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Defence Enzyme Activation and Enhancement of Quality Parameters by Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 w/w and Biocontrol Agent in Grapevine against *P.viticola*

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***Abstract* – Defence enzymes such as Phenol, peroxidase, Polyphenol oxidase and Phenyl alanine ammonia Lyase in grapevine (*Vitis vitefera*) in response to Azoxystrobin 25+Mancozeb 63% and biocontrol treated plants from 0 day to 15 days after treatment. The chemical fungicides induce more enzymes when compared to biocontrol agent treated plants. Two isoforms of peroxidase and three isoforms of Polyphenol oxidase were induced in grape plants upon treatment with Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 when compared to Azoxystrobin and Mancozeb when applied alone. The quality parameters such as reducing, non reducing and acidity has been increased when Azoxystrobin 25+Mancozeb 63% @0.36 percent applied plants from 1 day to 7 days.**

***Keywords* – Grapevine, Phenol, Polyphenoloxidase, Peroxidase, PAL.**

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. 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). It was first reported in India by Syndow and Butler (1912) from Pune, Maharashtra. Subsequently, the incidence of downy mildew was reported from different regions of India. In Tamil Nadu, it occurs in all grapevine belts (Ramakrishnan and Sundaram, 1955). Frequent fungicide and Inadequate fungicide application for the control of disease may leads to the resistence in crop plants.Therefore we considered alternative control measure using different concentration of Azoxystrobin+ mancozeb @ 0.24,0.30 and 0.36 per cent concentration giving an alternative control method by applying appropriate fungicide for the control of downy mildew

Azoxystrobin (Methyl *E*)-2-{2-[6-(2cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is one of the strobilurin class fungicide. It controls major diseases such as downy mildew of grapes (Archana, 2009: Sendhilvel, 2003). leaf spot (*Cercospora beticola*), powdery mildew (*Erysiphe betae*) of sugar beet, black spot (*Guignardia citricarpa*) of citrus, post-harvest rot (*Colletotrichum gloeosporioides*) of avocado (Slawecki et al .,2002 ;Anesiadis et al., 2003; Miles et al., 2004). The effectiveness of azoxystrobin in controlling the downy mildew is related to induction of plant defence compounds (Sundaravadana et al. 2007: Ling-yun *et al.* 2008: Anand, 2002. Azoxystrobin treated fruits recorded high Total Sugar content, Reducing sugar content, Non reducing sugar content and Acidity and Sugar Acid Ratio due to high metabolic transformation to soluble compound (Singh and Rajput, 1991: Archana, 2009)

The mancozeb (manganese ethylene bisdithiocarbamate (Polymeric) complex with zinc salt, is a broad spectrum contact dithiocarbamate group of fungicide with protective action. It is widely used on several crops including papaya anthracnose (Ahuya and Mohapatra,2010) and chilli anthracnose (Smitt,2010) The effectiveness of mancozeb in controlling the many diseases related to induction of plant defence compounds (Ahila devi,2009). The Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 w/w is being systemic and curative in nature controlling the downy mildew giving higher induction of defence enzymes and to increase the quality of grape fruits.

The objective of the present study is defence enzyme activation and enchancement of Quality parameters by new fungicide Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 w/w and biocontrol agent in Grapevine against downy mildew pathogen

II. MATERIALS AND METHODS

Estimation of total phenol

Sample preparation

Grapevine leaves were collected at 5, 10 and 15 days after inoculation with *P.viticola* from Glass house. One hundred mg were chopped into small bits; plunged into 80 percent ethanol on a boiling water bath for 10 min and cooled in running tap water. The extract was centrifuged at 1000 rpm for 10 min. The volume was made up to 25 ml with 80 per cent ethanol which was used for the assay of phenol.

Sample collection and enzyme extraction

The fungicide Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 at three concentration were compared with Mancozeb, Azoxystrobin, Hexaconazole, Metalaxyl+ Mancozeb and *P.fluorescens* for the induction of defence related enzymes. Grapevine plants in glass houses were sprayed with above treatments and were challenge inoculated separately with *P.viticola* The leaves samples were collected at 0, 1, 3, 5, 7 days after challenge inoculation and used for the study.

The leaf tissues were collected from plants and immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4 °C. The homogenate was centrifuged for 20 min at 10,000 rpm. The supernatant was used as enzyme extract for assaying of activities of phenol (Bray and Thorpe, (1954).) peroxidase (Hammerschmidt et al., 1982), poly phenol oxidase Mayer et al. (1965) and L-phenylalanine ammonia-lyase (PAL) (Dickerson et al., 1984).

