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CONTROL OF PHOMOPSIS CANE AND LEAF SPOT OF GRAPEVINE

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ABSTRACT: *Phomopsis cane and leaf spot of grapes is an economically important disease of grapes in many regions of the world. Efficacy of application of antioxidants, calcium salts and fungicides on control of phomopsis cane and leaf spot that caused by Phomopsis viticola, was examined under greenhouse conditions during 2010 growing season in Belco Egypte Company. Antioxidants were prepared and applied by using concentration 200 ppm, calcium salts were applied by using concentration 400 ppm and fungicide were applied by using concentration of recommended dose of the product company. All components were applied before and after artificial infection. All the tested antioxidants reduced the disease significantly, but ascorbic acid was gave the best result. The tested calcium salts decreased the disease significantly, but calcium phosphate was the best one. All the tested fungicides reduced significantly disease severity, generally the fungicide Filint gave the best result, followeds by Topas and Punch*

Key words: *Vitis vinifera , Phomopsis viticola, antioxidants, calcium salts and fungicides.*

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most ancient fruit crops known to humans. In Egypt, it is considered an important summer fruit because of its nutritive value, and also it is an important export crop, because of its high quality which are desirable in the world markets. Grapevine trees are liable to be attacked by many pathogens. Phomopsis cane and leaf spot of grapevine is one of the most important diseases which cause great losses in the yield (Kuo *et al.,* 1998; Saber, 1998; Lationovic *et al.,* 2004; Ellis and Erinik, 2008 and Kamal, Aida, 2008). *Phomopsis viticola* (Sacc.) is the causal agent of phomopsis cane and leaf spot of grapes (Sanseovic, 2002; Aroca *et al.,* 2008; Navarrete *et al.,* 2009 and Ypema *et al.,* 2011). The fungus can infect all green parts of the grapevine and overwinter in the bark of old canes. Dark brown lesions on the shoots and leaves are the most common symptoms of the disease. Cluster symptoms are usually first noticed as the fruit berries rot of fall to the ground (Schilder *et al.,* 2005; Ellis and Erinik, 2008 and Larignon *et al.,* 2009).

Phomopsis cane and leaf spot is the most difficult grapes disease to control. Therefore, this study aimed to study:

1. Reaction of different grape varieties and root stalk to *Phomopsis viticola*.
2. Control the dead-arm disease of grapevine (Phomopsis cane and leaf spot), by using antioxidants, calcium salts and different fungicides.

MATERIALS AND METHODS

Reaction of different grapevine cultivars to infection:

Five grape cultivars i.e., Ara, peraiem, utmroial, early sweat, Victoria and five root stalk i.e., Dog ridge, Freedom, Rekhtur, Polsen and Ramsey were evaluated against dead-arm disease. *Phonopsis viticola* isolates were grown on PDA for 10 days at 25°C, then 5 mm in diameter disk of fungus isolate was inoculated in dead cut of branch tissues of each cultivar. They tied strongly with adhesive tape. The seedlings were irrigated with tap water when necessary. Three replicates were used for each cultivar and four plants were used as check.

The radius of the dead-arm disease was evaluated on 15 transplants of each replicate as the scale:

0 = no symptoms.
1 = 1 – 15 mm canker area.
2 = 16 – 30 mm canker area.
3 = 31 – 45 mm canker area.
4 = up to 45 mm canker area.

The disease severity was calculated according to the following formula:
Disease severity (%) = [∑ (n×v)/ (N×V)] ×100
Where:
n = No. of transplants
v = the rate disease score
N = total No. of seedling investigated
V= highest disease severity rate canker area.

Disease Control:

Evaluation of calcium salts, some antioxidants and some fungicides to control Phonopsis cane and leaf spot of grapevine was carried out under greenhouse conditions.

Calcium salts:

Four calcium salts i.e., calcium phosphate, calcium carbonate, calcium chloride and calcium nitrate were applied under greenhouse conditions. 400 ppm solutions were prepared and sprayed before and after artificial infection. Three replicates for each calcium salt were used plus one group of four seedling was used as control. After 45 days, all of branches showing typical symptoms were estimated.

Antioxidants:

Five antioxidants were used in this study for controlling the dead-arm disease of grapevine, i.e., Ascorbic acid, Sodium benzoate, Boric acid, Citric acid and Oxalic acid at 200 ppm. Isolate No. 5 of the pathogen; the most virulent isolate; was used.

