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Efficacy of new ready-mix formulation of azoxystrobin and tebuconazole (Azoxystrobin 11% w/w + Tebuconazole 18.3% w/w SC) against downy mildew and powdery mildew disease and its safety assessment on grape

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Abstract
On field efficacy evaluation of a new ready-mix formulation of Strobulirin and Triazole group i.e. Azoxystrobin 11% + Tebuconazole 18.3% SC was done and two season experiment was conducted during 2014-15 and 2015-16 on grape variety Thomson seedless to evaluate the bio-efficacy of combination of fungicideagainst downy mildew and powdery mildew diseases. The results revealed that spraying of Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 - 1000 ml/ha at 40-50 days after forward pruning were found significantly on par with each other in reduction of PDI on leaves as well as berries and enhanced marketable yield of grapes which were followed by rest of the treatments. The safety assessment on grape plant revealed that Azoxystrobin 11% + Tebuconazole 18.3% SC did not show any phyto-toxicity symptoms even though it was applied at 1500 ml/ha. Collectively, the results of this study illustrate the unique property of Azoxystrobin 11% + Tebuconazole 18.3% SC in comparison to that of three protectant and systemic fungicides, and provide information on how Azoxystrobin 11% + Tebuconazole 18.3% SC should be best used in disease management programs for Downy and Powdery mildew diseases of grape.

Keywords: Grape, downy mildew, powdery mildew, Azoxystrobin, Tebuconazole

Introduction
Grape (*Vitisvinifera* L.) is one of the important fruit crops of India. However, most of the high yielding varieties are highly susceptible to downy mildew and powdery mildew diseases caused by *Plasmoparaviticola* and *Uncinulanecator*, respectively (Pearson and Goheen, 1988) [7]. These diseases affect leaves, shoots, flowers and berries causing huge losses both in quality and yield (Chadha and Shikhamany, 1999) [1]. In Telangana, Andhra Pradesh, Maharashtra and Karnataka, where 'two pruning-one yield' system of grape cultivation is practiced, risk of downy mildew infection on inflorescence and young bunch is very high during the first 55-60 days after pruning. Though the vines are susceptible to powdery mildew infection at all growth-stages, after the berry-softening stage, the pathogen does not infect berries but infects pedicels. Rains and heavy dew facilitate onset of downy mildew, while, powdery mildew incidence occurs when days are cloudy and nights are relatively warm. Maximum losses are incurred when the infection appears on bunches. The disease needs to be controlled before a powdery mass of spore inoculum develops in the vineyard.

This is found in most of the grape-growing areas of the world, including tropics even though several fungicides are known to control this disease effectively. There are a number of fungicides registered for management of downy and powdery mildews on grape in India. However, continuous use solo fungicides may be increase chances of developing resistance in pathogen. To prevent the development of fungicide resistant strains of these fungi, there is need to use combination product belongs from different groups. Keeping this factor in view an approach was made to evaluate the efficacy of new ready-mix formulation of Azoxystrobin and Tebuconazole (Azoxystrobin 11% w/w + Tebuconazole 18.3% w/w SC) against downy mildew and powdery mildew disease. Moreover, its safety assessment was also done to insure the safe use on grape crop.

Materials and Methods:

This experiment was conducted on grape growing season of 2014-15 & 2015-16 at Grape Research Station, Rajendranagar with 6 fungicidal treatments and 1 untreated control under Randomized Block Design (RBD) with 3 replications for bio-efficacy evaluation. Whereas, for phyto-toxicity evaluation 3 fungicidal treatments and 1 untreated control was adopted and trial was conducted after forward pruning during October 2014 to March 2015 and October 2015 to March 2016 and the age of crop is 3.5–4.5 year respectively. All the approved agronomic practices were adopted for cultivation of grape and a plot size of 20 sq. meter was selected for each replication. The spacing of grape vines were 9 ft x 5 ft (2.74 m x 1.52 m) and spraying was done with the hand operated knapsack sprayer fitted with hollow cone nozzle with a water volume of 500 l/ha. Application of fungicidal treatment were given just at the appearance of first symptom of disease in treatment plot and it was continued for three to four time in an interval of 10 days to insure perfect control of targeted disease.

First two spray was specifically for downy mildew as its appearance comes prior to powdery mildew with was continued further to insure complete check of downy mildew. Whereas, last two spay were targeted to powdery mildew. The detail of treatments is described below whereas, Annexure-I describe the schedule of spray application with respect to their targeted dose and number of application.