Gel electrophoresis studies

Peroxidase

To study the expression pattern of different iso forms of peroxidases in different treatments, activity gel electrophoresis was carried out. For native anionic polyacrylamide gel electrophoresis, resolving gel of 8 per cent acrylamide and stacking gel of 4% acrylamide were prepared (Laemmli, 1970) and further estimations were done by the procedure given by PO(Sindhu et al., 1984)and PPO (Jayaraman et al., 1987)

Effect of different fungicides and P.fluorescens on the quality of grapevine fruits

The influence of various spray treatments were studied with reference to Total sugars(Hedge and Hofrieter, 1962), Reducing sugars, Non-reducing sugars Somogyi (1952), Acidity and Sugar-acid ratio(Krishnamurthi et al., 1959) of grapevine fruits.

III. Results and Discussion

In the present observation, Azoxystrobin 8.3% + Mancozeb 64.75% at 0.36 per cent concentration was found to maximum phenol content after 15 days of inoculation with *P. viticola.* The samples from uninoculated plants sprayed with different treatments showed no much variation in the phenol content as compared to control. The results from the observations on the activity of peroxidase in the plant samples collected from different treatments revealed that challenge inoculation with *P.viticola* induced more enzyme activity upto 5 days when compared to control and decrease on 7 th day. In the uninoculated plants, different spray treatments were found to increase the activity of peroxidase compared to control. Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 at 0.36 percent found to show progressive increase upto 5th day of observation (0.860), PPO activity (0.892) on 5$^{th\ day}$ PAL (0.882).

The findings of the present study is supported by other reports. Jallel et al, (2008) reported that the total phenol content was increased with the triazole treatment both in the shoot and the tuber at all stages of growth of yam plant. Sundaravadana et al. (2007) reported that reduction of rice blast severity was mainly associated with induction of host defense mechanism by azoxystrobin. Increased the production of secondary metabolite – phenolic and lignification related enzymes, namely peroxidase, polyphenol oxidase and phenylalanine ammonia- lyase were observed in rice plants treated with azoxystrobin.

Native gel electrophoretic separation of crude peroxidase and poly phenol oxidase enzyme extracted from grapevine treated with Azoxystrobin 8.3 % w/w + Mancozeb 64.7 along with the standard fungicides, mancozeb 75% WP, azoxystrobin 23% SC, hexaconazole 2% SC and metalaxyl 8%+mancozeb 64% WP and *P.fluorescens* showed different peroxidase (PO) poly phenol oxidase(PPO) isoform patterns. The intensity of PO and PPO was more in Azoxystrobin 25+Mancozeb at 0.36 percent when compared to other treatments after challenge inoculation with *P.viticola*. The result on isozyme electrophoretic pattern of peroxidase and poly phenol oxidase revealed higher intensities of fraction in Azoxystrobin 25+Mancozeb at 0.36 percent.

Quality parameters in Grapevine

Total sugar content in fruits collected from all treatments after last spray declined with increasing days. Compared to control (10.87 per cent), total sugar content was higher in Azoxystrobin 8.3 % w/w + Mancozeb 64.7 at 0.36 percent treated fruits at 3 days after last spray. The maximum reducing sugar content of 6.92 per cent and Non reducing sugar found to be 11.57 on 3rd day after last spray. The application of all fungicides was found to increase the acidity of the fruits.

It was observed that in all the treatments, the increase in the acidity progressed upto 3rd day and showed a decline on the 4th day. However, the application of Azoxystrobin 8.3 % w/w + Mancozeb 64.7 at 0.36 percent found to induce acidity as a whole when compared to other treatments. Like sugar contents, sugar- acid ratio was also reduced with increasing days upto 4th day after last spray. Azoxystrobin 8.3 % w/w + Mancozeb 64.7 at 0.36 percent recorded the ratio of 38.76 and then it declined to the ratio of 37.78 at 4th day after the last spray. This was followed by other fungicides, mancozeb, azoxystrobin, hexaconazole and metalaxyl + mancozeb.

Archana (2009) reported that there were 22.9, 26.80, 2.63 and 28.5 per cent increase in total soluble solids, reducing sugars, non-reducing sugars and acidity in azoxystrobin 23 SC sprayed plots at 4 days after harvesting grape berries. Gadoury et al. (2003) reported that berries are reported to be susceptible for infection, until soluble solids levels (brix) reach 8 per cent, and established colonies are reported to sporulate until brix reach 15 per cent.

IV. Conclusion

Therefore based on the present results it can be concluded that the Azoxystrobin 8.3 % w/w + Mancozeb 64.7 @ 0.36 per cent concentration induce resistance compounds like Peroxidase, Polyphenol oxidase and

Phenylalanine ammonia Lyase and it could be used to increase the quality of the fruits.

