Isolation, Identification and Pathogenicity of Anthracnose of Grapevine and its Management by different chemicals

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Abstract: Grape (*Vitis venifera*) 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 Labotory of Mycology, Department of Plant Pathology, Sindh Agricultural University Tandojam, sindh, Pakistan, during 2017-2018 in order to properly manage Anthracnose disease of grape with systemic fungicides. 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 Diniconazol and Thiophanate-methyle at 200 ppm. Difenoconazol was significantly inferior against *Elsinoe ampelina*. Another field experiment was also conducted in which Diniconazole, Thiophanate methyl, Myclobutanil, Difenoconazole and Hexaconazole 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.

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Introduction

Grapes (*Vitis vinifera*) is the most important horticultural and well remunerative fruit crop. Grapevine belongs to "*Vitis*" genus and family *vitaceae*. Grapesvines are perennial fruit bearing crops which are economically very important. (Thach *et al*., 2008). 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. Grape is one of the most nourishing, delicious and clean fruit in the world. Freshly grapes juice have 15-25% carbohydrates, 70-86% water, organic acid is 0.4-1.6 percent, nitrogenous compounds 0.03-0.18%, mineral compounds 0.30.6% (Winkler, 1970). The vinifera grapes cultivars need long, warm- hot dry summer season and cool rainy winters for well development. So, two separate seasons of winter and summer are frequently found in Quetta and Chamman in the province of Balochistan are so suitable seasons for grapes production. Some regions of N.W.F.P and Punjab even have an almost appropriate weather for grapes culture; however the main problem is frequent raining during monsoon season on the time of ripening, which results in occurnce of fungal diseases. To get rid from these fungal diseases introduction of early ripening types of grapes or induction of earliness in grapes via cultural practices is needed. Anthracnose disease of grapevine, also called bird's eye rot or grapes black spot disease is more destructive disease of grapes which affects many areas of grapes production (Hopkins and Harris, 2000). Grapevine anthracnose disease is generally caused by *E. ampelina* fungus spp. Grape anthracnose disease cause more economical losses of grape crops in a frequent rainy area, because *E. ampelina* attack and infect all the aerial parts of the grapes, which induce different lesions on petioles, berries, rachises, peduncels, berries, leaves and shoots, and similarly cause early drop of berries and leaves. Those vineyards which are planted with the vulnerable grapesvine cultivars of anthracnose disease, then there the disease become so much difficult to manage properly and effectively even by the use of different fungicides each seven to

eleven days of interval (Emmett *et al*., 1981). In organic production, management of grapes anthracnose disease is especially challenging because in organic production the fungicides are not used. Regardless of type of production, organic or conventional, when once the disease is developed in vineyard, it cause yield losses and similarly the cost of different use of various fungicides make the yield of various grapes cultivars unprofitable which have susceptibility to the anthracnose disease. The Growth and production of grapesvine is however affected by biotic as well as abiotic factors. Pathogens such as viruses, viroids, bacteria, protozoa, parasitic plants, nematode and fungi which have the ability to attack and infect the grapesvines cause reduction in yields (Egan *et al*., 2008). Grapes anthracnose disease is particularly harmful to those vine cultivars which are commonly grown in rainy and humid areas (Mirica, 1988). Many lesions present on shoot, petioles, leaves, pedicels, berries and rachies and generally these lesions are basically in round shape which have brownish to black color margins with round or angular edges. In susceptible vine cultivars, growing points of green and young succulent tissues and shoots are frequently killed. Regular application of fungicides is essential to properly manage anthracnose disease of grapes. Yield losses and cost of different fungicides make it more difficult profitable production of grapes of the susceptible varieties in regions with warm and humid climates. 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 (Thind *et al.,* 2004). Surface moisture of the tissue is important for taking infection; however contradictory report is also present on best and optimum period (Zafar et al., 2017; Zafar et al., 2018; Rasheed et al., 2018). The conidia are produced within cane cankers during the month of May to August, which explain explosive nature of anthracnose disease of grapes, as the plant leaves are fully exposed toward primary and secondary inoculum for most parts of season (Carisse and Lefebvre, 2011).

Keeping in view the above facts, current research was conducted with the following

Objectives

- Isolation and identification of the causal agent from stem and leaf of infected grapesvine.
- Pathogenicity tests of most frequent fungi on grapesvine.
- Study of various means of dispersal of the disease causing agent under laboratory conditions.
- Effect of different fungicides on growth of disease causing agent and disease development under laboratory conditions.

In order to fulfill the objectives following line of work was adopted.

- Survey of growing areas of grapes in Balochistan as well as sample collection.
- Isolation, identification, purification and preservation of pathogen associated with stem and leaf of grapes.
- *In vitro and In vivo* evaluation of different fungicides against isolated pathogen.

Materials and methods

The present investigations were undertaken in the Department of Plant Pathology, Sindh Agricultural University Tandojam, sindh, Pakistan, during the year 2017 & 2018. The details of materials and methodologies followed during the course of investigation are described here under:

Survey

Extensive roving and periodic survey were conducted during the year 2017 and 2018 in different zone Balochistan. 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.

