

## Effect of Azoxystrobin 8.3%w/w + Mancozeb 68.75% on Powdery and Downy mildew Pathogens under *In vitro* condition

<sup>1</sup>Pannerselvam Ahila devi, Karikalan Vinothini<sup>1</sup> and <sup>1</sup>Velapagounder Prakasam  
Department of Plant Pathology, Centre for Plant Protection Studies Tamil Nadu Agricultural University,  
Coimbatore - 625 104

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**Abstract:** Field surveys were conducted in 3 major grapevine growing districts in Tamil Nadu viz., Coimbatore, Theni and Dindugal. The downy mildew infection on leaves was the highest (PDI 76.92 in Cumbum of Theni district. In the survey on the incidence of powdery mildew showed that the highest incidence of PDI on leaves, inflorescence and fruits in Cumbum of Theni with records of 65.49, 63.56, 61.23 percent. The pathogenicity test revealed that the downy mildew isolate D-TC1 of Theni district was found to be more virulent with highest PDI of (42.35). The powdery mildew isolate of Cumbum (P-TC1) of Theni district was found to be more virulent by recording the maximum PDI of (36.94). Among the different fungicide tested against spore germination of *P. viticola*, Azoxystrobin 8.3%w/w + Mancozeb 68.75 at 0.36 per cent recorded 89.26 % inhibition. The fungicide Azoxystrobin 8.3% + Mancozeb 68.75 in all three concentrations, was found to be superior in inhibiting the spore germination of *U. necator* by recording 90.27%, 89.91%, 80.70% respectively in 0.36, 0.30 and 0.24 % concentrations.

**Key words:** Grapevine – pathogenicity- Spore germination – Azoxystrobin – Mancozeb

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### I. Introduction

Grapevine (*Vitis vinifera* L.) is an important commercial fruit crop and one of the most widely cultivated crop in temperate, sub-tropical and tropical regions of the world. There are over 8,000 grape varieties in worldwide and grape appears in the top ten of the world's favourite fruits, along with tomatoes, mangos and bananas. Grapevine cultivation offers a great economic potential due to its higher yield and monetary returns owing to the export to Gulf, European countries and to some extent West Asian countries. Grape is basically a sub-tropical crop. However, in India, grapes are cultivated for their excellence also under tropical conditions. In world they are cultivated in an area of 7.2 m ha with a total production of 67.32 m tonnes during 2011. In India, grapevine is grown in an area of 111 thousand ha. India is the 12<sup>th</sup> largest grape producing country in the world with the production of 1235.00 thousand tonnes. (Indian Horticulture Database -2011[1]). Production of grapevines is threatened by biotic (viruses, bacteria, fungi and insects) and abiotic stresses (i.e. drought, winter cold). From these stresses fungal infections reduce mostly the yield and damage fruit and wine quality. Fungal diseases viz., downy mildew *Plasmopara viticola*, Powdery mildew *Uncinula necator* and Anthracnose *Elsinoe ampelina* are found to be the major constraints in grapevine cultivation.

Among the fungal diseases, downy mildew caused by *P. viticola* is the most destructive and explosive disease of grapevine. It has been recorded in 91 countries from temperate to tropical zones (CMI, 1988[2]). Powdery mildew caused by *U. necator* is an another important endemic fungal diseases on commercial grapevine varieties and it is not possible to harvest good quality fruits without prophylactic measures (Rao, 1991[3]). Recently several fungicides viz., propiconazole, fenarimol, bupirimate, penconazole, dimethomorph, triademeton, pyrazophos, hexaconazole, chlorothalonil and flusilazole were introduced in India for control of powdery mildew pathogens. In addition, several new molecules are also tested against downy mildew pathogens, most important ones are metalxyl and Fosetyl-Al group of fungicides. Ravikumar (2007[4]) reported that the application of Ridomil Gold 68 WG (metalaxyl M + mancozeb 4+64WG) @ 0.25 per cent reduced the incidence of downy mildew in grapevine up to 71 per cent. But there are certain strains of Oomycetous fungi, which are resistant to metalaxyl. So new fungicide molecule with novel mode of action is needed to replace the failing compounds.

