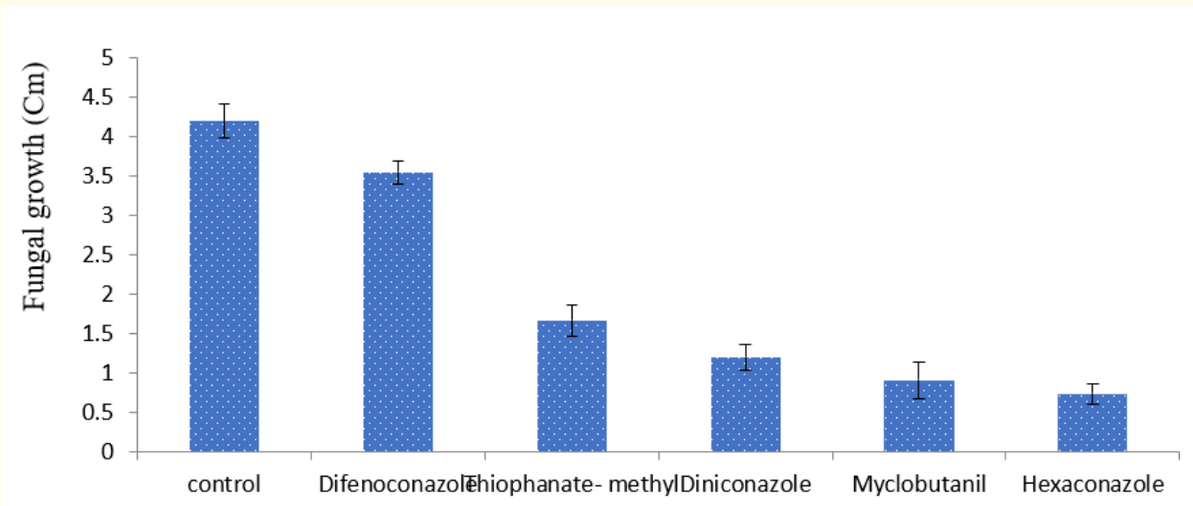

Figure 1: *Effect of different systemic fungicides against the colony growth (cm) of Sphaceloma ampelinum.*

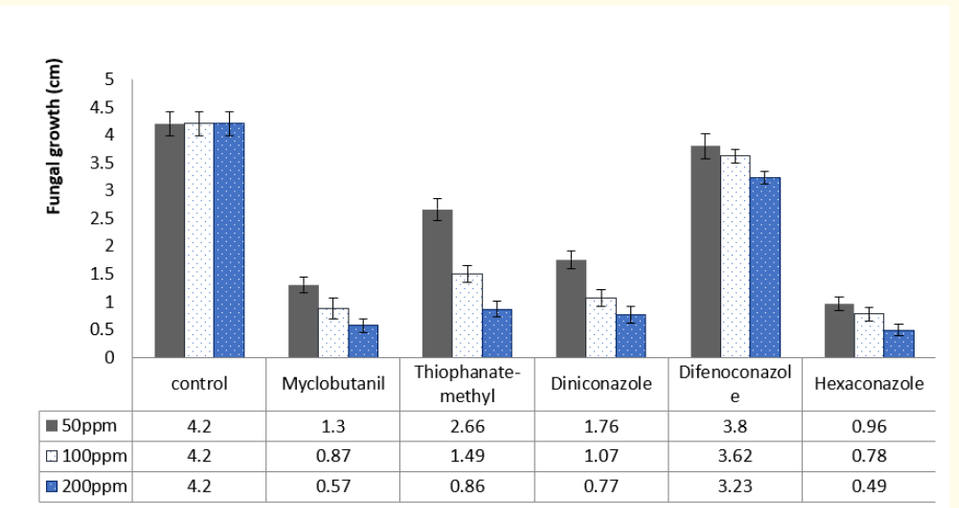

Figure 2: *Effect of different concentrations of fungicides against the colony growth (cm) of Sphaceloma ampelinum.*

In-vitro evaluation of different plant extracts by applying poisoned food technique against the mycelial growth of *Sphaceloma ampelinum*

Plant extracts garlic, ginger, onion and neem at three different concentrations 10%, 20% and 30% were evaluated. However, when the growth of fungus in response to various concentrations of plant extracts at an incubation period of 7 days at 25°C compared based on means of all plant extracts concentration (Table 6), garlic, onion, ginger, and neem showed 24.87%, 18.65%, 35.23%, 26.42% reduction in the growth of fungal mycelium respectively.

