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# *In-vitro* evaluation of selected fungicides against *Elsinoe ampelina* causing anthracnose of grape

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**Abstract**
Anthracnose is a major disease of grape in sub-tropical region for which non chemical management is not found to be much effective causing economical losses. In order to reduce the losses and maximize the yield without making much harm to the eco system, an attempt was made to study the efficacy of five systemic, four non systemic and five combi fungicides at different concentration in inhibiting the mycelial growth of *Elsinoe ampelina* under *in-vitro* condition. Among all, used fungicides, Hexaconazole (100%) was found most effective treatment in inhibition of fungal mycelium followed by Carbendazim (97.32%) which was found significantly superior over rest of the treatments. Least mycelial inhibition was found in the treatments involving copper oxychloride (55.04%).

**Keywords:** Anthracnose, *Elsinoe ampelina*, fungicides, grapes

## Introduction
Grape (*Vitis vinifera* L.) is botanically a berry, is among the ancient plants on earth and is one among the most consumed fruit crop worldwide. Being the major producer and ranked 9th position in grape production (Shikamany, 2001; Gade *et al*., 2014) [7, 2], Indian grape production suffers from abiotic and biotic stresses. Biotic stress such as anthracnose is a major fungal infection caused by *Elsinoe ampelina*, also referred as "Bird's eye spot" following powdery mildew and downy mildew.

Pliny from Italy was the first to refer the disease in the 1st century of Christian era and later reported by Burrill during 1886 from Illinois, U.S.A. It was witnessed that the disease was existing in Europe for centuries and was introduced into the United States through cuttings / young vines from European grapes. The pathogen was first noticed in 1903 near Pune in India (Butler, 1905) [1]. The disease is widely predominant in Maharashtra, Karnataka, Punjab, Haryana and Andhra Pradesh.

The pathogen affects all the above ground parts in the green stage, mostly on the new shoots and fruits. Small spherical to irregular, 2-6 mm dia. in size, dark brown spot appear on leaves which future turn gray in the middle and dark brown at the borders. The central necrotic tissue often falls off leaving a shot-hole presence. In severe cases the complete driving of leaves is also noticed. On shoots and tendrils, small isolated brown spots develop which stretch to from ovoid, slightly depressed lesions. Later, the central area of the abrasion develop into ashy-gray colour bordered by darker rim. The affected young branch may be limited in growth as well as shedding of inflorescence / flower buds takes place due to infection. On berries, typical bird's eye spot symptoms appear having violet to gray center and dark brown margins.

In the recent years, there has been major thrust on residue free grape production. For this, botanicals and bio agents are being used, which are not must effective in managing the disease profitably. In view of this, different fungicides and there combination at different concentration were tested for their efficacy and to fit in with the management schedule of the disease suitably.

## Materials and Methods
Collection, isolation and maintenance of *Elsinoe ampelina:* Grape leaves and young branch of Chenin Blanc and Chardonnay which had symptoms of anthracnose pathogen were poised from the grape vineyard of Alpine Wineries Private Limited, Mysore and other wine grape growing regions of Karnataka and Maharashtra during the survey and were used for isolation of the fungus under *in-vitro* condition. The diseased samples were washed thoroughly under the tap water and allowed to dry in shade under laboratory conditions.

The infected portion along with some healthy part was cut into small pieces, and superficially sanitized and then transferred aseptically to Petri plates containing Potato Dextrose Agar (PDA) media. The inoculated Petri plates were then incubated at 28 ± 1˚ C and progress of pathogen was observed periodically. The pure colonies that developed from these infected leaf/stem bits was transferred onto the PDA slants aseptically. The fungus was sub cultured on PDA slants and allowed to grow at 28±1˚C for one week and such slants were preserved in refrigerator at 4˚C and were renewed once in two months. The preserved culture was used for further experiments.

**Evaluation of different fungicides against *Elsinoe ampelina* under *in-vitro* condition**

Evaluation of four each of non–systemic and systemic fungicides were conceded for their usefulness to inhibit the mycelial growth of *Elsinoe ampelina* by "poisoned food technique" as described by Schmitz (1930) [6]. The experiment was carried out using Completely Randomized Designs (CRD) and the data will be analyzed statistically.

The non-systemic fungicides were used at 0.1, 0.2 and 0.3 per cent concentration while the systemic fungicides were tested at 0.05, 0.10 and 0.15 percent concentration (Table 1). The requisite quantities of fungicides were added aseptically to 60 ml PDA medium which were cooled to 45 ºC so as to give required concentrations. Twenty ml of the poisoned medium was poured into sterile Petri plates. After solidification, the plates were then inoculated with five mm mycelial discs cut by a sterile cork borer from 18 days old culture of the test fungus and incubated at 28± 1 ºC. Three replications were maintained for each treatment. The PDA without any fungicides served as control. The radial growth of the colony was recorded when maximum growth was absorbed in control and it was calculated by using the following formula (Vincent, 1927) [8].