Azoxystrobin is a systemic fungicide. It is absorbed through the roots and translocated in the xylem to the stems and leaves, or through leaf surfaces to the leaf tips and growing edges. Bartlett *et al.*, 2001[5] reported that the huge impact of the strobilurins on agriculture is well exemplified by the development of azoxystrobin which has now been registered for use on a broad spectrum of fungal diseases on 84 different crops in 71 countries, representing over 400 crop/disease systems

Mancozeb is a coordination product of Zinc ion and Manganese ethylene bis-dithio-carbamate. This compound has a negligible vapour pressure, therefore it has a low potential to volatilize into the air. In water, mancozeb can be quickly hydrolyzed with a half-life of less than 2 days. Mancozeb is used to protect many fruit, vegetable, nut and field crops against a wide spectrum of diseases, including potato blight, leaf spot, scab (on apples

and pears) and rust (on roses). It is also used for seed treatment of cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax and cereal grains Berg (1988).[6]

In the quest for find newer and more efficacious molecules, the present investigation was carried out using a new formulation. A combination of Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 w/w) of United Phosphorus Limited, Mumbai

The efficacy of the combined action may be much higher against the fungal pathogens with no risk of development of resistance against fungicides. With this background, the present study was carried out envisaging the Survey for the incidence of downy mildew and powdery mildew diseases of Grapevine in Tamil Nadu and to study the efficacy of Azoxystrobin 8.3 % + Mancozeb 64.7 against powdery and downy mildew pathogens.

## **II. Materials and methods**

### **1. Survey for the incidence of downy mildew and powdery mildew in Tamil Nadu**

An intensive survey was conducted during 2011 in some of the grapevine growing districts of Tamil Nadu viz., Coimbatore, Dindugal and Theni to assess the severity of the disease incidence of downy mildew and powdery mildew and per cent disease index was calculated as per the standard grade chart given by Jamdar and Desai (1997)[7]

#### **1.1 Raising of grapevine plants**

The variety Muscat, which is found to be susceptible to both downy mildew and powdery mildew was used for all studies in this investigation.

The grapevine variety Muscat was raised in pots by vegetative propagation under glass house conditions. The cuttings were collected from mature canes of healthy and virus – free vines. Depending upon the length of internodes in a cultivar, mature wood was cut into pieces of about 25-30 cm long which contained atleast four buds. The thickness of cuttings was 7-8 mm dia (pencil thickness). The lower end of the cuttings was soaked in 50 ppm of Rootex (Indole Butyric Acid) for 24 h. Then the cuttings were planted in mud pots containing red soil, sand and farmyard manure at 3:1:1 ratio. The pots were watered periodically.

#### **1.2 Collection and maintenance of inoculum of *Plasmopara viticola* and *Uncinula necator* in glass house**

Grapevine leaves showing typical downy mildew and powdery mildew symptoms were collected from different districts of Tamil Nadu. In the case of downy mildew sporangial suspension was prepared by harvesting the diseased leaves and flooding the leaves with sterile distilled water. The process was repeated three times to obtain sufficient inoculum. In the case of *U.necator* conidial suspension was prepared as described above. The sporangial and conidial suspension was strained separately through two layers of cheese cloth and centrifuged twice at 4000 rpm for 30 min. The sporangial and conidial concentrations were adjusted to  $5 \times 10^6$  per ml with sterile distilled water and used for maintaining the downy mildew and powdery mildew inoculum in glass house. To maintain the sufficient inoculum, grape vine plants in the glass house were sprayed with spore suspension, after making slight pin pricks. They were then covered with polythene bag for 24 h to maintain high humidity for disease development (Vimala, 2005)[8] The different isolates of downy mildew fungus was maintained for further studies.

#### **1.3 Pathogenicity of *P.viticola* and *U.necator* in grapevine leaves**

Sporangial suspension of downy mildew and conidial suspension of powdery mildew was prepared by scrapping the sporangia and conidia from the infected leaves of grapes. The sporangial and conidial suspension concentration was adjusted to  $5 \times 10^6$  per ml using a haemocytometer. These suspensions were sprayed separately on healthy leaves of grapevine in the Plant Pathology glass house. The plants were covered by polythene bag for 24 hours to maintain high humidity for disease development. The development of the disease was observed after 10 days of inoculation and PDI was worked out to identify the best isolate for further study.