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Table 1: Changes in the different defence enzymes in grapevine leaves challenge with inoculation of *P.viticola*

Treatment	Phenol mg/g of fresh Tissue*	Peroxidase(PO) Absorbance (420 ηm) min^{-1} g^{-1} at different days after inoculation*	Peroxidase(PPO) Absorbance (495 ηm) min^{-1} g^{-1} at different days after inoculation*	Phenylalanine ammonia lyase (PAL) Absorbance (290 ηm) min^{-1} g^{-1} at different days after inoculation*
Azoxystrobin 8.3 % + Mancozeb 64.7 (99.6+800) g ai ha^{-1} (0.24%)	0.827^{b}	0.672^{ab}	0.767^{b}	0.769^{b}
Azoxystrobin 8.3 % w/w + Mancozeb 64.7 (124.5+1000) g ai ha^{-1} (0.30)%	0.845^{ab}	0.716^{a}	0.882^{a}	0.873^{a}
Azoxystrobin 8.3 % w/w + Mancozeb 64.7 (149.4+1200) g ai ha^{-1} (0.36%)	0.881^{a}	0.720^{a}	0.889^{a}	0.879^{a}
Mancozeb 75% WP 1500 g ai ha^{-1} (0.40%)	0.658^{d}	0.452^{d}	0.421^{e}	0.590^{e}
Azoxystrobin 23% SC 125 g ai ha^{-1} (0.10%)	0.723^{c}	0.506^{c}	0.504^{d}	0.696^{cd}
Hexaconazole 2% SC 60 g ai ha^{-1} (0.60%)	0.600^{f}	0.403^{e}	0.360^{f}	0.489^{f}
Metalaxyl 8% + Mancozeb 64% WP 2000 g ai ha^{-1} (0.50%)	0.687^{e}	0.491^{cd}	0.580^{c}	0.643^{de}
P.fluorescens (0.20%)	0.733^{cd}	0.649^{b}	0.758^{b}	0.746^{bc}
Control	0.494^{g}	0.332^{f}	0.310^{g}	0.380^{g}

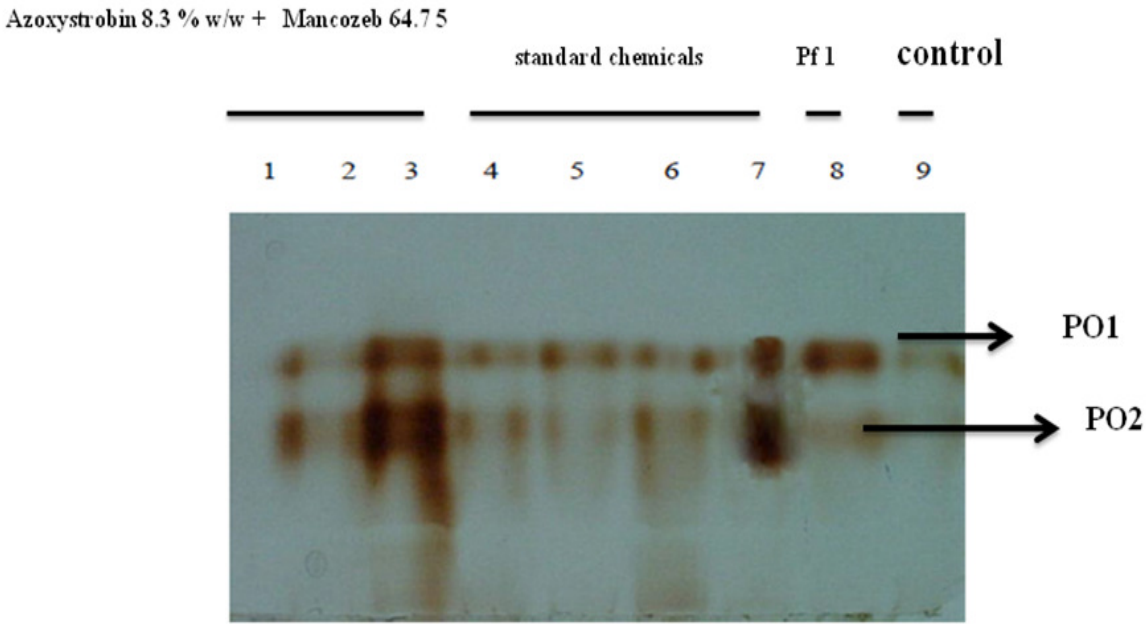
*Mean of three replications
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 2: Effect of different fungicides and *P.fluorescens* on the sugar and acid content in grapevine fruits

