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EFFICACY OF FOLIAR SPRAYS OF STROBILURIN AND TRIAZOLE FUNGICIDES IN CONTROLLING POWDERY MILDEW DISEASE ON GRAPEVINES

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ABSTRACT

The present study showed that strobilurin and triazole fungicides were effective in controlling *Uncinula necator* (Schwein) Burrill, which cause the economically more important powdery mildew of grapevines. Laboratory experiment exhibited that concentration of 50 g /100L water of Bellis was very effective against conidial production, being 89.0% inhibition. Concentration of Flint, Amistar Top and Cabrio Top at 20,75 and 100 g or ml /100L water inhibited conidial production by 85, 84 and 83% , respectively. Ten days after the third application in field grown grapevines, powdery mildew disease was significantly suppressed on vines treated with Bellis at 50 g/100L water, with the infected leaf area, being 1.2cm and provided 92.85% reduction . Amistar Top followed by Flint, Collis and Caprio Top had similar effective on vines. The post infection foliar application of strobilurin and triazole fungicides inhibited the development of powdery mildew disease on leaves and fruit clusters. During 2008 and 2009 seasons , Bellis was the best fungicide, showed significant suppression on leaves and fruit clusters provided higher efficacy more than other treatments and the control .In this respect, application of strobilurin fungicides with different concentration were highly effective and more than triazole fungicides in preventing the infection on leaves and fruit clusters .

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INTRODUCTION

Grape powdery mildew caused by *Uncinula necator* (Schwein) burtii can infect all green tissues of grapevines. It is an annually re-occurring disease in vineyards of all major viticultural areas. Leaf infection adversely affects vine growth, yield and winter hardiness. Cluster infection can seriously affect yield and quality of table grapes. Severe epidemics in successive years reduce the vigor and productivity of grapevines Pearson and Goheen (1988) and Gabriele *et al* (1997) mentioned that Kresoxim -methyl (Collis fungicide) was applied at a range of concentrations and at different times before and after inoculation, in glass-house trials under moderate relative humidity (60%) and all pre-infectional applications completely inhibited conidial germination. Least efficacies were observed with detached leaves at high humidity in Petri dishes. Post-infectional application of at least 8mg a.i /L. inhibited sporulation and mycelial growth and 67mg a.i /L caused partial collapse of surface structures. Penconazole (Triazole fungicide) at 17mg a.i /L did not inhibit germination, but prevented hyphal development and caused growth distortion with hyphal tip swelling.

Myclobutanil is a triazole fungicide with a mode of action similar to that of sterol demethylation inhibiting (DMI) fungicides. These materials are potent inhibitors of ergosterol biosynthesis in many pathogenic fungi. Among DMI fungicides, triadimefon was the first registered for the control of grapevine powdery mildew in Europe and in the USA (Erickson and Wilcox, 1997).

Trifloxystrobin a new strobilurin fungicide is active against a wide range of fungal plant pathogens. Trifloxystrobin (Flint) is effective for controlling the ascomycete *U. necator* which causes the economically more important powdery mildew of grapevines. The fungicide was very effective against conidial germination of *U.necator in vitro* and inhibited mycelial growth and sporulation of the fungus *In vivo*. trifloxystrobin was as suppressive as sulphur and superior to sterol inhibitor fungicides when applied to heavily powdery mildew infected leaves and fruit clusters of field grown grapevines (Moshe Reuveni, 2001).

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Paul *et al* (2007) demonstrated that fungicide spray application of Kresoxim methyl (65-70mg/L) or myclobutanil (24mg/L) at full flowering provided the best control of cluster powdery mildew on grapevine cultivars. Therefore, fungicides treatment applied during flowering appeared to be essential to control grapevine powdery mildew.

The objective of this research was to study the efficacy of strobilurin and triazole fungicides in controlling powdery mildew in field grown grapevines.

MATERIAL AND METHODS

Experimental fungicides:

Different systemic fungicides were evaluated to study their effect against powdery mildew disease of grapevine caused by *Uncinula necator* during 2008 and 2009 seasons.