Collection of disease samples

Grape leaves and shoots having typical symptoms of anthracnose, collected from the grape vineyards from grape growing areas of Balochistan during the survey. The samples then carefully putted inside polythene bags in order to protect the samples from dehydration. The collected diseased samples were then brought to the Mycology Lab, Department of Plant Pathology, Sindh Agricultural University Tandojam, sindh, Pakistan.

Isolation of pathogen

For the isolation of 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 bloting paper and such bits were then transferred aseptically to Petri plates containing potato dextrose agar (PDA) medium. The inoculated Petri plates were then incubated at 28±1^{o}C and growth of the fungus was observed periodically. The pure colonies that developed from these infected leaves and stem bits were then transferred on the PDA slants aseptically.

Identification of pathogen

For the identification of pathogen, we made different slides from five to seven days old pathogen

cultures, and then these slides were examined under compound microscope in mycology lab in order to identify the pathogen. The pathogen was morphologically identified uner microscope. The morphological characteristics of the causal organism were studied on PDA media under artificial conditions. The important characters studied were as under:

Hyphae - shape, septation, width and colour Conidia - size, shape and type of fruiting body.

Fig. 1: Grape leaf and stem showing Anthracnose disease symptom

Pathogenicity

To prove the pathogenicity of isolated pathogen, detached leaf technique and detached twig method was used to confirm Koch's postulates.

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× 104 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. The organism was reisolated from these artificially inoculated leaves and the culture so obtained was compared with the original culture.

Detached twig method

Healthy grape vine twigs bearing leaves were identified from healthy grapevine cultivar Anab-e-shahi and cutted with sterilized pruning scissor, surface sterlized and immediately put in sterilized conical flask containing water. Then such twigs were inoculated with a spore suspension of 2×104 conidia/ml by ordinary baby sprayer, in ten different sterilized conical glass flasks and incubated in humid chamber. Uninoculated twigs sprayed with sterilized water were also maintained as control. Observations were made at regular intervals for symptom development. The organism was reisolated from these artificially inoculated twigs and the culture so obtained was compared with the original culture.

Management trial

***In vitro* evaluation of fungicides against the mycelial growth of *Sphaceloma ampelinum* [telemorph: *Elsinoe ampelina*] by applying poisoned food technique**

Five different fungicides namely thiophenate 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 (Nene and Thapliyal. 1993). 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. The fungicides were thoroughly mixed with the medium by shaking with hands before autoclaving. After mixing the fungicides autoclave was done. About 15 ml of sterilized medium was poured in each 9 cm sterilized petridish. 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 incubited at 28 C and data on the radial colony diameter was recorded after 4-5 days of incubation when the 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 petridishes.

Inhibition of radial growth was computed based on colony diameter on control plate using the

following formula as stated by Sundar *et al.* (1995).

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

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

***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,* during the year of 2017-2018 in the research area of plant pathology department. 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 pipetted out or weighted with the help of micro-balance and properly dissolved in water in order to get desired concentrations of these chemicals. Spraying was properly done with the help of handheld sprayer which was manually operated. The systemic fungicides which were used against the anthracnose disease of grapes are given below:

Treatments	Fungitoxicant
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 Chatta (1992), similarly PDI (percent disease intensity) was recorded by using the Wheeler's farmula (1969). The obtained data was statistically analyzed.

Disease incidence

The disease incidence was recorded by counting the numbers of infected or diseased units over total number of units multiply by hundred, as the formula for recording the disease incidence is given below:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased unit}}{\text{Total number observed}} \times 100$$

Disease intensity

The disease intensity was recorded by visual observations using 0-4 scale (Fig. 2) adopted by Chatta (1992) with slight modifications. The Chatta scale is given below.

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

Per cent disease intensity (PDI) was recorded by using formula:

$$\text{PDI} = \frac{\text{Sum total of numerical rating}}{\text{Total units observed} \times \text{Maximum numerical value}} \times 100$$

Results and discussion

The results of the experiments undertaken during the year 2017-2018 on the Isolation, Idendification and Pathogenicity of anthracnose of grapevine caused by *Spaceloma ampelinum* (telemorph: *Elisinoe ampelina*) and its possible management are presented as under.

Symptomatology

Symptomatology of anthracnose of grapes under natural conditions of infection in field was observed on unsprayed vines of susceptible grape cv. Anab-e-shahi during the year 2017 and 2018. During the periodic observations of marked vine, the initial symptoms were noticed in the first week of May. The first evidence of the disease appeared on the young leaves having small, circular to irregular dark brown spots gradually attaining 1-2 mm dia. in size. As the disease developed the central portion of these spots turned grayish with the dark brown margins. Eventually the central necrotic gray tissue dropped out causing typical shot hole symptoms on the leaves. In severe cases the complete drying of the leaves was noticed. On the petioles and tendrils of such affected leaves/shoots light brown circular spots were noticed that became elliptical sunken necrotic cankers in the later stage of disease development which rapidly grew in size. These sunken lesions produced ashy gray center with a dark rim in advanced stage completely

girdling the canes. Often such shoots exhibited weak restricted growth. The infection extending to rachis and pedicels of the inflorescence resulted in shriveling and drying up of the inflorescence and eventual shedding of the floral buds. On berries the lesions were initially circular, light brown that turned dark brown to black spots resembling bird's eye. The center of the lesion on the berries was initially violet which later turned to ashy gray with dark margins. In advance stage, disease produced sour rot in berry.