Treatment	Non-Reducing sugar content	Acid content	Sugar acid ratio
	Different Days after last spray*(percent)		
Azoxystrobin 8.3 % w/w + Mancozeb 64.7 (99.6+800) g ai ha^{-1} (0.24%)	7.39	0.42	37.52
Azoxystrobin 8.3 % w/w + Mancozeb 64.7 (124.5+1000) g ai ha^{-1} (0.30)%	8.78	0.46	39.14
Azoxystrobin 8.3 % w/w + Mancozeb 64.7 (149.4+1200) g ai ha^{-1} (0.36%)	8.86	0.48	39.96
Mancozeb 75% WP 1500 g ai ha^{-1} (0.40%)	6.76	0.33	34.08
Azoxystrobin23%SC 125 g ai ha^{-1} (0.10%)	7.08	0.34	34.50
Hexaconazole 2% SC 60 g ai ha^{-1} (0.60%)	5.91	0.30	33.36
Metalaxyl 8% + Mancozeb 64% WP 2000 g ai ha^{-1} (0.50%)	6.79	0.38	36.67
P.fluorescens (0.20%)	6.32	0.32	35.87
Control	5.44	0.25	31.35

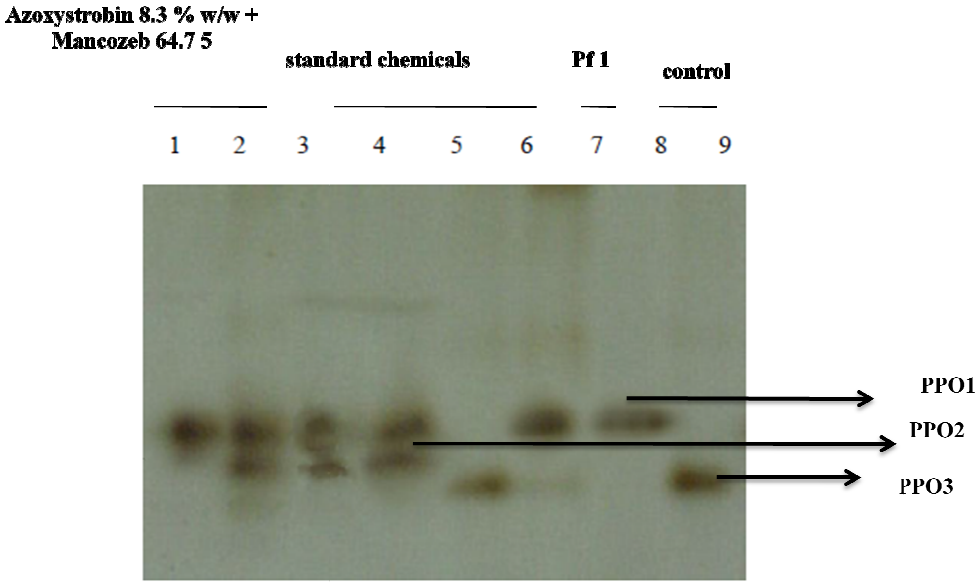
*Mean of three replications
Values in parentheses are arcsine-transformed values
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Plate 1: Peroxidase isozymes in protein extracts from grapewine leaves treated with Azoxystrobin 8.3% w/w + Manocozeb 64.75 and P.*fluorescens* against P.*viticola*.



Peroxidasae isoenzymes in protein extracts from grapevine leaves treated with Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 (Lane 1-3),standard chemicals(Mancozeb, azoxystrobin, Hexaconazole, Metalaxyl+ mancozeb)-Lane 4-7and *Pseudomonas fluorescens*- (Pf1)lane 8 control – Lane 9. Fifty microgram equivalent of protein was loaded on each lane in a non denaturing gel. Following electrophoresis, the gel was incubated for 30 min with 0.1 M pottasium phosphate buffer, pH7

Plate 2: Polyphenol oxidase isozymes in protein extracts from grapewine leaves treated with Azoxystrobin 8.3 % W/W + Mancozeb 64.7 5 and *P.fluorescens* against *P.viticola.*



Polyphenol oxidase isoenzymes in protein extracts from grapevine leaves treated with Azoxystrobin 8.3 % w/w + Mancozeb 64.7 5 (Lane 1-3),standard chemicals(Mancozeb, azoxystrobin, Hexaconazole, Metalaxyl+ mancozeb)-Lane 4-7and *Pseudomonas fluorescens*- (Pf1)lane 8 control – Lane 9. Fifty microgram equivalent of protein was loaded on each lane in a non denaturing gel. Following electrophoresis, the gel was incubated for 30 min with 0.1 M pottasium phosphate buffer, pH7

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