#### **1.4 Evaluation of different fungicides**

A new formulation of Azoxystrobin 8.3%w/w + Mancozeb 68.75% w/w of United Phosphorus Limited, Mumbai was used for all the studies.

##### **1.4.1 Efficacy of Azoxystrobin 8.3%w/w + Mancozeb 68.75% and other fungicides against sporangial germination of *P.viticola***

In order to compare the efficacy of Azoxystrobin 8.3%w/w + Mancozeb 68.75% and other fungicides for the control of downy mildew of grapevine, preliminary screening of the fungicides was made. Leaves showing downy mildew growth were tapped at 5 AM to dislodge the preformed sporangia and were collected in a petri dish containing sterile water. A drop of fungicides UPF 509 at different concentration 0.24%,0.3%,0.36% and other fungicides

Mancozeb at 0.4%, Azoxystrobin at 0.1%, Hexaconazole at 0.6% and Metalaxyl+ Mancozeb at 0.5% was placed individually in a cavity slide and allowed to air dry and then a drop of the sporangial suspension ( $5 \times 10^6$  sporangia/ ml) was added and incubated at 20°C for six hours in moist Petri dishes under darkness. The sporangial suspension in sterile water served as control. The treatments were replicated thrice. Observations on the sporangial germination were recorded after six hours of incubation by counting the total number of sporangia and number of sporangia germinated in each microscopic field. Three such microscopic fields were observed and the mean per cent sporangial germination and per cent inhibition of sporangial germination were worked out (CSFT, 1943).[9]

#### 1.4.2 Efficacy of Azoxystrobin 8.3%w/w + Mancozeb 68.75% and other fungicides against conidial germination of *U. necator*

The efficacy of Azoxystrobin 8.3%w/w + Mancozeb 68.75% and other fungicide against the conidial germination of *U. necator* was assessed by Detached leaf technique (Varalakshmi *et al.*, 1999)[10]. Grapevine leaves were washed in sterile distilled water and air dried. Fungicide UPF 509 at different concentration 0.24%, 0.3%, 0.36% and other fungicides Mancozeb at 0.4%, Azoxystrobin at 0.1%, Hexaconazole at 0.6% and Metalaxyl+ Mancozeb at 0.5% were placed individually on the adaxial surface of the leaf and the droplets were evenly spread with a fine camel hairbrush and allowed to air dry. The treated leaves were inoculated with the conidia of *U. necator* ( $5 \times 10^6$  conidia/ ml). The leaves sprayed with the conidial suspension alone served as control. Three leaves from each treatment were transferred to a Petri dish with their petioles dipped in water and incubated at 20°C. After 72 h, the leaves were observed under the microscope (equipped with fine light arrangement) for conidial germination. The total and germinated conidia were counted in three microscopic fields and per cent inhibition of conidial germination was calculated.

#### 1.5 Statistical analysis

The data generated from various experiments of this study were statistically analyzed by DMRT with IRRISTAT software. The data with per cent values were subjected to arc sine transformation

