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Short communication

Bio-efficacy of Aureofungin-sol in control of downy and powdery mildews in grape

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ABSTRACT

Bio-efficacy of Aureofungin-sol, an antifungal antibiotic, for control of downy mildew and powdery mildew of grape was evaluated during October 2008 - April 2009 fruiting season in vineyards at three locations in Maharashtra. Four to nine sprays of Aureofungin-sol, @ 0.108, 0.163 and 0.217 g/l, starting from 12-16 days to 46-75 days after fruit pruning gave good control of downy mildew on leaves and bunches, and increased harvestable yield over the Control. Similarly, four sprays of Aureofungin-sol @ 0.108 g/l at 11 to 20 days' interval at 65 days after pruning provided complete control of powdery mildew on leaves and bunches. No residue of Aureofungin-sol was found in harvest samples above the limit of detection (0.1 mg/kg).

Key words: *Plasmopara viticola*, *Uncinula necator*, Sharad Seedless, Tas-A-Ganesh, harvestable yield, (Cymoxanil+Mancozeb) 72WP, Azoxystrobin 23SC

Grape (*Vitis vinifera* L.) is one of the important fruit crops of India. However, it is highly susceptible to downy mildew and powdery mildew caused by *Plasmopara viticola* and *Uncinula necator*, respectively (Pearson and Goheen, 1988). These diseases affect leaves, shoots, flowers and berries causing huge losses both in quality and yield (Chadha and Shikhamany, 1999). In Maharashtra, Andhra Pradesh and Karnataka, where 'two pruning - one yield' system of grape cultivation is practised, 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.

There are a number of registered systemic and non-systemic fungicides for use on grape in India for management of downy and powdery mildews (nrcgrapes.nic.in/zipfiles/Pesticide List.pdf.). However, to avoid build up of fungicide residues, as well as to prevent development of fungicide-resistant strains of these fungi, there is a need for a large number of effective molecules that can be used judicially in the spray schedule (Sawant *et al*, 2007). Aureofungin-sol is a broad-spectrum antifungal antibiotic, developed and manufactured in India. It is an improved formulation of Aureofungin, containing 46.15% Aureofungin technical and 53.85% solubilizing agent.

Potential of Aureofungin in control of downy and powdery mildews of grape was reported over forty years ago by Kadkol and Gopalkrishnan (1971) and Sinha *et al* (1970), respectively. While Kadkol and Gopalkrishnan (1971) used detached infected leaves for their studies on downy mildew, Sinha *et al* (1970) conducted their experiments on hybrid grape seedlings. There is no report of field efficacy of Aureofungin, and no practice of its use in commercial vineyards. The present study was conducted to evaluate bio-efficacy of Aureofungin-sol, an improved formulation of Aureofungin, for controlling downy and powdery mildews of grape at different locations of Maharashtra. The field trial was conducted during fruiting season and terminal residues of Aureofungin-sol were checked at harvest to confirm food-safety.

Bio-efficacy

Trials for control of downy mildew were conducted during October 2008 - April 2009 fruiting season on 'Sharad Seedless' trained to the Bower system at Walwa (Dist.

Sangli) and Golegaon (Dist. Pune), and on 'Tas-A-Ganesh' (Thompson Seedless) trained on extended Y trellises at the research farm of NRC for Grapes (NRCG) (Dist. Pune). The trial for control of powdery mildew was conducted at NRCG. Trials were laid out in RBD with four replications consisting of 12 vines each. Data were recorded on two vines placed in the centre, while the rest were treated as guard vines. Test sample of Aureofungin-sol 46.15% S.P. was obtained from M/s Hindustan Antibiotics Ltd., Pune. Weather data was recorded and disease-risk was predicted using 'Logic' developed at NRCG, Pune. Fungicide sprays were given whenever weather conditions appeared to be favourable for development of the diseases. At NRCG, the first four sprays were aimed at control of downy mildew, and the subsequent four sprays for control of powdery mildew. Cymoxanil + Mancozeb 72 WP (8%+ 64%) and Azoxystrobin 23SC were used as positive controls in downy mildew and powdery mildew trials, respectively. Water volume used for sprays was about 1000 lha^{-1}.