Treatment Details

T. No.	Treatment	Dosage(g or ml)/ ha	
		a. i.	Formulation
T1	Azoxystrobin 11% +Tebuconazole 18.3% SC	55+91.5	500
T2	Azoxystrobin 11% +Tebuconazole 18.3% SC	82.5+137.25	750
T3	Azoxystrobin 11% +Tebuconazole 18.3% SC	110+183	1000
T4	Azoxystrobin 23 %SC	125	500
T5	Tebuconazole 25.9% EC	187.5	750
T6	Kresoxim-methyl 44.3% SC	350	700
T7	Untreated control (No spray)	-	-
*T8	Azoxystrobin 11% +Tebuconazole 18.3% SC (x)	82.5+137.25	750
*T9	Azoxystrobin 11% +Tebuconazole 18.3% SC (2x)	165+274.5	1500
*T10	Azoxystrobin 11% +Tebuconazole 18.3% SC (4x)	330+549	3000
*T11	Untreated control (Water spray only)	-	-

Note:* Treatments were used for phytotoxicity evaluation.

Annexure-I: The treatments were imposed as per details of spray schedules given below.

Treatments adopted	:	6+1+2+1= 10
T1	:	Three to four foliar sprays of Azoxystrobin 11% +Tebuconazole 18.3% SC @ 500 ml/ha after forward pruning at 10 days interval.
T2	:	Three to four foliar sprays of Azoxystrobin 11% +Tebuconazole 18.3% SC @ 750 ml/ha after forward pruning at 10 days interval.
T3	:	Three to four foliar sprays of Azoxystrobin 11% +Tebuconazole 18.3% SC @ 1000 ml/ha after forward pruning at 10 days interval.
T4	:	Three to four foliar sprays of Azoxystrobin 23%SC @ 500 ml/ha after forward pruning at 10 days interval.
T5	:	Three to four foliar sprays of Tebuconazole 25.9% EC@ 750 ml/ha after forward pruning at 10 days interval
T6	:	Three to four foliar sprays of Kresoxim-methyl 44.3% SC@ 700 ml/ha after forward pruning at 10 days interval
T7	:	Untreated control (No spray)
T8	:	Three to fourfoliar sprays of Azoxystrobin 11% +Tebuconazole 18.3% SC @ 750 ml/ha after forward pruning for phyto-toxicity test
T9	:	Three to fourfoliar sprays of Azoxystrobin 11% +Tebuconazole 18.3% SC @ 1500 ml/ha after forward pruning for phyto-toxicity test
T10	:	Three to four foliar sprays of Azoxystrobin 11% +Tebuconazole 18.3% SC @ 3000 ml/ha after forward pruning for phyto-toxicity test
T11	:	Untreated control (Water spray only)

A. Bio-efficacy observations for incidence of downy mildew were recorded on 30 leaves randomly selected /treatment before treatments and before 2nd, 3rd, 4th spray treatment and 10 days after last spray and on 30 bunches/treatment at harvest by adopting 0-4 scale:

0 = No disease

1 = 1 to 25% leaf area infected by the disease or bunch infestation.

2 = 26 to 50% leaf area infected by the disease or bunch infestation

3 = 51 to75% leaf area infected by the disease or bunch infestation.

4 = 76-100% leaf area infected by the disease or bunch infestation.

B. Bio-efficacy observations for incidence of powdery mildew were recorded on 30 leaves randomly selected /treatment before treatments and before 2nd, 3rd, 4th spray treatment and 10 days after last spray by adopting 0-4 scale:

0 = No disease

1 = 1-24% leaf area infected

2 = 25-50% leaf area infected

3 = 51-75% leaf area infected

4 = 76-100% leaf area infected

The data thus recorded was calculated into Percent Disease Index (PDI) by using the following formula:

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of leaves/bunches observed x Maximum rating scale}}$$

The data generated from various experiments of this study were statistically analyzed by the procedure described by Gomez and Gomez, (1984) [4]. The data with per cent values were subjected to arcsine transformation.

C. Recorded the marketable yield at the time of harvest after removal of infested rachis and berries from bunches per ha (t).

D. Phyto-toxicity observations on safety assessment of the test fungicide was done on the basis of visual observation and test fungicide at their recommended dose as well as its higher dosages (as detailed in Treatment details table) were also recorded separately. The observations on 0, 1, 3, 5, 7 and 10 days after each spraying were recorded for chlorosis, wilting, vein clearing, epinasty, hyponasty, necrosis and scorching and the standard 0-10 scale was used to assess phyto-toxicity parameters. The scale is as below.

Score	Phytotoxicity (%)
0	0-00
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

Results and Discussion

Experiment details indicated that all treatments were found significantly superior over control for PDI on leaves, berries and enhanced marketable yield/vine. There was gradual reduction in PDI in all the fungicidal treatments after each spray treatment but an increase was recorded in untreated control.