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

**Where**

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment

**Results and Discussion**

**Evaluation of fungicides against *E. ampelina* under *in-vitro* condition:** Five systemic, four non systemic and five combi fungicides were evaluated at 3 different concentrations under *in-vitro* condition for their effectiveness in inhibiting mycelial growth of *E. ampelina* using poisoned food technique. The per cent inhibition over control was worked out based on the mycelial growth in control plates.

**Systemic fungicides**

All the fungicides evaluated were significantly superior over the control with respect to per cent mycelial inhibition. Amongst the systemic fungicides tested at three concentrations (0.05%, 0.1% and 0.15%) complete fungal inhibition was recorded in treatments comprising hexaconazole at all the concentration, Carbendazim at 0.10 and 0.15% concentration and propiconazole at 0.15% concentration which found significantly superior over rest of the treatments. The minimum inhibition of mycelial growth was witnessed in Difenconazole (77.65%) at 0.05% (Table 2). Irrespective of concentration of fungicides tested, the treatment involving hexaconazole recorded maximum mean% mycelial inhibition (100%) followed by carbendazim (97.32%) and propiconazole (94.04%) and least% mycelial inhibition (86.13%) was recorded with Difenconazole.

**Non Systemic fungicides**

Amongst the non-systemic fungicides examined at three concentrations (0.1%, 0.2% and 0.3%). The maximum fungal reticence was recorded in treatments comprising mancozeb at all concentration which found significantly greater over rest of the treatments and it was followed by 85.68% and 83.66% inhibition by chlorothalonil at 0.3 and 0.2%, respectively. The least inhibition of mycelial growth was observed in Copper Oxy Chloride (45.29%) at 0.1% (Table 3). Irrespective of concentration of fungicides tested, the treatment involving mancozeb recorded maximum mean% mycelial inhibition (91.67%) followed by chlorothalonil (81.38%) and least% mycelial inhibition (55.04%) was recorded in Copper Oxy Chloride at 0.1%.

**Combi fungicides**

Amongst the five combi-fungicides tested at three concentrations (0.05%, 0.1% and 0.2%) complete mycelial inhibition was documented in treatment with Captan 70% + Hexaconazole 5% (Taqat) and Hexaconazole 4% + Zineb 68% (Avatar) at all concentration and was found significantly superior over all other treatments. The least% inhibition of mycelial growth (77.65) was witnessed with the blend of Trycyclazole 18% + Mancozeb 62% (75.73) and Carboxin 37.5% + Thiram 37.5% at 0.05% concentration. Irrespective of concentration of fungicides tested (Table 4), the treatment involving Hexaconazole 18% + zineb 68% and captan 70% + hexaconazole 5% recorded maximum mean per cent mycelial inhibition (100%) which was found to be at par with Carbendazim 12% + Mancozeb 63% (98.34%) and least percent mycelial inhibition (82.55%) was recorded in Carboxin 37.5% + Thiram 37.5% WP.

In the present research, 5 systemic fungicides (0.05%, 0.1% and 0.15%), four non systemic fungicides (0.1%, 0.2% and 0.3%) and five combi products (0.05%, 0.1% and 0.2%) were examined for their antifungal action against anthracnose of grapes. The systemic fungicides of hexaconazole at all the concentration, carbendazim at 0.10 and 0.15 per cent concentration and propiconazole at 0.15 per cent concentration were showed complete inhibition of mycelial growth and non-systemic fungicides of mancozeb at all concentrations and chlorothalonil at 0.2 and 0.3 per cent were showed maximum inhibition and combi product of Captan 70% + Hexaconazole 5% (Taqat) and Hexaconazole 4% + Zineb 68% (Avatar) at all concentration showed complete inhibition of mycelia and these results were confined with the findings of Gawade *et al*. (2009) [3] who reported that carbendazim found the highest mean inhibition (90.59%) of mycelial growth of *Colletotrichum truncatum*, followed by Propiconazole (87.95%), Hexaconazole (86.15%), Difenconazole (84.81%) and Chlorothalonil (70.23%).

Similar results were found by Ramani *et al*. (2015) [5] who reported that, carbendazim 50 per cent WP at 50 ppm, copper oxychloride 50 per cent WP at 500 ppm and combination of carbendazim 12 per cent WP + mancozeb 63 per cent WP at 100 ppm concentrations were found to be utmost effective.

Parvathy and Girija (2016) [4] reported that maximum mycelial inhibition was obtained with tebuconazole (0.1%) and combination fungicide mancozeb+ carbendazim (0.1%) which gave 100% growth inhibition over the control among the seven fungicides tested against colletotrichum gloeosporioides causing anthracnose of black pepper.