Amistar 25%SC (azoxystrobin), Amistar top 32.5%SC (difenoconazole + azoxystrobin), Collis 30%SC (kresoxim-methyl + boscalid), Bellis 38%WG (pyraclostrobin + boscalid), Cabrio Top 60% WG (pyraclostrobin + metiram) and Flint 50%WG (trifloxystrobin) are new strobilurin fungicides which used in all experiments.

The sterol inhibitor (SI) fungicides, Score 25%EC (difenoconazole) and Punch 40%EC (flusilazole) were used in alternation as a standard treatments in laboratory and field experiments when the efficacy against powdery mildew was evaluated.

Pathogen:

Isolate of *U.necator* obtained from infected leaves of Red Romi cv at Assiut region, Egypt, were maintained on reinfected vines in growth chamber 24-26C and 60-70%RH. Inoculum of *U.necator* was obtained from freshly sporulating colonies, 10-15 days after inoculation .

Leaf disc inoculation with *U.necator* :

Leaf disc technique was used to evaluate the efficacy of different fungicides. Ten leaf discs (10 mm in diameter) were cut with a cork

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borer from healthy leaves of Red Romi grape cultivar in vineyard in Assiut region and placed upper surface in Petri dishes (10cm in diameter). containing filter paper moistend with 5ml of sterile distilled water. Each treatment was replicated ten times. Inoculum was obtained from freshly sporulating colonies and prepared by placing infected grape leaves into Petri dishes. Conidia were shaken onto the upper surface of ten leaf discs in each Petri dish. After inoculation, the leaf discs and the inner surfaces of the Petri dishes covers were sprayed with various concentrations of strobilurin and sterol inhibitor fungicides. For disease development, the Petri dishes with discs were incubated in a growth chamber at 24-26C. After 10 days from inoculation, conidia were detected in the check treatment (unsprayed leaf discs). Powdery mildew conidia were rated according to 0-4 scale described by Reuveni (1998), then the infected and uninfected leaf discs were counted.

Control of powdery mildew in orchard :

A field experiment conducted in 2008 season, using table grape cv. Red Romi in a commercial vineyard. Ten treatment, consisting of untreated check, Amistar at 50ml/100Lwater, 75ml Amistar Top, 50ml Collis, 50ml Score, 3ml Punch, 30 and 50g Bellis, 100g Cabrio Top and 20g Flint/100L.water were evaluated. Fungicides were sprayed three times on 10 June, 15 days intervals, when symptoms of powdery mildew were evident on leaves, 10 days later after the third application, leaves were evaluated for the disease incidence and counted for sporulating leaves and the infected area was measured.

Suppression of grape powdery mildew in the orchard :

Another experiments were conducted in 2008 and 2009 seasons to compare the suppressive effect of strobilurin against powdery mildew with those of sterol inhibitor fungicides . Ten treatments were applied to cv. Red Romi grape (15 years old) in commercial vineyard. They were; an untreated control, 50 ml Amistar /100 L. water, Amistar Top (75ml /100 L.w), Collis (50ml /100L.w), Score (50ml /100L.w), Punch (3 ml /100 L.w), Bellis (30and 50 g /100L.w), Cabrio Top (100g/100L.w) and Flint (20g /100L.w). Vines in this experiment remained untreated against powdery mildew until the initiation of this

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experiment, to allow disease development. Fungicides were sprayed three times (15 days intervals) on leaves and clusters, on June 10 and 25 days and July 10 days in each season, when powdery mildew symptoms were evident on leaves and in fruit clusters. The experiment was in complete randomized block design with three replicates for each treatment. Each replicate contained five trees. Ratings of disease using a scale of 0-4 (Reuveni and Reuveni, 1995) were made on July 25, and converted to disease index according to the equation suggested by Townse and Heuberger (1943).