Analysis of variance clearly shows that there is a significant interaction between concentration and mycelial growth with increasing the plant extracts concentration (Table 6).

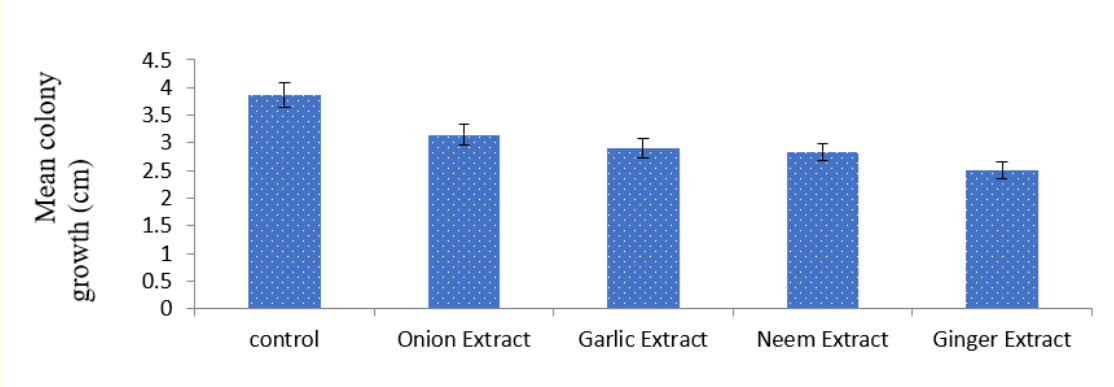

Figure 3: *Effect of different plant extracts against the colony growth (cm) of Sphaceloma ampelinum grapevine anthracnose pathogen.*

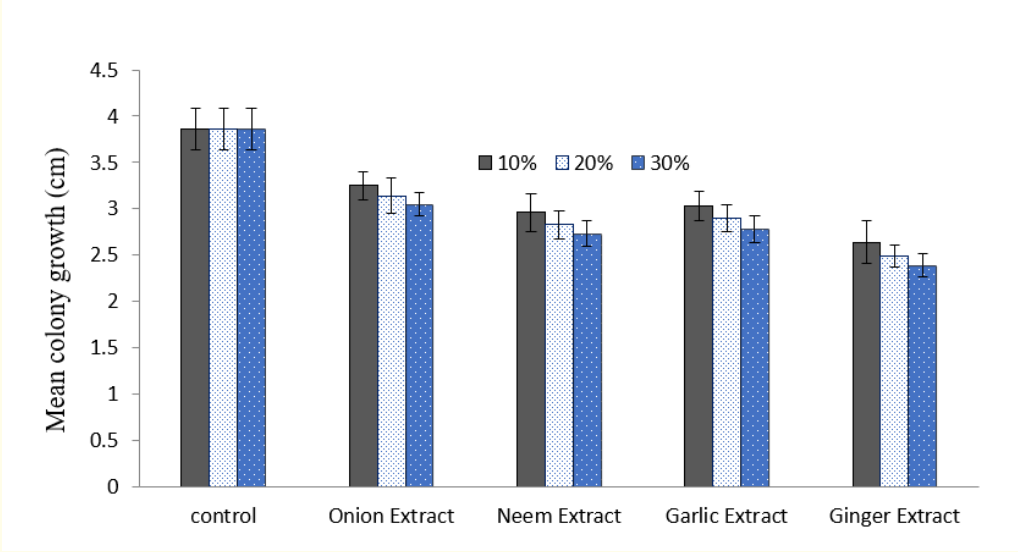

Figure 4: *Effect of different concentration of plant extracts against the colony growth (cm) of Sphaceloma ampelinum grapevine anthracnose pathogen.*