**Table 1:** Details of treatments (fungicides)

| Sr. No. | Common name | Trade name |
|---|---|---|
| Systemic fungicides | | |
| 1 | Carbendazim | Bavistin 50 WP |
| 2 | Difenconazole | Score 25 EC |
| 3 | Hexaconazole | Contaf 5 EC |
| 4 | Propiconazole | Tilt 25 EC |
| 5 | Teboconazole | Folicure 250 EC |
| **Non systemic fungicides** | | |
| 1 | Capton | Captaf 75 WP |
| 2 | Copper oxy chloride | Blitox 50 WP |
| 3 | Chlorothalonil | Kavach 75 WP |
| 4 | Mancozeb | Indofil M-45 |
| **Combi products** | | |
| 1 | Captan 70% + Hexaconazole 5% WP | Taqat |
| 2 | Carbendazim 12% +Mancozeb 63% WP | Saaf |
| 3 | Carboxin 37.5% + Thiram 37.5%WP | Vitavax power |
| 4 | Hexaconazole 4% + Zineb 68% WP | Avatar |
| 5 | Trycyclazole 18% + Mancozeb 62% WP | Merger |

**Table 2:** Effect of systemic fungicides against *Elsinoe ampelina* under *in-vitro* condition

| Systemic fungicides | % inhibition of outward growth over control | | | Mean |
|---|---|---|---|---|
| | Concentration (%) | | | |
| | 0.05 | 0.1 | 0.15 | |
| Carbendazim | 91.96 (73.47) | 100.00 (90.00) | 100.00 (90.00) | 97.32 (84.49) |
| Difenconazole | 77.65 (61.86) | 89.04 (70.68) | 91.70 (73.27) | 86.13 (68.60) |
| Hexaconazole | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| Propiconazole | 89.28 (70.85) | 92.85 (74.45) | 100.00 (90.00) | 94.04 (78.43) |
| Teboconazole | 85.67 (67.77) | 89.71 (71.26) | 91.96 (73.47) | 89.11 (70.83) |
| Mean | 88.91 (72.79) | 94.32 (79.28) | 96.72 (83.35) | |
| | S.Em± | | CD at 1% | |
| Treatments (T) | 0.99 | | 3.90 | |
| Concentrations (C) | 0.72 | | 2.80 | |
| T × C | 1.78 | | 7.02 | |

**Table 3:** Effect of non-systemic fungicides against *Elsinoe ampelina* under *in-vitro* condition

| Non Systemic fungicides | Per cent inhibition of radial growth over control | | | Mean |
|---|---|---|---|---|
| | Concentration (ppm) | | | |
| | 0.1 | 0.2 | 0.3 | |
| Captan | 55.68 (48.24) | 62.05 (51.98) | 66.67 (54.71) | 61.46 (51.64) |
| Chlorothalonil | 74.81 (59.89) | 83.66 (66.17) | 85.68 (67.48) | 81.38 (64.51) |
| Copper Oxy Chloride | 45.29 (42.29) | 57.78 (49.46) | 62.05 (51.98) | 55.04 (47.91) |
| Mancozeb | 88.71 (70.02) | 91.29 (73.01) | 95.03 (77.17) | 91.67 (73.40) |
| Mean | 66.12 (55.11) | 73.69 (60.15) | 77.36 (62.83) | |
| | S.Em± | | CD at 1% | |
| Treatments (T) | 0.12 | | 0.47 | |
| Concentrations (C) | 0.10 | | 0.41 | |
| T × C | 0.21 | | 0.82 | |

**Table 4:** Effect of combi fungicides against *Elsinoe ampelina* under *in-vitro* condition

| Combi fungicides | Per cent inhibition of radial growth over control | | | Mean |
|---|---|---|---|---|
| | Concentration (%) | | | |
| | 0.05 | 0.10 | 0.2 | |
| Captan 70% + Hexaconazole 5% WP (Taqat) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| Carbendazim 12% +Mancozeb 63% WP (Saaf) | 95.03 (77.17) | 100.00 (90.00) | 100.00 (90.00) | 98.34 (85.72) |
| Carboxin 37.5% + Thiram 37.5%WP (Vitavax power) | 77.65 (61.86) | 82.72 (65.41) | 87.28 (69.08) | 82.55 (64.11) |
| Hexaconazole 4% + Zineb 68% WP (Avatar) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| Trycyclazole 18% + Mancozeb 62% WP (Merger) | 75.73 (60.46) | 87.28 (69.08) | 88.72 (70.03) | 83.91 (66.52) |
| Mean | 89.68 (75.89) | 94.00 (80.90) | 95.20 (81.82) | |
| | S.Em± | | CD at 1% | |
| Treatments (T) | 0.24 | | 0.94 | |
| Concentrations (C) | 0.19 | | 0.73 | |
| T × C | 0.42 | | 0.94 | |

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