**Table: 1. Survey for the incidence of downy mildew and powdery mildew in major grapevine growing areas of Tamil Nadu**

Name of the district	Name of the village	Downy mildew			Powdery mildew		
		Percent disease Index*					
		Leaves	Inflorescence	Fruits	Leaves	Inflorescence	Fruits
Coimbatore	Mathampatty	71.13 <sup>b</sup> (57.48)	69.94 <sup>b</sup> (56.73)	67.81 <sup>b</sup> (55.43)	60.91 <sup>bc</sup> (51.30)	59.34 <sup>b</sup> (50.36)	57.34 <sup>b</sup> (49.20)
Coimbatore	Theeethipalayam	65.14 <sup>d</sup> (53.79)	61.21 <sup>d</sup> (51.47)	59.18 <sup>d</sup> (50.24)	54.95 <sup>c</sup> (47.81)	53.28 <sup>d</sup> (46.83)	50.18 <sup>d</sup> (45.06)
Coimbatore	Theenampalayam	73.26 <sup>b</sup> (58.82)	70.32 <sup>b</sup> (56.98)	68.15 <sup>b</sup> (55.61)	61.56 <sup>b</sup> (51.65)	60.02 <sup>b</sup> (50.77)	57.49 <sup>b</sup> (49.26)
Coimbatore	Thondamuthur	70.62 <sup>c</sup> (57.17)	69.49 <sup>b</sup> (56.42)	67.24 <sup>b</sup> (55.06)	60.38 <sup>c</sup> (50.94)	58.19 <sup>bc</sup> (49.66)	56.14 <sup>bc</sup> (48.50)
Theni	Cumbum	76.92 <sup>a</sup> (61.27)	75.15 <sup>a</sup> (60.07)	72.32 <sup>a</sup> (58.24)	65.49 <sup>a</sup> (53.97)	63.56 <sup>a</sup> (52.83)	61.23 <sup>a</sup> (51.47)
Theni	Odapatty	75.63 <sup>a</sup> (60.40)	73.29 <sup>a</sup> (58.82)	71.69 <sup>a</sup> (57.80)	62.38 <sup>b</sup> (52.12)	60.12 <sup>b</sup> (50.83)	58.34 <sup>b</sup> (49.78)
Theni	Surulipatty	69.72 <sup>c</sup> (56.60)	66.53 <sup>c</sup> (54.63)	64.63 <sup>c</sup> (53.49)	58.94 <sup>d</sup> (50.13)	58.04 <sup>c</sup> (49.60)	54.82 <sup>c</sup> (47.75)
Dindugal	Uthupatty	40.19 <sup>f</sup> (39.29)	35.24 <sup>f</sup> (36.39)	32.47 <sup>f</sup> (34.70)	38.65 <sup>e</sup> (38.41)	37.21 <sup>f</sup> (37.58)	36.64 <sup>e</sup> (37.23)
Dindugal	Chakkainayakanur	68.21 <sup>c</sup> (55.67)	62.53 <sup>d</sup> (52.24)	60.78 <sup>d</sup> (51.18)	57.24 <sup>d</sup> (49.14)	57.09 <sup>c</sup> (49.02)	53.24 <sup>c</sup> (46.83)
Dindugal	Chinnalapatty	45.71 <sup>e</sup> (42.53)	40.92 <sup>e</sup> (39.76)	38.24 <sup>e</sup> (38.17)	51.18 <sup>f</sup> (45.63)	49.68 <sup>e</sup> (44.77)	49.35 <sup>d</sup> (44.60)

\*Mean of three replications

Values in parentheses are arcsine-transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

**Table 2. Pathogenicity tests with different isolates of *P.viticola* and *U.necator* under glass house condition**

Name of district	Name of village	Downy mildew		Powdery mildew	
		Isolate	PDI on leaves*	Isolates	PDI on leaves*
Coimbatore	Mathampatty	D-CM1	31.15 <sup>c</sup> (33.89)	P-CM1	28.54 <sup>c</sup> (32.27)
Coimbatore	Theeethipalayam	D-CT2	19.29 <sup>f</sup> (25.99)	P-CT2	15.48 <sup>e</sup> (23.11)
Coimbatore	Theenampalayam	D-CT3	35.21 <sup>b</sup> (36.39)	P-CT3	31.46 <sup>b</sup> (34.08)
Coimbatore	Thondamuthur	D-CT4	26.56 <sup>d</sup> (30.98)	P-CT4	25.28 <sup>d</sup> (30.13)
Theni	Cumbum	D-TC1	42.35 <sup>a</sup> (40.57)	P-TC1	36.94 <sup>a</sup> (37.41)
Theni	Odapatty	D-TO2	40.16 <sup>a</sup> (39.29)	P-TO2	34.28 <sup>a</sup> (35.79)
Theni	Surulipatty	D-TS3	25.28 <sup>d</sup> (30.13)	P-TS3	22.91 <sup>e</sup> (28.59)
Dindugal	Uthupatty	D-DU1	14.94 <sup>e</sup> (22.71)	P-DU1	10.54 <sup>h</sup> (18.91)
Dindugal	Chakkainayakanur	D-DC2	22.18 <sup>e</sup> (28.04)	P-DC2	19.45 <sup>f</sup> (26.13)
Dindugal	Chinnalapatty	D-DC3	16.54 <sup>e</sup> (23.97)	P-DC3	12.86 <sup>h</sup> (20.96)