Disease incidence on leaves and bunches was recorded on 0-4 rating scale, where 0= nil, 1= trace to 25, 2= 26 to 50, 3= 51 to 75, and 4= more than 75 % leaf area infected (Horsfall and Heuberger, 1942). The ratings were recorded for ten leaves and two bunches on ten randomly-selected canes per vine. Observations on incidence of downy mildew were recorded periodically, while that on powdery mildew were recorded after the fifth spray. Per cent Disease Index (PDI) was calculated as per McKinney (1923). All data were subjected to ANOVA (Panse and Sukhatme, 1989). The PDI data were transformed using Arcsine transformation. Results found significant at P=0.05 only are discussed.

Aureofungin-sol residue:

Aureofungin-sol was applied at a standard dose of 0.163gl^{-1} and double dose of 0.325gl^{-1} to vines as three consequent foliar sprays at 50, 35 and 20 days before harvest. Berry samples of two kg were collected at random from treated and control plots 1 h after the third spray and at harvest.

The entire sample (2kg) was crushed in a grinder and 5g of the homogenized sample was taken in a centrifuge tube to which 5 ml ethanol and 5g anhydrous sodium sulphate were added. The mixture was homogenized for 2 min. at 15000rpm and then centrifuged for 5 min. at 5000rpm. The supernatant was collected in another 50ml centrifuge tube. The extraction was repeated twice by adding 5ml ethanol. The supernatant was collected in the same tube, mixed thoroughly and centrifuged again. The supernatant was evaporated at 35^{o}C in a rotary evaporator. The aqueous phase remaining after evaporation was made up to 5ml with a mixture of 10% sodium carbonate solution in water and ethanol (1:9 v/v). From the final extract, 2ml was transferred to an Eppendorf tube and centrifuged at 10000rpm for 5 min. The clear supernatant was filtered through 0.2µm membrane-filter, and 10µl of this was subsequently injected in to UPLC- DAD for estimation of residues.

The analytical method was validated for per cent recovery. Recovery was measured by fortifying untreated blank matrix at 0.25mg/kg (LOQ) and 1.0mg/kg, in six replicates. Recovery was above 80% at both the levels. The limit of detection (LOD) was 0.1mg/kg and limit of quantification (LOQ) was 0.25 mg/l. All field samples were analyzed by the same method.

Residue analysis was done using Acquity Ultra Performance LC with Photodiode Array Detector, Waters Corporation. Chromatographic separation of the analyte from matrix compounds was achieved using C18 column (Acquity UPLC ®BEH C18-1.7µ (100mm x 2.1mm)). The mobile phase composed of (A) 0.1% formic acid, and (B) methanol, in a gradient program and detection was performed at 380nm. The characteristic UV-Visible spectrum (200-500nm) of Aureofungin was used for confirmation.

Bio-efficacy for control of downy mildew

At Walwa: The vineyard was pruned on 10th October and treatments were applied six times, from 24th October to 18th November 2009. On leaves, the first symptom of downy mildew was observed on 28th October in the untreated (negative) control, and the disease progressed further to cause cent percent infection by 21st November (Table 1). PDI in the positive control, i.e., Cymoxanil + Mancozeb 72 WP (8 %+ 64 %), ranged from 0.00 to 5.31, which was significantly less than negative control for the corresponding date of observation. During this period, Aureofungin-sol treatments recorded PDI in the range of 0.00 - 15.56. In the first observation, PDI in the highest concentration of Aureofungin-sol (0.217) was less than in the negative control and on par with positive control. In the last observation, PDI was reduced to 0.00 in all the treatments of Aureofungin-sol, indicating total control of the disease on par with that obtained with fungicide mixture of Cymoxanil + Mancozeb 72 WP.