RESULTS AND DISCUSSION

Effect of strobilurin and triazole fungicides on conidia production of *U. necator* on leaf discs :

Strobilurin and triazole fungicides were used to study their effect on production of conidia of *U. necator* *in vitro*. Strobilurin had a partial effect on conidia production of the tested fungus. Almost, similar level of inhibition of conidia production was found on leaf discs treated with triazole fungicides and inoculated with conidia of *U. necator*. There was a clear reduction in the percentage of infected leaf discs at different concentrations of strobilurin as shown in Table 1. Bellis fungicide at 50 g/100 L water had the highest effect of inhibition, whereas, conidial production were inhibited by 89.0%. Concentrations of Flint, Amistar Top and Cabrio Top at 20 g, 75 ml and 100 g/100 L water inhibited conidial production by 85.0, 84.0 and 83.0%, respectively. At the same time, Collis, Score and Amistar each at 50 ml/100 L water gave the moderate effect of inhibition, whatever, conidial inhibition reached 82.0, 81.0 and 80.0% compared with the check treatment, whereas the percentage of infected leaf discs reached it 100%. On the other hand, Punch at 3 ml and Bellis at 30 g/100 L water gave the less effect of conidial inhibition under *in vitro* conditions.

Bellis at 50 g / 100 L water were the superior fungicide which inhibited disease development on leaf discs and provided significant protection up to 89.0%, relative to check treatment, followed by Flint and Amistar Top. The obtained data show that strobilurin is an

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important fungicides, are active as foliar spray against powdery mildew disease in grapevines under vitro and field conditions.

Strobilurin strongly inhibited conidial production and spore germination of *U. necator*, with potential to reduce initial infection and spread of the pathogen (Margot *et al* , 1998 and Tally *et al* , 1998).

Table 1 : Production of conidia of *U. necator* on 100 leaf discs of Red Romi grapevines treated with strobilurin and triazole fungicides

Fungicides	Concentration with ml or g /100L water	Number of Leaf discs in each sporulation				
		0	1	2	3	4
Amistar 25%SC	50ml	80	15	5	-	-
Amistar Top 32.50SC	75	84	12	4	-	-
Collis 30% SC	50	82	13	3	2	-
Score 25% EC	50	81	9	6	4	-
Punch 40% EC	3	78	13	5	4	-
Bellis 38% WG	30g	75	13	8	4	-
Bellis 38% WG	50	89	11	-	-	-
Cabrio top 60%WG	100	83	12	3	2	-
Flint 50%WG	20	85	9	3	3	-
Check	0.0	-	17	18	27	38

Control of grape powdery mildew in the orchard:

Three foliar application of strobilurin or triazole fungicides significantly reduced the percentage of sporulating leaves and infected leaf area on the mildewed leaves of Red Romi grape cv .as shown in Table 2.

Ten days after the third applications, powdery mildew disease was significantly suppressed on vines treated with Bellis at 50 g /100L water , with the infected leaf area , being 1.2 cm and provided 92.85% reduction in infected leaf area, while it increased up to 16.8 cm on

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untreated vines . Applications of 75ml Amistar Top /100L water ,20 g Flint,50 ml Collis and 100g Cabrio Top were similarly effective on vines, with the infected leaf area being 1.5,1.7 and 1.8cm and exhibited 91.07 , 89.88 and 89.28% reduction in infected leaf area , respectively compared with the check nontreated vines . A treatment with 50 ml Amistar /100L water gave moderate effect, with the infected leaf area being 2.0cm and provided 88.09% inhibition of powdery mildew compared with the nontreated vines . In this respect , 50ml Score/100L water, 3 ml Punch and 30g Bellis gave the less effect in controlling the disease and showed 86.90,85.71and 84.52% inhibition, respectively as compared with the nontreated vines. The field data presented here exhibited that post infection foliar applications of strobilurin in the orchard inhibited development of powdery mildew in grapevines and provided 92.85% protection compared with the check . The inhibitory effect was mainly expressed as reduction in infected leaf area , thus, more effectively eradicating the disease incidence. . Strobilurin was very effective, as well as triazole fungicides in reducing the percentage of sporulation leaves and infected leaf area of vines . These results are in agreement with Godet *et al* (1997) who mentioned that Amistar (azoxystrobin) has a favourable environment profile leading to a high efficacy against grape diseases, which gave effective control including powdery and downy mildew diseases.

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Table 2: Efficacy of strobilurin and triazole fungicides in controlling powdery mildew in field grown grapevines during 2008 season.