Source of variance	DF	SS	MS	F	P
Concentration	2	0.25	0.12	688.32	0.0000**
T	4	9.20	2.30	12334.2	0.0001*
TxC	8	0.06	0.008	45.11	0.0000**
Error	30	0.005	0.0001		
Total	44	9.53			

Table 4: *ANOVA of plant extracts used under lab condition.*

***: Highly significant at P ≤ 0.05%; *: Significant at P ≤ 0.05%.*

Plant Extracts	Mean Mycelial Growth in cm	Homogeneous Groups	Fungal inhibition Percentage over control
Control	3.86	A	
Onion	3.14	B	18.65
Garlic	2.90	C	24.87
Neem	2.84	D	26.42
Ginger	2.50	E	35.23

Table 5: *The mean colony growth (cm) of Sphaceloma ampelinum under lab condition at 25 C on PDA with one of the four plant extracts at three different concentrations.*

Mean values in this column having similar letters do not differ significantly as determined by LSD test (P ≤ 0.05).

Plant Extracts	Mean colony growth (cm) at various concentrations		
	10%	20%	30%
Ginger	2.64 k	2.49 l	2.39 m
Onion	3.25 b	3.14 c	3.05 d
Neem	2.96 f	2.83 h	2.73 j
Garlic	3.03 e	2.90 g	2.78 i
Control	3.86 a	3.86 a	3.86 a

Table 6: *LSD All-Pair-wise comparisons test of Mean colony growth (cm) for concentration* treatment.*

Mean values in this column having similar letters do not differ significantly as determined by LSD test (P ≤ 0.05).

Conclusion

Fungicides Diniconazole, Thiophanate-methyl, Myclobutanil, Difenoconazole and Hexaconazole at three different concentrations as 50 ppm, 100 ppm and 200 ppm and four plant extracts such as Onion, Garlic, Neem and Ginger extract at 10%, 20% and 30% concentrations were evaluated with poisoned food technique. Generally, the mycelial growth of *Sphaceloma ampelinum* significantly decreases with increasing of plant extracts and fungicides concentrations. Hexaconazole and Myclobutanil had been found to be highly effective in descending order in inhibiting colony growth of *Sphaceloma ampelinum* as they reduced 82.38%, 78.33% mycelial growth respectively over control, followed by Diniconazole and Thiophanate-methyl. While Difenoconazole was found to be less effective and reduced 15.47% mycelial growth of the test pathogen as compared to control. Similarly among four plant extracts, *Zingiber officinale* was found to be highly

effective in descending order in inhibiting the colony growth of *Sphaceloma ampelinum* as they reduced 35.23% reduction in the mycelial growth of *Sphaceloma ampelinum* followed by neem *(Azadirachta indica*) and garlic *(Allium sativum*) as they reduced 26.42% and 24.87% mycelial growth of the pathogen respectively, while onion*(Allium cepa*) has been found to be less effective and reduced 18.65% Pathogens mycelia growth over control. Using plant extracts, we can surely get a good result as our investigation shows a reliable outcome and more research is required to check out many other plants extracts. We can easily analyze our economical losses in addition to pollution factors which are damaging for the crops as well as human being. The approach of integrated management should include minimum use of chemicals to check the population of pathogen; it will encourage the use of biological control agents in order to decrease the inoculums population, cultural practices modification. Different scientific communities are involved similarly innovative strategies in order to properly overcome and manage plant disease. Considering the outcomes of this research investigation, this study actually designed in order to compare the potential lethal effects of plant extracts with synthetic chemicals which is least investigated in Pakistan.