Disease-incidence on bunches, recorded 15th December (65 days after pruning) onwards, showed cent per cent infection in the control on all four dates of observation. But, PDI in all Aureofungin-sol treatments was in the range of 0.00 to 2.19 (Table 1). At any date, PDI in all the three concentrations of Aureofungin-sol was on par with and significantly less than in the negative control. In the first two observations, PDI in Aureofungin-sol treatments was on par with the positive control; but, subsequently, PDI in Aureofungin-sol 0.217ml/l was significantly less than in the (Cymoxanil + Mancozeb) 72 WP treatment, indicating excellent control of downy mildew by Aureofungin-sol. Harvestable yield in the negative control was nil, as all bunches were affected with downy mildew. Aureofungin-sol treatments recorded yields in the range of 21.55 - 23.15 kg/nine, which were on par with each other and with yields in the positive control (Cymoxanil + Mancozeb 72 WP) (Table 1).

At Golegaon: Until the first week of December, 2008 i.e., up to 41 days after pruning, environmental conditions were not very favorable for downy mildew appearance; therefore, only three preventive sprays were given. However, in the first week of December, weather turned conducive for disease-development due to rains; therefore four, more sprays were given between 8th and 22nd December. Cent percent PDI was recorded on leaves as well as bunches in the negative control in both the observations (Table 2); but, in Aureofungin-sol treatments, PDI was significantly lower (31.06 to 49.81 on leaves, and 52.50 to 64.69 on bunches). Even Cymoxanil+Mancozeb 72 WP treatment did not provide complete control of the disease and showed 27.31 and 33.44 PDI on leaves, and 20.00 to 28.75 on bunches. Harvestable yield in the negative control was nil, as all bunches were affected with downy mildew. Bunches in the positive control (fungicide treatment) also showed high PDI, because of which the yield was low. Harvestable yields in Aureofungin-sol treatments were significantly higher than in the negative control, though less than in the positive control (Table 2).

At Pune: Relatively low downy mildew incidence was observed and PDI in the negative control was in the range of 8.50 to 9.69 on leaves and 20.63 to 27.50 on bunches. PDI in Aureofungin-sol at all three concentrations was significantly less than that in the negative control on any day of observation (Table 3). PDI at different doses of Aureofungin-sol was on par with each other, except that PDI in Aureofungin-sol at 0.217g/l dose was significantly less than PDI at 0.108g/l dose on leaves on the last date of observation; and on bunches, on second day of observation.

At all the locations, the antifungal antibiotic Aureofungin-sol, at 0.108 to 0.217g/l showed good control of downy mildew on leaves and bunches compared to the untreated control, and showed increase in harvestable yield. The fungicide was tested at very high disease-pressure at Walwa and Golegoan, where, there was cent percent loss in yield due to downy mildew in the negative control vines. At all these three locations, seven to nine sprays were required to provide adequate protection, while, at Pune, the disease-pressure was not high, and only four sprays sufficed for satisfactory control of the disease.

Table 1. Bio-efficacy of Aureofungin-sol for the control of downy mildew on Sharad Seedless at Walwa