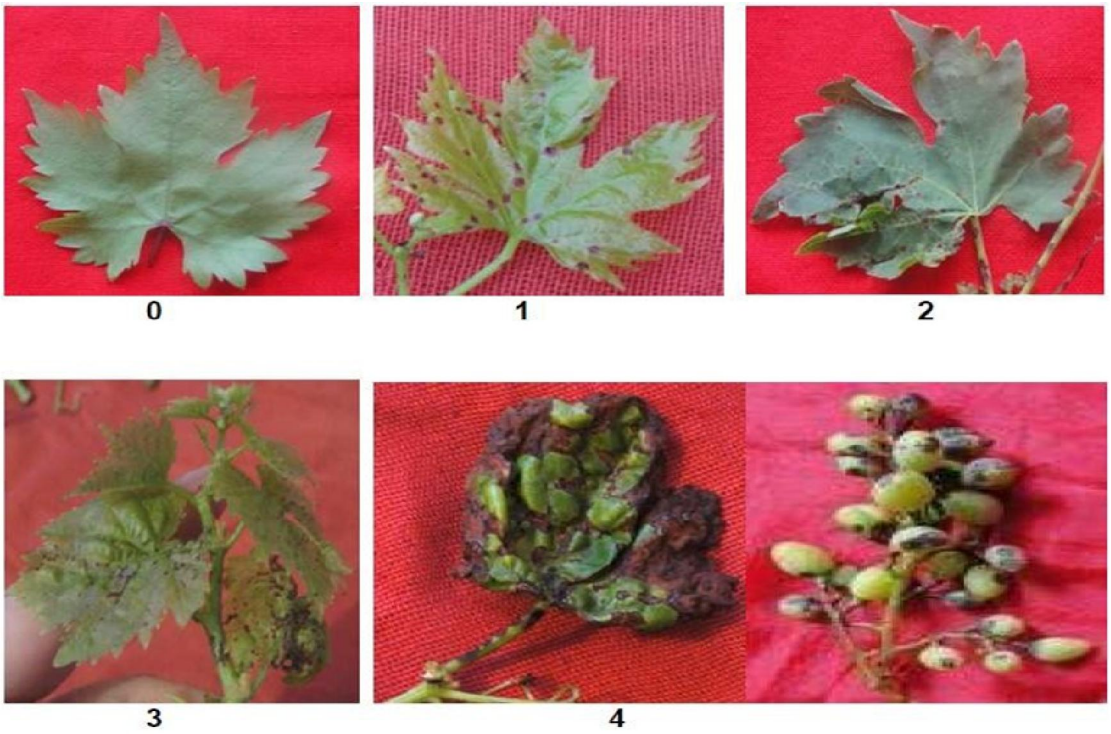

Fig. 2: Scale (0-4) used for assessment of grapes anthracnose intensity

Fig. 3: Development of anthracnose disease symptoms on grape leaves, stems and berries.

Isolation and Idenification of causal pathogen

Disease samples collected from the surveyed areas during the months of May-June 2017- 2018 were brought to laboratory for isolation as well as identification of the causal pathogen following the procedure described under Materials and Methods. The results revealed that S*phaceloma ampelinum* de Bary (telemorph: *Elisinoe ampelina* Shear) was yielded from the diseased samples.

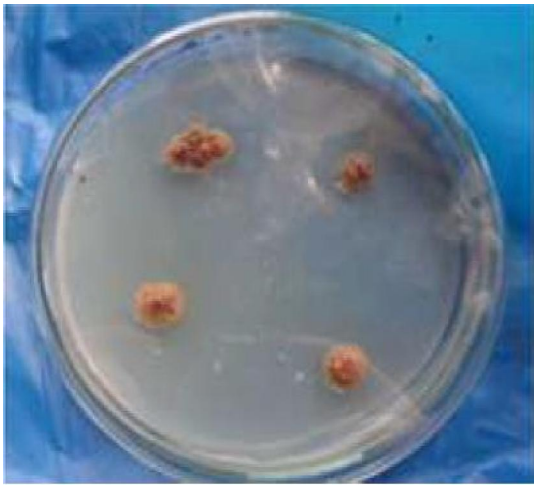

Fig. 4: Colony of *Sphaceloma ampelinum* (telemorph: *Elsinoe ampelina*) on PDA

Morphological characters of the fungus

The various morphological characters of the pathogen as observed on PDA were as under:

Colony appeared as circular mound with irregular margins. Mounds were brick red in colour. *Sphaceloma ampelinum* was found to be very slow growing with the colony size of 30.66 mm attained after 40 days of incubation. Mycelium was branched, septate, hyaline- pale brown measuring 3.02 µm in width. The fungus sporulated moderately <20-50 spores/microscopic field (450x). Fruiting body was disc shaped acervulus measuring 60µ in width and 35µ breadth at 450x. Conidiophores were infrequently formed. Pale brown- hyaline, single celled, and circular to oblong measuring 5.6-8.9 × 2.3-4.5 µm conidia. No perfect or sexual stage of the fungus was found.