Bibliography

1. Australian Wine and Brandy Corporation. "Annual Report" (2009-2010).
2. Anonymous. "Food and Agriculture Organization. Govt. of Pakistan". FAO year Book Production 51 (1997).
3. Anderson HW. "Diseases of fruit crops". McGraw-Hill New York (1956): 377-382.
4. Begum F., *et al*. "Integrated control of seedling mortality of lentil caused by *Sclerotium rolfsii*". *Bangladesh Journal of Plant Pathology's* 23 (2006): 60-65.
5. Chadha KL. "Indian viticulture scenario. "International symposium on grapes production and proceesing". *Baramati India* 12 (2006).
6. Commenil P., *et al*. "Purification and properties of an extracellular lipase from the fungus *Botrytis cinerea*". *Lipids* 30 (1995): 351-356.
7. Carisse O., *et al*. "A model to estimate the amount of primary inoculums of *Elsinoë ampelina*". *Plant Disease* 95 (2011):1167-1171.
8. Chatta SK. "Biology, perpetuation and control of grape vine anthracnose caused by *Sphaceloma ampelinum*". Sprinagar 33 (1992).
9. Emmett RW., *et al*. "Fungicide evaluation for control of grapes vine black spot or anthracnose". *Fungicide and Nematicide Tests* 36 (1981): 40.
10. Egan MJ., *et al*. "Genomes, free radicals and plant cell invasion: recent developments in plant pathogenic fungi". *Current Opinion in Plant Biology* 11 (2008): 367-372.
11. Ferreira RB., *et al*. "Engineering grapes vine for increased resistance to fungal pathogens without compromising wine stability". *Trends in Biotechnology* 22 (2004): 168-173.
12. Gupta JS., *et al*. "Effect of metabolites from different host plants on conidial germination of *Colletotrichum graminicola* and *C. capsici*". *Geobios* 8 (1981): 226-228.
13. Hopkins DL., *et al*. "A greenhouse method for screening grapes vine seedlings for resistance to anthracnose". *Horticultural Science* 35 (2000): 89-91.
14. Hopkins DL., *et al*. "Fungicidal control of bunch grapes diseases in Florida". *Proceedings of the Florida State Horticultural Society* 86 (1973): 329-333.
15. Kars I., *et al*. "Necrotizing activity of five *Botrytis cinerea* endopolygalacturonases produced in *Pichia pastoris*". *The Plant Journal* 43 (2005): 213-225.

16. Mirica IIRC., *et al*. "Anthracnose: Compendium of Grapes Diseases". American Phyto-pathological Society St. Paul, MN (1988): 18-19.

17. Nene YL., *et al*. "Fungicides in plant disease control". Oxford and IBH publishing Co (1993): 691.

18. Pampanagouda B. "Studies on anthracnose of grapes caused by *Sphaceloma ampelinum*". Thesis submitted to the University of Agricultural Sciences Dharwad (2000):113.

19. Suhag LS., *et al*. "Third intern Symposium". Subtrop Trop Hort Bangalore (1972): 207-208.

20. Sastry MNL., *et al*. "Efficiency of some fungicides in the control of anthracnose of grapes vine". *Pesticides* 23 (1989): 29-30.

21. Sundar AR., *et al*. "*In vitro* Antagonism of *Trichoderma* spp. against two Fungal Pathogens of Castor". *Indian Journal of Plant Protection* 23 (1995): 152-155.

22. Suhag LS., *et al*. "Epidemiology of grapes vine anthracnose caused by *Sphaceloma ampelinumin*". *North Indian Phytopathology* 30 (1977): 460-465.

23. Thind SK., *et al*. "Epidemiology of powdery mildew, downy mildew and Anthracnose diseases of grapes vine". *Kluwer Academic Publishers Netherlands I* (2004): 621-638.

24. Tanaka S., *et al*. "Studies on the grapes anthracnose *Elsinoe ampelina* cultural aspects of the fungus". *Bulletin of the Faculty of Agriculture* 25 (1974): 917-946.

25. Winkler AJ., *et al*. "General Viticulture second edition". Berkley Univ. of Calif. Press (1947).

26. Winkler AJ. "General viticulture". Berkeley Los Angeles London: Uni. Of Calif. Press (1970).

27. Wheeler BEJ. "An Introduction to plant diseases". John Wiley and Sons Limited, London (1969): 301.

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