At final spray treatment during year 2014-15, all the fungicidal treatments recorded significantly less PDI of Downy mildew on leaf or vine in comparison to untreated control (Table 1). In treatments, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded minimum PDI i.e. 7.47 which was at par with Azoxystrobin 11% +Tebuconazole 18.3% SC @ 750 ml/ha (7.93 PDI) followed by rest of the treatments. Similar results were obtained during year 2015-16. In treatments, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded minimum PDI i.e. 6.77 which was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha (6.83 PDI). Pooled data from the two years individual observations it was found that Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded least PDI i.e. 7.12 and was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha which recorded 7.38 PDI.

In case of Powdery mildew disease all the fungicidal treatments gave good performance as compared to untreated control during year 2014-15. In treatments, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded minimum PDI i.e. 1.10 which was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha (1.20 PDI) followed by rest of the treatments. Similar results were obtained during year 2015-16. In treatments, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded minimum PDI i.e. 1.27 which was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha (1.30 PDI). Pooled data from the two years individual observations it was found that Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded least PDI i.e. 1.19 and was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha which recorded 1.25 PDI.

Both the diseases were observed on grape bunches also during year 2015-16 (Table 1). All the fungicidal treatments were showing better control on bunches. However, pooled results showed that significantly least Downy mildew and Powdery mildew disease was recorded in Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha i.e. 0.99 and 0.59 per cent, respectively and Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha i.e. 1.07 and 0.62 per cent, respectively and both were at par with each other.

During year 2014-15, all the fungicidal treatments recorded significantly less PDI of Downy mildew in comparison to untreated control (Table 1). In treatments, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded minimum PDI i.e. 7.47 which was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha (7.93 PDI), but it was at par with. Followed by Azoxystrobin 11% + Tebuconazole 18.3% SC @ 500 ml/ha (10.40 PDI), Kresoxim-methyl 44.3% SC @ 700 ml/ha (10.40 PDI), Tebuconazole 25.9% EC @ 750 ml/ha (10.27 PDI) and Azoxystrobin 23% SC @ 500 ml/ha (10.60 PDI).

All fungicidal treatments recorded significantly more yield in comparison to untreated control (Table 1). In treatments, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha recorded significantly more berry yield i.e. 26.23 t/ha and was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha with berry yield 24.33 t/ha during 2014-15 (Table 1). Similarly during 2015-16, Significantly more yield recorded in Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha i.e. 27.16 t/ha which was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha i.e. 25.15 t/ha.

The pooled data of two years also showed higher grape berry yield in Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha i.e. 26.70 t/ha which was at par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha i.e. 24.74 t/ha (Table 1).

There was no any phyto-toxicity symptoms i.e. chlorosis, wilting, vein clearing, epinasty, hyponasty, necrosis and scorching observed on any of the treatments even at higher dose of Azoxystrobin 11% + Tebuconazole 18.3% SC i.e. at 1500 and 3000 ml/ha (Table 2).

The results also showed that Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 ml/ha (x dose) and even at 1500 ml/ha (2x dose) and 3000 ml/ha (4x dose) did not show any phytotoxicity symptoms. Similarly all the other fungicides tested also did not exhibit any phytotoxicity symptoms. It can be summarized that Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750-1000 ml/ha is highly effective in reducing the powdery mildew disease in grapes without causing any phytotoxicity.

Azoxystrobin provided 100 per cent disease control when applied 1 to 5 days before inoculation while post-infection applications of azoxystrobin had little effect on the incidence of disease which reduced colony area by 47 per cent (Wong and Wilcox, 2001) [9]. Powdery Mildew of grape vine can be controlled by several groups of single-site fungicides, e.g. Quinone outside Inhibitors (QoI), sterol demethylation inhibitors (DMI), and methyl Benz imidazole carbamates. The QoIs are a group of fungicides that can be used to control Powdery mildew. QoIs control plant pathogens by inhibiting mitochondrial respiration leading to ATP deficiency (Gisi *et al*., 2002) [2]. Effective control of powdery mildew can be obtained by timely dusting with dinocap (0.2%) or carbendazim (0.1%) or folicur (0.05%) (Mallikarjun *et al*., 2008) [6]. Godwin *et al*. (1992) [7] and Wilcox *et al*. (1999) reported that azoxystrobin is the only currently available fungicide to provide effective control of powdery mildew (*U. necator*), which are most important fungal diseases of grapevine. Hundekar *et al*., (2008) [5] reported that the fungicide Kresoxim methyl @ 300 to 350 g a.i. per ha was found to be effective in reducing the severities of both powdery mildew as well as downy mildew.