4.5 Pathogenicity

The conidial suspension of isolated fungus was inoculated on the susceptible grape cultivar, Anab-e-shahi following "detached leaf technique" and "detached twig method" as described in the Materials and Methods. The characteristics symptoms appeared on the grape leaves after six days of inoculation showing minute brown specks initially along the margins of the leaf lamina that gradually developed into dark brown lesions with white/gray center. Reisolations were carried out from these lesions and comparisons were done with the original culture to confirm the identity of the pathogen. The pathogen *Sphaceloma ampelinum* de Bary (Shear) so confirmed by the pathogenicity test and authenticated literature was taken for further study.

Fig. 5: Colony of *Sphaceloma ampelinum* (telemorph: *Elsinoe ampelina*)

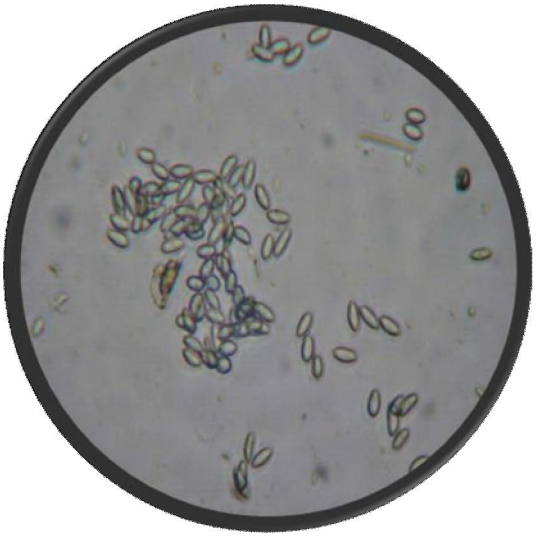

Fig. 6: Spores of *Sphaceloma ampelinum* (telemorph: *Elsinoe ampelina*)