Treatment	Dose	PDI on leaves				PDI on bunches				Harvestable
	g / l	28/10/08	4/11/08	18/11/08	21/11/08	15/12/08	17/12/08	19/12//08	22/12/08	yield (kg/ vine)
Aureofungin-sol	0.108	1.19 (4.39) bc	1.06 (4.89)	10.44 (18.72)b	0.00 (0.00)a	0.00 (0.00)a	1.25 (5.48) a	2.19 (7.34) ab	2.19 (7.34) ab	23.15
Aureofungin-sol	0.163	0.25 (1.43) abc	0.94 (4.80)	15.56 (23.19)c	0.00 (0.00)a	0.00 (0.00)a	1.25 (5.48) a	1.56 (6.00) ab	1.88 (6.67) ab	22.15
Aureofungin-sol	0.217	0.13 (1.01) ab	0.31 (2.74)	14.94 (22.69)c	0.00 (0.00)a	0.00 (0.00)a	0.94 (3.88) a	0.94 (3.88) a	1.25 (4.39) a	21.55
(Cymoxanil + Mancozeb) 72 WP	2.500	0.00 (0.00) a	0.38 (1.76)	5.31 (13.28)a	0.00 (0.00)a	0.00 (0.00)a	1.56 (7.09) a	2.50 (8.94) b	2.81 (9.61) b	21.40
Control	——	0.69 (4.58) c	1.56 (6.06)	35.25 (36.40)d	100.00 (90.00)b	100.00 (90.00)b	100.00 (90.00) b	100.00 (90.00) c	100.00 (90.00) c	0.00
SEm ±		1.13	1.93	1.00	0.00	0.00	1.34	1.56	1.62	1.07
CD (*P*=0.05)		3.47	NS	3.08	0.00	0.00	4.13	4.81	5.01	3.43
CV (%)		98.60	95.32	8.76	0.00	0.00	11.95	13.45	13.77	9.72

Figures in the parentheses are Arsine transformed values of percentages.

Table 2. Bio-efficacy of Aureofungin-sol for the control of downy mildew on Sharad Seedless at Golegoan

Treatment	Dose	PDI of downy mildew on leaves		PDI of downy mildew on bunches		Harvestable
	g / l	8/12/2008	23/12/08	8/12/2008	23/12/08	yield (kg/ vine)
Aureofungin-sol	0.108	49.81 (44.87)d	43.56 (41.28)d	60.63 (51.14)b	64.69 (53.55)c	1.73
Aureofungin-sol	0.163	42.50 (40.67)c	36.50 (37.14)c	59.69 (50.62)b	61.25 (51.50)bc	1.65
Aureofungin-sol	0.217	38.38 (38.26)b	31.06 (33.85)b	52.50 (46.42)b	54.69 (47.69)b	1.86
(Cymoxanil + Mancozeb) 72 WP	2.500	33.44 (35.31)a	27.31 (31.49)a	20.00 (26.40)a	28.75 (32.39)a	3.11
Control	——	100.00 (90.00)e	100.00 (90.00)e	100.00 (90.00)c	100.00 (90.00)d	0.00
SEm ±	0.50	0.41	2.63	1.28	0.07	
CD (*P*=0.05)	1.55	1.27	8.11	3.93	0.21	
CV (%)	2.02	1.76	8.99	4.63	8.31	

Figures in the parentheses indicate arcsine transformed values of percentages

Table 3. Bio-efficacy of Aureofungin-sol for the control of downy mildew on Tas-A-Ganesh at Pune

Treatment	Dose	PDI of downy mildew on leaves			PDI of downy mildew on bunches		
	g / l	20/12/08	27/12/08	19/01/09	20/12/08	27/12/08	19/01/09
Aureofungin-sol	0.108	0.63 (4.51)a	0.50 (3.98)a	0.25 (2.86)c	3.76 (11.00)a	3.13 (10.05)b	1.88 (6.82)b
Aureofungin-sol	0.163	0.63 (4.41)a	0.45 (3.76)a	0.08 (1.36)ab	2.50 (7.78)a	1.88 (6.82)ab	1.88 (6.82)b
Aureofungin-sol	0.217	0.50 (3.98)a	0.44 (3.76)a	0.00 (0.00)a	1.88 (6.82)a	0.63 (2.27)a	0.63 (2.27)ab
(Cymoxanil + Mancozeb) 72 WP	2.500	0.31 (3.16)a	0.25 (2.86)a	0.19 (2.15)bc	2.50 (9.09)a	1.25 (4.55)ab	0.00 (0.00)a
Control	———	8.50 (16.92)b	9.18 (17.62)b	9.69 (18.11)d	20.63 (26.95)b	25.00 (29.95)c	27.50 (31.60)c
SEm ±		0.46	0.38	0.46	1.63	1.81	1.92
CD (*P*=0.05)		1.43	1.16	1.41	5.00	5.58	5.93
CV (%)		14.09	11.83	18.66	26.36	33.77	40.50