Fungicides:

Ten fungicides i.e., Flowable sulfer, Champion, Filint, Topas, Punch, Peals, Ridomil gold, Somieat, Koside 101 and Rubigan (Table 1), were used to study their effect on grapevine dead-arm incidence. The highest susceptible cultivar (superior seedless) was used in this experiment. Branches of this cultivar were inoculated with *Phomopsis viticola* separately, then sprayed with the tested fungicides at the same time of inoculation as well as before 2 days of spraying with fungicides and 3, 7, 15 and 35 days after spraying with fungicides. Generally, four seedlings of two years old rotted vines under greenhouse conditions were used as replicates. The length of diseased parts of branches was measured after 45 days from artificial infection.

Statistical analysis:

All data obtained were subjected to the proper statistical analysis for each experiment using the SAS (2004) statistical software. Comparisons were made following Duncan's LSD (0.05).

Table (1). List of used fungicides, their commercial name, active ingredient recommended dose and pre-harvest ingredient (P.H.I.).

Commercial name	Active ingredient	Recommended dose	P.H.I* (day)
Flowable sulfur	Sulfur 80%	250 cm / 100 l.w	7
Champion	Copper oxycolride 77%	150 g / 100 l	7
Filint	Alastervjelloran 50%	20 g / 100 l	21
Topas	Penconazole 10%	25 cm / 100 l.w	21
Punch	Fluzilazal 40%	3 cm / 100 l.w	21
Peals	Pyraclostrobin 12.8% + Boscalid 25.2%	50 gm / 100 l.w	15
Ridomil gold	Metalaxylm mancozeb 72%	250 g / 100 l	7
Somieat	Danokonasul 5%	35 cm / 100 t	14
Koside 101	Cuprichyd roxide 77%	150 g / 100 l	7
Rubigan	Fenarimol 12%	25 cm / 100 l	21

* Pre-harvest ingredient.

RESULTS AND DISCUSSION

1. Reaction of different grape verities and root stalk to *Phomopsis viticola*:

The results in Table (2a) clear that early sweet seedless cv. at 45 days was found to be the highest susceptible one to eye infection (3.33 disease severity), followed by Victori seedless (2.91 disease severity). Praiem seedless recorded the least eye infection severity (2.5 disease severity).

Infection of shoots (3 cm) results show that early sweet seedless and Utimroial seedless were found to be the highest susceptible varieties, severity of the infection of each was 3. In contrast, Praiem seedless recorded the least disease severity (2). Infection of shoots; 6, 10 and 20 cm; showed that, the Utmroial seedless, Praiem and Utmroial seedless gave 2.75, 1.41 and 0.41 disease severity, respectively. Ara, Victoria and Victoria seedless gave 1.75, 0.50 and 0.00 disease severity, respectively. These results are in agreement with Saber (1997), Kuo *et al.* (1998), Lationovic *et al.* (2004) and Kamal Aida (2008).

As for root stalk reaction to the fungus (Table 2b). Data revealed that, Dog Ridge at 45 day was found to be the highest susceptible one to eye infection giving the highest disease severity (3.5). Freedom recorded that the least eye infection severity (2.33).

Table (2 a). Reaction of different grapes to *Phomopsis viticola*.

Variety	Infection mode / disease severity					Control	Mean
	Inf. eye	Shoot 3 cm	6 cm.	10 cm.	20 cm.		
Ara	2.75[a]*	2.58[a]	1.75[b]	1.25[a]	0.08[a]	0	1.68
Praiem	2.5[a]	2[a]	2.16[ab]	1.41[a]	0.16[a]	0	1.64
Utmroial	2.83[a]	3[a]	2.75[a]	0.91[a]	0.41[a]	0	1.99
Early sweet	3.33[a]	3[a]	2.25[ab]	1.33[a]	0.33[a]	0	2.046
Victoria	2.91[a]	2.91[a]	2.75[a]	0.5[a]	0[a]	0	2.014
LSD at 0.05	1.39	1.0339	0.894	0.8169	0.4571	0	

* The means followed by the same letters are not significantly different according to LSD at 5%.

Table (2 b). Reaction of different grapes (root stalk) to *Phomopsis viticola*.

Root stalk	Infection mode / disease severity					Control	Mean
	Inf. eye	Shoot 3 cm	6 cm.	10 cm.	20 cm.		
Ramse (solt crik)	2.83[a]*	2.91[a]	2.16[a]	1.66[a]	0.16[a]	0	1.94
Polsen (1103)	3.16[a]	3.16[a]	2.41[a]	1.50[a]	0.16[a]	0	2.078
Rekhtur (110)	2.91[a]	3.66[a]	2.58[a]	1.58[a]	0.33[a]	0	2.012
Freedum	2.33[a]	3.25[a]	2.58[a]	1.91[a]	0.33[a]	0	2.012
Dog Ridge	3.5[a]	3.25[a]	2.41[a]	1.58[a]	0.33[a]	0	2.21
LSD at 0.05	1.3188	0.8237	0.6558	0.6281	0.4028	0	

* The means followed by the same letters are not significantly different according to LSD at 5%.