Fungicides	Concentration with ml or g /100L water	Sporulating leaves %	Infected leaf area (cm)	Protection %
Amistar 25%SC	50ml	18.0	2.0	88.09
Amistar top32.5%SC	75	14.0	1.5	91.07
Collis 30%SC	50	19.0	1.8	89.28
Score 25%EC	50	21.0	2.2	86.90
Punch 40%EC	3	24.0	2.4	85.71
Bellis 38%WG	30g	26.0	2.6	84.52
Bellis 38%WG	50	11.0	1.2	92.85
Cabrio Top 60%WG	100	19.0	1.8	89.28
Flint 50%WG	20	18.0	1.7	89.88
Check	0.0	83.0	16.8	-
L.S.D at 5%		4.35	0.22	-

Effect of foliar application with strobilurin and triazole fungicides in controlling powdery mildew disease:

Different concentrations of strobilurin and triazole fungicides were evaluated for their effectiveness against powdery mildew disease of Red Romi grape during 2008 and 2009 seasons . Three post-infection foliar applications of strobilurin and triazole fungicides inhibited the development of the disease on leaves and fruit clusters.

Data presented in Table 3 demonstrated that treatment with 50g/100L water Bellis showed significant suppression , being 6.20 and 4.10% on leaves and fruit clusters, respectively and provided 90.92 and 92.57% efficacy. While, disease severity increase in nontreated vines, reached 68.32 and 55.20% , respectively. Applications of Amistar Top , Collis and Amistar at concentrations of both 75 and 50ml/100L water were highly effective , but not significantly and more effective than triazole fungicides in preventing the infection on leaves and fruit clusters, being 7.66,8.10 and 8.44% on leaves and 5.83,6.75 and 6.90% on fruit clusters, reaching efficacy 88.78, 88.14 and 87.64 on leaves and 89.43,87.77 and 87.50% efficacy on fruit

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clusters, respectively. Cabrio Top followed by Flint and Score at concentrations 100,20 and 50g or ml/100L water gave the moderate effect , but not significantly in preventing the infection with powdery mildew on leaves and fruit clusters . While ,Punch and Bellis at concentrations 3ml and 30g/100L water were the least effective of the treatment in suppressing the disease during 2008 season.

Table 3: Evaluation of strobilurin and triazole fungicides against powdery mildew disease of grapevine under natural conditions during 2008 season:

Fungicides	Concentration with ml or g/100L water	Severity of infection on Leaves	Efficacy %	Severity of infection on berries	Efficacy %
Amistar 25%SC	50ml	8.44	87.64	6.90	87.50
AmistarTop 32.5%SC	75	7.66	88.78	5.83	89.43
Collis 30%SC	50	8.10	88.14	6.75	87.77
Score 25%EC	50	9.65	85.87	7.25	86.86
Punch 40%EC	3	10.55	84.55	8.34	84.89
Bellis 38%WG	30g	11.10	83.75	8.65	84.32
Bellis 38%WG	50	6.20	90.92	4.10	92.57
Cabrio Top 60%Wg	100	9.10	86.68	7.65	86.14
Flint50%Wg	20	9.25	86.46	7.39	86.61
Check	0.0	68.32	-	55.20	-
L.S.D at 5%		1.32	-	1.08	-

Also, it was clear from the data in Table 4, that all strobilurin and triazole fungicides significantly reduced the disease severity on leaves and fruit clusters during 2009 season. At the same time, Bellis at 50g/100L water gave the best results in controlling the disease, whereas the disease severity was 5.80 and 3.77% on leaves and fruit clusters of grapevines of this experiment, while the disease severity increased in nontreated vines, reaching 57.20 and 46.88%, respectively.

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Table 4: Evaluation of strobilurin and triazole fungicides against powdery mildew disease of grapevines under natural conditions during 2009 season.