\*Mean of three replications

Values in parentheses are arcsine-transformed values

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

**Table 3. Efficacy of Azoxystrobin 8.3%w/w + Mancozeb 68.75% and other fungicides against spore germination of *Plasmopara viticola* and *Uncinula necator***

Treatment	<i>P.viticola</i>		<i>U.necator</i>	
	Percent germination*	Percent reduction over Control	Percent germination*	Percent reduction over control
Azoxystrobin 8.3% + Mancozeb 68.75% (99.6+800) g ai ha <sup>-1</sup> (0.24%)	21.31 <sup>b</sup> (27.48)	77.39	18.34 <sup>b</sup> (25.35)	80.70
Azoxystrobin 8.3% + Mancozeb 68.75% (124.5+1000) g ai ha <sup>-1</sup> (0.30%)	10.46 <sup>a</sup> (18.86)	88.90	9.58 <sup>a</sup> (18.02)	89.91
Azoxystrobin 8.3% + Mancozeb 68.75% (149.4+1200) g ai ha <sup>-1</sup> (0.36%)	10.12 <sup>a</sup> (18.54)	89.26	9.24 <sup>a</sup> (17.69)	90.27
Mancozeb 75% WP 1500 g ai ha <sup>-1</sup> (0.40%)	35.31 <sup>de</sup> (36.45)	62.54	22.52 <sup>bc</sup> (28.32)	76.30
Azoxystrobin 23% SC 125 g ai ha <sup>-1</sup> (0.10%)	26.34 <sup>bc</sup> (30.87)	72.06	27.61 <sup>cd</sup> (31.69)	70.94
Hexaconazole 2% SC 60 g ai ha <sup>-1</sup> (0.60%)	42.12 <sup>c</sup> (40.46)	55.32	39.21 <sup>c</sup> (38.76)	58.73
Metalaxyl 8% + Mancozeb 64% WP 2000 g ai ha <sup>-1</sup> (0.50%)	31.25 <sup>cd</sup> (33.98)	66.85	32.42 <sup>de</sup> (34.70)	65.88
Control	94.28 <sup>f</sup> (77.18)	-	95.03 <sup>f</sup> (78.75)	-

### III. Results

#### 2. Survey and Pathogenicity for Powdery and Downy mildew pathogens

Field surveys were conducted in 3 major grapevine growing districts in Tamil Nadu viz., Coimbatore, Theni and Dindugal during 2011 and observations of the incidence of downy mildew and powdery mildew were recorded and Percent Disease Index (PDI) was worked out.

##### 2.1 Downy mildew (*Plasmopara viticola*)

The results from the surveys showed that the highest infection of downy mildew with PDI 76.92 was recorded in Cumbum of Theni district, followed by Odapatty (75.63), and Theenampalayam (73.26) of Coimbatore. The same scenario was noticed on the incidences of inflorescences and fruits wherein, the Cumbum of Theni recorded (75.15) followed by Odapatty (73.29) and Theenampalayam (70.32) of Coimbatore in inflorescences. In the case of fruits also, Cumbum and Odapatty of Theni district recorded PDIs of 72.32, 71.69 percent, followed by Theenampalayam of Coimbatore (68.15).

## **2.2 Efficacy of Azoxystrobin 8.3%w/w + Mancozeb 68.75% and other fungicides against the spore germination of *P.viticola* and *U.necator***

From the results, it was inferred from the data that Azoxystrobin 8.3%w/w + Mancozeb 68.75% was found to be more effective than other four fungicides in inhibiting the sporangial germination of *P.viticola* at 0.36 per cent concentration as it recorded 89.26 % inhibition as compared to 88.90 and 77.39 in 0.30% and 0.24% concentrations respectively. Among other fungicides, azoxystrobin was found to show 72.06 percent inhibition followed by mancozeb which recorded 62.54%, whereas the least inhibition of 55.32 was recorded in hexaconazole. The combination of metalaxyl+ mancozeb was found to be more effective when compared to mancozeb alone as it recorded 66.85 per cent reduction as compared to 62.54 per cent in mancozeb. The similar findings were also reported by several workers.