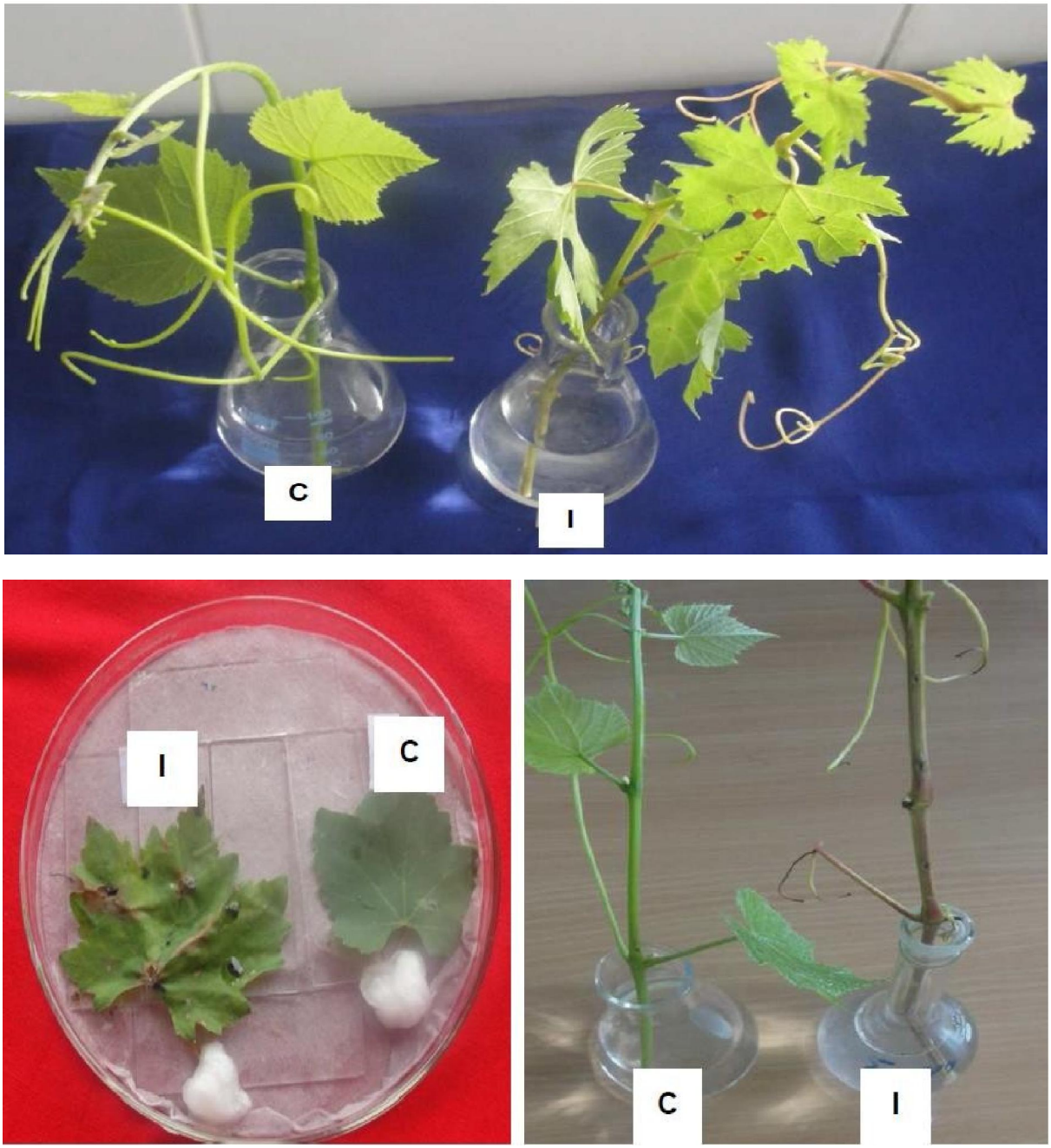

Fig. 7 Pathogencity test of *Sphaceloma ampelinum*
I = inoculated
C = uninoculated

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

Fungitoxic effect of five fungicides viz Diniconazole, Thiophanate-methyl, Myclobutanil, Difenoconazole and Hexaconazole at three different concentrations as 50ppm, 100ppm and 200ppm were evaluated in case of *in vitro* condition through poisoned food technique. The results of five fungicides against *Sphaceloma ampelinum* have given in the table 2 and 3 respectively. The effects of five fungicides were found to be greatly varied (Table 2 and 3). Generally there was a significant reduction in the mycelial growth of *Sphaceloma ampelinum* with increase of fungicides concentrations. However, when the fungus growth at various concentrations of fungicides at the incubation period of 5 days at 25°C compared on comparison basis of 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.

The data on the effect of various concentrations of different fungicides (Hexaconazole, Diniconazole, Thiophanate-methyl, Myclobutanil and Difenoconazole) is given in Table 4.3. The plates without fungicides served as control. All of the fungicides inhibited the mycelial growth of *Sphaceloma ampelinum* invariably. After five days of incubation, Hexaconazole was found to be the most effective in reducing the mycelial growth of *Sphaceloma ampelinum* with disease inhibition zone vale of 0.49cm at the concentration of 200ppm followed by Myclobutanil, Diniconazole, Thiophanate-methyl and Difenoconazol with disease inhibition zone vale of 0.57cm, 0.77cm, 0.86cm and 3.23cm at the same concentration (200 ppm).

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

Table. 1.: ANOVA of Systemic fungicides used under lab condition

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			

**Highly significant at P ≤ 0.05%

Table. 2.: LSD All-pairwise comparisons test of mean myclial growth (cm) for treatment

S.N	**Treatment**	**Mean Myclial 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	

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

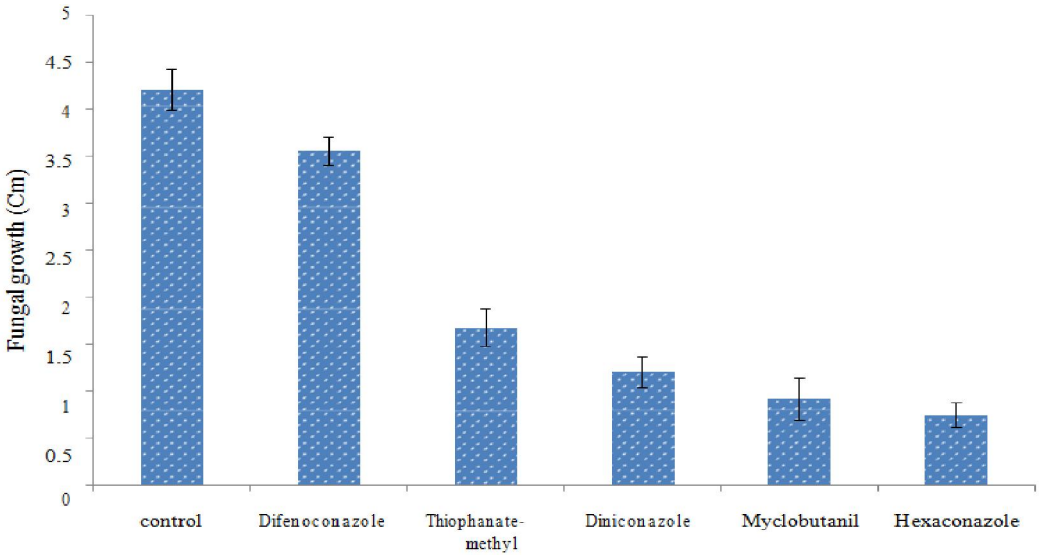

Fig 8: Effect of different Systemic fungicides against the colony growth (cm) of ***Sphaceloma ampelinum***

Table. 3.: LSD All-pairwise comparisons test of mean myclial growth (cm) for concentration*treatment

Treatments	Mean colony growth (cm) at various concentration		
	50ppm	100ppm	200ppm
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