Table 1: Effect of different fungicide on the management of Downy mildew and Powdery mildew disease as well as yield of grape.

Treatments	Downy mildew PDI, Powdery mildew PDI & Marketable Yield/vine (Kg) 2014-15					Downy mildew PDI, Powdery mildew PDI & Marketable Yield/vine (Kg) 2015-16					Pooled analysis of 2014-15 & 2015-16				
	Downy mildew PDI (On leaf)	Powdery mildew PDI (On leaf)	Downy mildew PDI (On bunches)	Powdery mildew PDI (On bunches)	Marketable yield (t/ha)	Downy mildew PDI (On leaf)	Powdery mildew PDI (On leaf)	Downy mildew PDI (On bunches)	Powdery mildew PDI (On bunches)	Marketable yield (t/ha)	Downy mildew PDI (On leaf)	Powdery mildew PDI (On leaf)	Downy mildew PDI (On bunches)	Powdery mildew PDI (On bunches)	Marketable yield (t/ha)
T1	10.40 (18.81)	2.13 (8.40)	4.80 (12.57)	2.73 (9.38)	19.55	9.53 (17.98)	2.17 (8.46)	3.57 (10.73)	3.30 (10.02)	20.52	9.97 (18.40)	2.15 (8.43)	4.19 (11.81)	3.02 (10.00)	20.04
T2	7.93 (16.35)	1.20 (6.28)	1.10 (6.01)	0.73 (4.90)	24.33	6.83 (15.14)	1.30 (6.54)	1.03 (5.83)	0.54 (4.04)	25.15	7.38 (15.76)	1.25 (6.42)	1.07 (5.92)	0.62 (4.50)	24.74
T3	7.47 (15.85)	1.10 (6.01)	1.00 (5.74)	0.70 (4.79)	26.23	6.77 (15.01)	1.27 (6.44)	0.97 (5.64)	0.47 (3.91)	27.16	7.12 (15.48)	1.19 (6.25)	0.99 (5.70)	0.59 (4.39)	26.70
T4	10.60 (19.00)	2.23 (8.59)	3.60 (10.75)	1.63 (7.05)	20.83	9.43 (17.89)	2.10 (8.33)	1.87 (7.85)	0.90 (5.44)	21.83	10.02 (18.45)	2.17 (8.46)	2.74 (9.52)	1.27 (6.46)	21.33
T5	10.27 (18.69)	2.30 (8.72)	3.63 (10.72)	1.33 (6.54)	18.87	9.47 (17.92)	2.20 (8.53)	1.90 (7.92)	0.93 (5.54)	20.00	9.87 (18.31)	2.25 (8.63)	2.77 (9.57)	1.13 (6.10)	19.44
T6	10.40 (18.81)	2.20 (8.52)	3.43 (10.54)	1.67 (7.15)	20.90	9.37 (17.82)	2.13 (8.40)	1.90 (7.92)	0.90 (5.44)	22.24	9.89 (18.33)	2.17 (8.46)	2.67 (9.40)	1.29 (6.51)	21.57
T7	28.90 (32.51)	28.67 (32.37)	11.50 (19.82)	6.93 (15.27)	15.59	28.57 (32.27)	27.00 (31.26)	8.73 (17.19)	6.83 (15.15)	16.53	28.74 (32.42)	27.84 (31.85)	10.12 (18.55)	6.88 (15.21)	16.06
SEM ±	0.64	0.67	1.16	0.84	0.70	0.64	0.62	0.60	0.43	0.65	0.65	0.64	0.90	0.66	0.68
CD at 5%	2.00	2.08	3.58	2.61	2.16	1.97	1.92	1.86	1.35	2.00	1.96	1.93	2.71	1.99	2.05

Table 2: Crop safety assessment of Azoxystrobin 11% +Tebuconazole 18.3% SC @ 750 ml/ha, 1500 ml/ha, 3000 ml/ha and Untreated control on Grape during 2014-15 & 2015-16

Treatments	Dosage (g or ml)/Ha		Chlorosis& Scorching (Days after spray)						Vein clearing& Wilting (Days after spray)						Epinasty & Hyponasty (Days after spray)						Necrosis (Days after spray)					
	a.i.	Formulation	0	1	3	5	7	10	0	1	3	5	7	10	0	1	3	5	7	10	0	1	3	5	7	10
Azoxystrobin 11% + Tebuconazole 18.3% SC	82.5 + 137.25	750	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Azoxystrobin 11% + Tebuconazole 18.3% SC	165 + 274.5	1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Azoxystrobin 11% + Tebuconazole 18.3% SC	330 + 549	3000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Untreated control	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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