Figures in the parentheses indicate arcsine transformed values of percentages

Table 4. Bio-efficacy of Aureofungin-sol for the control of powdery mildew on Tas-A-Ganesh at Pune

Treatment	Dose(per l)	PDI of powdery mildew on leaves			PDI of powdery mildew on bunches			Harvestable
		15/01/09	6/02/09	28/02/09	15/01/09	6/02/09	28/02/09	yield (kg/vine)
Aureofungin-sol	0.108 g	0.75 (4.93)a	0.38 (3.46)b	0.00 (0.00)a	2.50 (9.09)a	1.88 (6.82)a	0.00 (0.00)a	4.76
Aureofungin-sol	0.163 g	0.69 (4.70)a	0.38 (3.46)b	0.00 (0.00)a	1.88 (6.82)a	1.25 (4.55)a	0.00 (0.00)a	5.46
Aureofungin-sol	0.216 g	0.56 (4.28)a	0.06 (0.72)a	0.00 (0.00)a	1.25 (4.55)a	0.63 (2.27)a	0.00 (0.00)a	5.71
Azoxystrobin 23 SC	1.00 ml	0.56 (4.28)a	0.38 (3.46)b	0.00 (0.00)a	1.86 (6.82)a	1.89 (6.82)a	0.00 (0.00)a	5.66
Control	—-	8.25 (16.68)b	9.13 (17.57)c	9.88 (18.24)b	16.25 (23.75)b	18.13 (25.15)b	18.75 (25.55)b	3.79
SEm ±	0.32	0.41	0.45	1.64	1.54	0.69	0.18	
CD (*P*=0.05)	0.99	1.25	1.39	5.06	4.74	2.13	0.55	
CV (%)	9.28	14.21	24.74	32.18	33.70	27.10	7.08	

Figures in the parentheses indicate arcsine transformed values of percentages

Bio-efficacy for control of powdery mildew

PDI of powdery mildew at all three concentrations of Aureofungin-sol was significantly less than in the negative control at all three dates of observation on both leaves and bunches (Table 4). PDI in Aureofungin-sol treatments was on par with PDI in the positive control, i.e., Azoxystrobin 23 SC, at all dates of observation. PDI in all treatments was reduced to zero on the last date of observation indicating, that, no active powdery mildew was present on both leaves or bunches in vines treated with Aureofungin-sol at all three concentrations, while, in the control, it was 9.88 on leaves and 18.75 on the bunch. Harvestable yields in all Aureofungin-sol treatments were significantly higher than those in the negative control, and were on par with (0.108 and 0.163 doses) or more (at 0.217 dose) than in the positive control (Table 4).

Studies suggest that even at the lowest dose of 0.108g/l, Aureofungin-sol was able to control powdery mildew as effectively as the systemic fungicide Azoxystrobin. Therefore, for control of powdery mildew, even a dose as low as this can be recommended. Earlier studies indicate that sprays of Aureofungin are effective in mitigating anthracnose infection in the field and increasing grape yield (Bedi *et al*, 1969). Hence, this chemical has a potential to minimize infection by three important grape diseases in India. Spray of Aureofungin-sol at all three concentrations tested did not result in any visible phytotoxic effects.

Residue analysis

In the zero-day samples, for the standard dose, average residue was found to be 0.74 ±0.02mg/kg grapes. For the double dose, it was 1.34±0.01mg/kg grapes. No residue was found in samples above the limit of detection (0.1mg/kg).

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