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

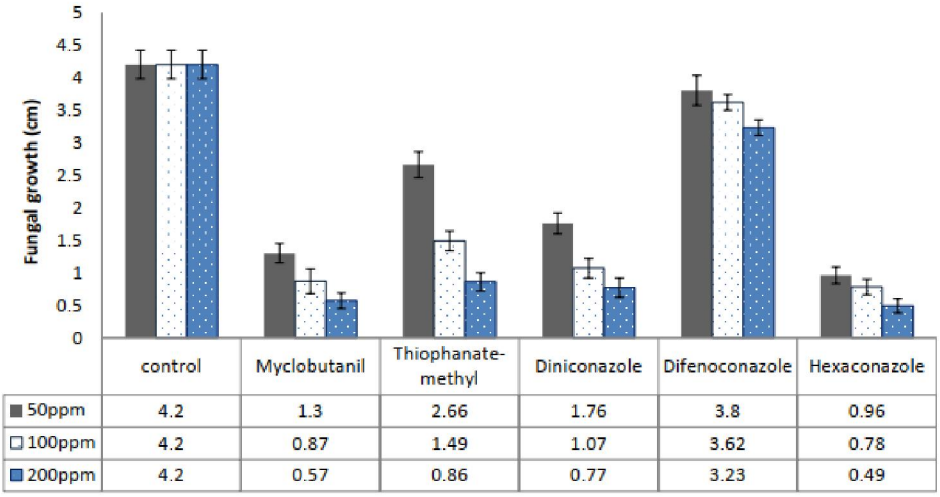

Fig 4.3: Effect of different concentrations of fungicides against the colony growth (cm) of *Sphaceloma ampelinum*

Table. 4.: ANOVA Table for disease incidence under field condition (used five systemic fungicides)

Source of variance	DF	SS	MS	F	P
Concentration	2	646.7	323.34	347.73	0.0000**
Treatments	5	20354.7	4070.93	4378.00	0.0000**
Week	2	4920.5	2460.24	2645.81	0.0000**
CxT	10	411.3	41.13	44.23	0.0000**
CxW	4	25.6	6.40	6.88	0.0001*
TxW	10	4522.3	452.23	486.34	0.0000**
CxTxW	20	43.4	2.17	2.33	0.0029*
Error	106	98.6	0.93		
Total	161	31083.0			

** = Highly significant at $P \leq 0.05\%$
* = Significant at $P \leq 0.05\%$

Table. 5.: LSD All-Pairwise comparisons Tests of disease incidence for treatment

Treatments	Mean Disease Incidence %	Homogeneous Groups
Control	75.701	A
Difenoconazole	48.37	B
Thiophanate-methyl	47.96	BC
Diniconazole	47.56	CD
Myclobutanil	47.13	D
Hexaconazole	40.93	E

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

Greenhouse evaluation of different fungicides against anthracnose disease of grapes by foliar spray

Five fungicides namely Diniconazole, Thiophanate-methyl, Myclobutanil, Difenaconazole and Hexaconazole were further evaluated to check their efficiency in greenhouse condition in order to control anthracnose disease of grape. Three sprays of three different concentrations had done at seven days interval and the data on disease incidence was recorded after seven days interval for each fungicide which was sprayed on the diseased grapes plant. The data showed that fungitoxic effect of different concentrations of treatments in reducing of anthracnose disease of grapes had been found to be greatly varied from each other. (Table 5 and 6).

Among all fungicides, Hexaconazole and Myclobutanil were found to be the most effective fungicides in controlling the disease incidence of anthracnose as compare to other, while Difenaconazole was found to be less effective in controlling anthracnose disease incidence.

When the three concentrations compared on the basis of means of disease incidence after 3rd spray, it showed that 3rd concentration of Hexaconazole and Diniconazole was found to be highly effective in the reducing Anthracnose disease of grapes followed by Myclobutanil, Thiophanate-methyl and Difenaconazole (Table 6).

Analysis of variance showed that there was no any statistical difference between the effectiveness of Diniconazole and Difenaconazole at 1st concentration, Diniconazole, Thiophanate-methyl and Difenaconazole at 2nd concentration and similarly Diniconazole and Myclobutanil were statistically equally effective at 3rd concentration (Table 6).

Table # 6: LSD All-Pairwise comparisons Tests of disease incidence for concentration*treatment

S.N	Treatments	Mean Disease incidence (%) at various concentrations		
		0.25%	0.5%	1%
1	Diniconazole	51.44 de	47.58 f	43.68 ij
2	Thiophanate- methyl	51.87 d	47.77 f	44.23 i
3	Myclobutanil	50.67 e	46.58 g	44.14 ij
4	Difenoconazole	51.53 de	48.29 f	45.30 h
5	Hexaconazole	43.28 j	40.88 k	38.62 l
6	Control	73.81 c	76.00 b	77.28 a

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

Table.7: LSD All-Pairwise comparisons Tests of disease incidence for treatment*weeks

S.N	Treatments	Mean Disease incidence (%) at three different weeks		
		Week 1	Week 2	Week 3
1	Diniconazole	57.74 e	48.91 g	36.04 l
2	Thiophanate-methyl	60.21 d	46.85 h	36.81 kl
3	Myclobutanil	55.61 f	48.16 g	37.62 k
4	Difenoconazole	57.18 e	48.89 g	39.04 j
5	Hexaconazole	48.38 g	40.23 i	34.16 m
6	Control	68.81 c	75.01 b	83.27 a