Fungicides	Concentration with ml org /100L water	Severity of infection on leaves	Efficacy %	Severity of infection on berries	Efficacy %
Amistar 25%Sc	50ml	7.60	86.71	5.48	88.31
Amistar Top 32.5%Sc	75	7.40	87.06	4.79	89.78
Collis 30%sc	50	7.90	86.18	5.87	87.47
Score25%Ec	50	9.15	84.0	6.23	86.71
Punch40%Ec	3	9.70	83.04	7.39	84.23
Bellis 38%wg	30g	9.10	84.09	6.13	86.92
Bellis38% wg	50	5.8	89.86	3.77	91.95
Cabrio Top 60%wg	100	8.0	86.01	5.62	88.01
Flint 50%wg	20	8.10	85.83	6.13	86.92
Check	0.0	57.20	-	46.88	-
L.S.D at 50%		1.04	-	1.03	-

The disease severity on vines treated with Amistar Top, Amistar and Collis showed nonsignificant suppression, more effective than triazole fungicides in preventing the infection on the leaves. Also, the same trend was observed on fruit clusters, reaching 4.79, 5.48 and 5.87%, respectively. Cabrio Top at 100g/100L water, followed by Flint at 20g/100L water were effective in decreasing disease severity on leaves and fruit clusters compared with the check treatment during 2009 season. Score and Punch as triazole fungicides were the least effective of the treatments in suppressing the disease. Score was not significantly, more effective than Punch in suppressing powdery mildew disease on leaves and fruit cluster generally; it could be concluded that Bellis 38%WG and Amistar Top 32.5% SC were the best fungicides in controlling powdery mildew disease during 2008 and 2009 seasons.

Fungicides are the most important control measures on susceptible vines grown in region with high disease pressure. So, systemic fungicides such strobilurin and triazole are very active against powdery mildew disease. These fungicides penetrate the

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leaves and have three principal advantage, the active substance is not removed by rainfall, treatment is curative and vegetation formed after treatment is protected as well as the yield was increased. These results are in agreement with Reuveni (2009) who mentioned that trifloxystrobin is active against *U.necator* and provide an excellent control against this fungus which causes powdery mildews in apple, mango, grapes and nectarine trees.

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تأثير الرش بمبيدات ستروبيليورين وتريازول في مقاومة مرض البياض الدقيقي علي العنب

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أظهرت الدراسة أن مبيدات ستروبيليورين وتريازول ذات تأثير فعال في مقاومة مرض البياض الدقيقي وهو من الأمراض الاقتصادية الهامة والتي تصيب العنب في البستان.

وأوضحت الدراسة العملية أن استخدام مبيد بيليز ٣٨ % بتركيز ٥٠ جم / ١٠٠ لتر ماء أعطى تأثيراً عالياً حيث ثبت إنتاج الجراثيم بنسبة ٨٩%.

التركيزات المستخدمة من مبيدات فلينت ، أميستار توب ، كبريوتوب ثبتت إنتاج الجراثيم الكونيدية للمرض بنسبة ٨٤،٨٥، ٨٣% علي التوالي . بعد عشرة أيام من الرش الثالثة علي أشجار العنب ، حدث تثبيط معنوي للإصابة عند استخدام مبيد بيليز ٣٨% بتركيز ٥٠ جم/١٠٠ لتر ماء ، وكان متوسط المساحة المصابة من الأوراق ١،٢ سم وحدث اختزال في نسبة الإصابة وصل إلى ٩٢،٨٥ % ولقد أعطت مبيدات فلينت ، كوليز ، كبريوتوب بالتركيزات المستخدمة نفس التأثير .

أدي استخدام مبيدات الإستروبيليورين والتريازول رشا علي العنب بعد ظهور الإصابة إلي تثبيط الإصابة علي الأوراق وعلي الثمار . في موسم ٢٠٠٨ و ٢٠٠٩ م فقد كان مبيد بيليز ٣٨% أفضل المبيدات المستخدمة ضد الإصابة بالمرض علي الأوراق والثمار حيث أدي إلي تثبيط معنوي للإصابة مقارنة بالمعاملات الأخرى والكنترول .

ولقد أظهرت الدراسة أن مبيدات استروبيليورين أعطت تأثيراً عالياً مقارنة بمبيدات تريازول في منع حدوث الإصابة علي الأوراق وعلي الثمار .