## **2.3 Efficacy of Azoxystrobin 8.3%w/w + Mancozeb 68.75% and other fungicides against the spore germination of *U.necator***

The studies on the effect of fungicides on the conidial germination of *U.necator* also yielded the same result. The new fungicide, Azoxystrobin 8.3% + Mancozeb 68.75% in all three concentrations, was found to be superior in inhibiting the spore germination by recording 90.27%, 89.91%, 80.70% respectively in 0.36, 0.30 and 0.24 % concentrations. Mancozeb recorded 76.30 per cent inhibition, followed by azoxystrobin (70.94). The least inhibition of 58.73 was recorded in hexaconazole. The combination of metalaxyl+mancozeb was found to be less effective when compared to mancozeb alone, as it recorded 65.88 per cent reduction as compared to 76.30 per cent in mancozeb. The similar findings were also reported by several workers.

## **IV. Discussion**

Practically no survey reports was available on the incidence of downy mildew disease in different districts of Tamil Nadu and this seems to be the new study. However, In India the disease was first reported by Syndow and Butler, 1912[11] from Pune, Maharashtra. Subsequently the incidence of downy mildew was reported from different places in India. First time Ramakrishnan and Sundaram (1955) [12] reported the occurrence of this disease in Tamil Nadu. Field surveys were conducted indicated that the powdery mildew infection with highest incidence of PDI on leaves, inflorescence and fruits were observed in Cumbum of Theni with records of 65.49, 63.56, 61.23 percent respectively. This was followed by Odapatty of Theni 62.38, 60.12, 58.34 respectively on leaves, inflorescence and fruits.

In Coimbatore, the highest incidences were recorded in Theenampalayam with the incidences of 61.56, 60.02 and 57.49 percent respectively on leaves, inflorescences and fruits. In general, it was observed that among the three locations, Dindugal district recorded less incidence of downy mildew and powdery mildew when compared to other districts. Practically no survey report was available on the incidence of powdery mildew in different districts of Tamil Nadu and this seems to be the new study. However reports from other parts of India were reviewed. Sohi (1983)[13] reported that in South India, the loss in yield was reported upto 50 per cent due to infection by this disease. Sendhil Vel (2003)[14] studied the *in vitro* efficacy of azoxystrobin against the spore germination of downy mildew of grapes and found that even at a concentration of 100 ppm, it was able to reduce the germination up to 90 percent, with an increasing concentration of the chemical (250, 500, 750 and 1000 ppm) complete inhibition of germination. Nithyameenakshi *et al.* (2006)[15] reported that *in vitro* study of spore germination revealed that azoxystrobin at 0.05% arrest the spore propagules of downy mildew, powdery mildew and anthracnose of grapes.

Archana (2009)[16] reported the extent of inhibition of sporangial germination increased with the increase in concentration of fungicides. Among them, Azoxystrobin (23 SC) completely inhibited the sporangial germination of *P. viticola* at 300 ppm onwards. Similarly, Azoxystrobin (23 SC) recorded cent per cent inhibition of conidial germination at a concentration of 250 ppm and above. Azoxystrobin provided cent per cent control of downy mildew in grapes, when applied 1 to 5 days before inoculation and 85 per cent mean reduction of resporulation from diseased tissue, when applied 6 days after inoculation (Wong and Wicox, 2001)[17]. Azoxystrobin inhibited the conidia to germinate and formation of appressoria in black rot (*Guignardia bidwelli*) pathogen of grape (Hoffman and Wilcox, 2003)[18].

Ravikumar (1998)[19] reported that penconazole (0.1 %) difenconazole (0.1 %), nimbicidin (0.3 %) and neemark (0.3 %) were effective against *Sphaerotheca pannosa* in laboratory conditions using agar plate method. Dhruj *et al.* (2000)[20] reported that hexaconazole, penconazole and wettable sulphur were found effective against powdery mildew of fenugreek.

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