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

Table. 8: LSD All-Pairwise comparisons Tests of disease incidence for concentration*treatment*week

S. N	Treatments	Mean Disease incidence (%) at various weeks								
		Week 1			Week 2			Week 3		
		0.25 %	0.5 %	1 %	0.25 %	0.5 %	1 %	0.25 %	0.5 %	1 %
1	Diniconazole	61.17 i	57.0 6 k	55.0 0 lm	52.34 n	49.1 6 pq	45.2 4 s	40.81 u	36.5 2 xy	30.7 9 C
2	Thiophanate- methyl	64.11 h	60.0 6 ij	56.4 6 kl	51.32 no	46.6 0 rs	42.6 4 t	40.18 uv	36.6 6 x	33.5 8 za
3	Myclobutanil	57.57 k	55.2 6 lm	54.0 1 m	51.45 no	47.7 6 qr	45.2 9 s	43.01 t	36.7 4 x	33.1 3 ab
4	Difenoconazol e	59.44 j	57.2 9 k	54.8 1 m	51.76 n	48.8 7 pq	46.0 5 s	43.38 t	38.7 1 vw	35.0 4 xy
5	Hexaconazole	50.11 op	48.6 7 pq	46.3 7 rs	43.37 t	39.4 4 uvw	37.8 9 wx	36.37 xy	34.5 3 yz	31.6 0 bc
6	Control	66.55 g	69.4 4 f	70.4 4 f	73.36 e	75.3 6 d	76.3 2 d	81.51 c	83.2 2 b	85.0 7 A

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

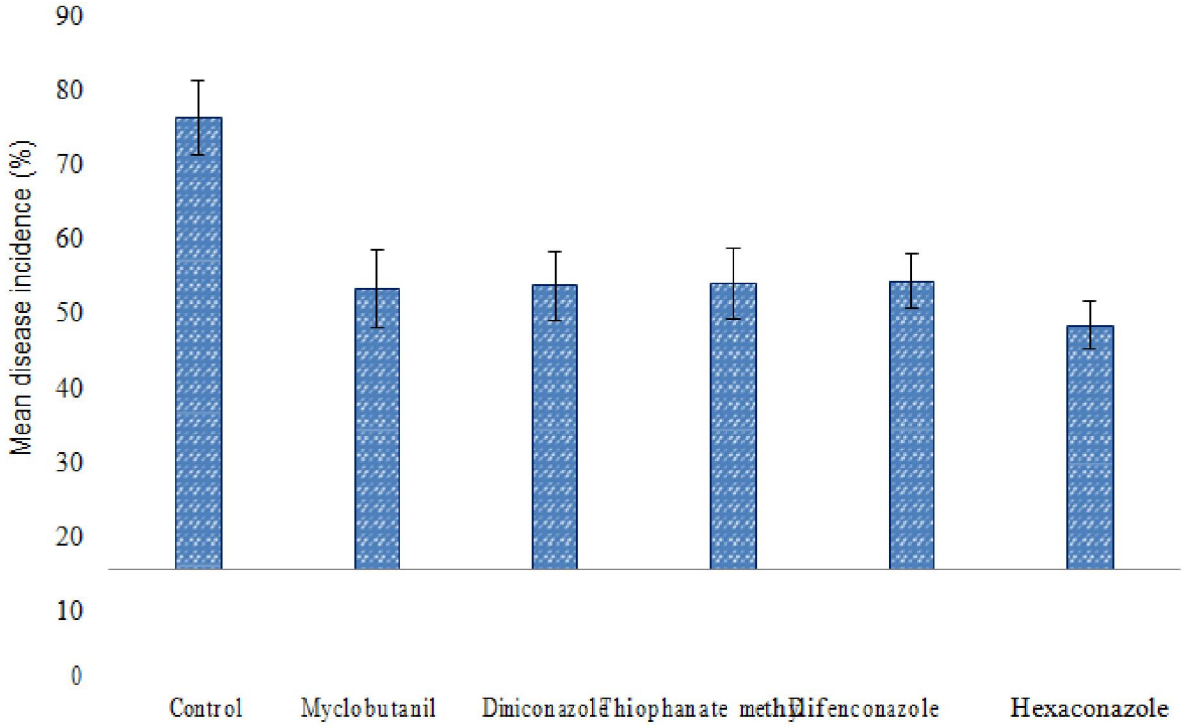

Fig 10: Effect of fungicides on percent Disease Incidence under greenhouse condition

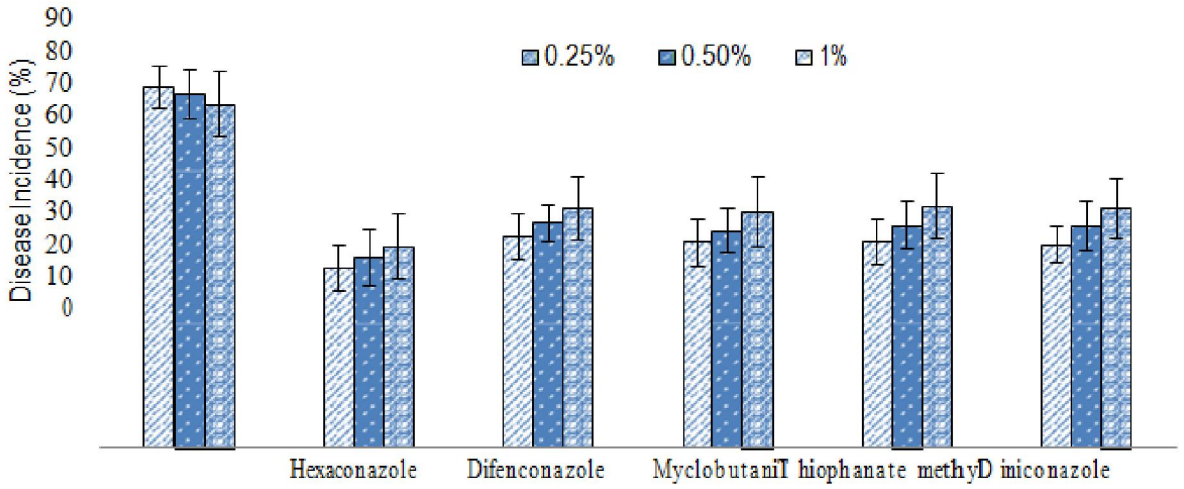

Fig 11: Effect of different concentration of fungicides on percent Disease Incidence under greenhouse condition

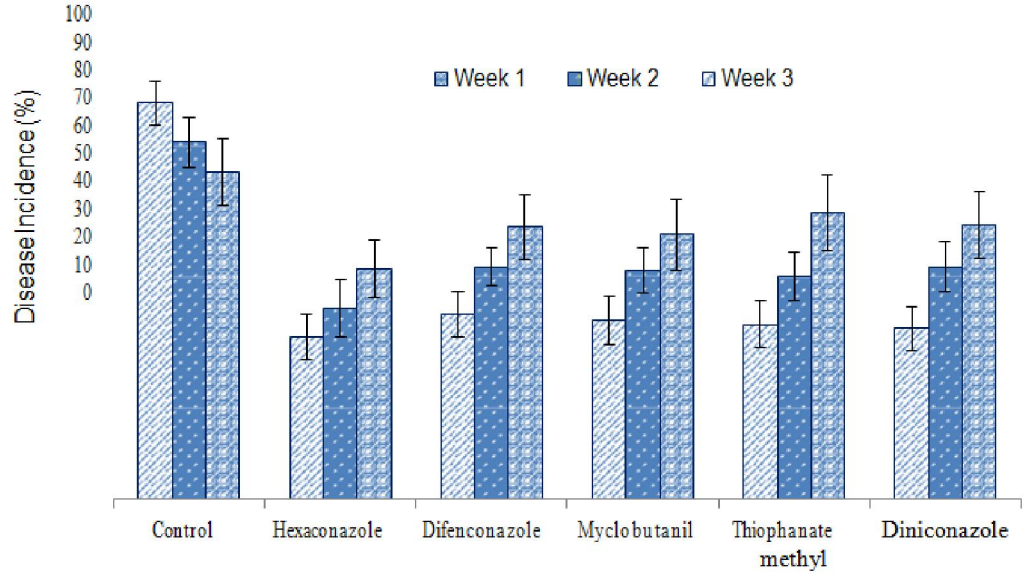

Fig 12: Effect of weeks and fungicides on Percent Disease Incidence under greenhouse condition

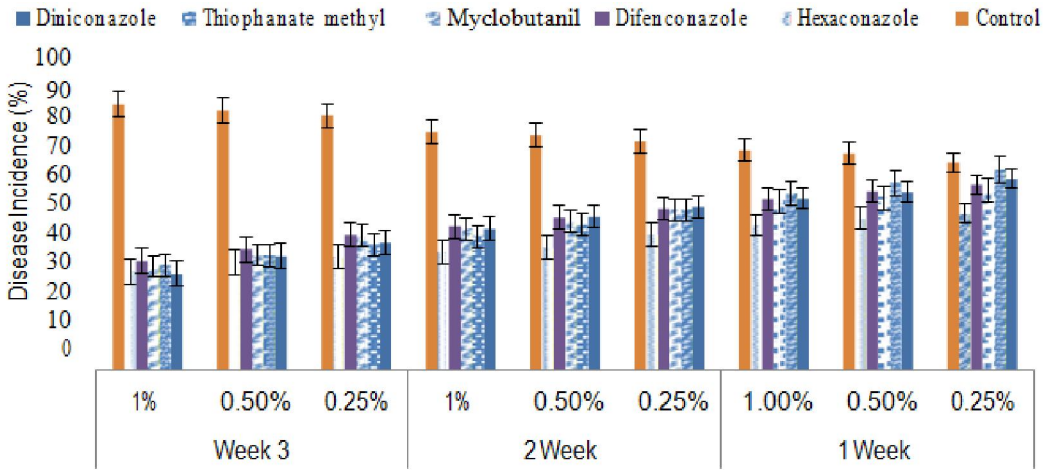

Fig13: Effect of different concentrations of fungicides on percent Disease Incidence under greenhouse condition

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