Infection of 3 cm shoots results show that Rekhtur 110 was found to be highest susceptible varieties (3.66 disease severity). In contrast, Ramse (salt crik) recorded the lowest disease severity (2.91). Infection of shoots 6 and 10 cm results recorded that the highest disease severity was found on Rekhtur 110 and Freedum (2.58 and 1.91, respectively). In contrast Ramse and Polsen 1103 showed the least disease severity (2.16 and 1.50, respectively). Dog Ridge, Freedum were susceptible in infection of shoots 20 cm, their disease severity was 0.33. But the Ramse and Polsen recorded that the least degree of disease severity (0.16) among the root stalk tested. These results are in agreement with Cazelles *et al.* (1991).

Calcium salts:

Data in Table (3) show that calcium phosphate is the most effective salt in controlling dead-arm disease caused by *Phomopsis viticola* in case of spraying seedling before (0.25 disease severity). Calcium nitrate and calcium chloride were less effective (1.25 disease severity). On the other hand, in case of spraying seedling with calcium salts after artificial infection with 3, 7 and 15 days, the best salt and the more effected salt was calcium phosphate, during the 3, 7 and 15 days (0.25, 0.75 and 1.25, respectively). In contrast the least effective on *Phomopsis viticola* was calcium chloride and calcium carbonate during the 3, 7 and 15 days (1.75, 2.00 and 2.25, respectively). But in case of the 35 days there aren't any significant between the four calcium sorts, in companion with control. Noyes and Hancock (1981) and Trazilbo *et al.* (2009) studied that incidence and severity of weight mould were significant were used with application of calcium chloride and calcium sulfate.

Table (3). Effect of four calcium salts on dead-arm disease of grapevine (Superior) by *Phomopsis viticola* under greenhouse conditions.

Calcium salts	Disease severity % on grapevine seedlings				
	Days before inoculation	Days after inoculation			
	2	3	7	15	35
Calcium phosphate	0.25^{b}*	0.25^{b}	0.75^{a}	1.25^{a}	2.25^{a}
Calcium chloride	01.25^{b}	1.75^{ab}	2.00^{a}	2.25^{a}	2.75^{a}
Calcium carbonat	0.75^{b}	1.75^{b}	2.00^{a}	2.25^{a}	2.00^{a}
Calcium nitrate	1.25^{ab}	0.75^{b}	2.5^{a}	2.00^{a}	2.5^{a}
Control (untreated)	2.75^{a}•	2.75^{a}	2.75^{a}	2.75^{a}	2.75^{a}
LSD at 0.05	1.82	1.81	2.00	1.92	1.81

The means followed by the same letters are not significantly different according to LSD at 5%.
* Mean of 3 replicates, data were recorded 45 days after inoculation.
• Mean of 4 replicates, data were recorded 45 days after inoculation.

Antioxidant:

Data in Table (4) illustrated that ascorbic acid is the most effective antioxidant in the control of the dead-arm disease and gave the lowest disease severity incidence (1.75), sodium benzoate and citric acid were less effective. In case of spraying seedling three days after artificial infection ascorbic acid was the most effective antioxidants (2.0 disease severity). In contrast, the least effective one on mycillial growth of *Phomopsis viticola* was sodium Benzoate (3.00). On the other hand, ascorbic acid and oxalic acid were the most effective antioxidants in case of sprayed seedling after artificial infection with 7 days (2.25 disease severity), but the least effective one on *Phomopsis viticola* was citric acid (3.50 disease severity). On the other side, in case of spraying seedling after artificial infection with 15 and 35 days, oxalic acid and ascorbic acid were the best and the most effective against *Phomopsis viticola* (2.5 and 2.00, respectively). In contrast, boric acid was the least effective one. In general, there were no significant differences among the tested antioxidants. These results were confirmed those obtained by Galal *et al.* (2002), Saber *et al.* (2003) and Kim *et al.* (2008). They reported that salicylic acid, ascorbic acid and oxalic acid were gave the best effects as the decreased the incidence and severity of fruit rots.

Fungicides:

Data in Table (5) indicate that all fungicides tested to control dead-arm disease of grapevine seedling were highly effective in case of spraying before artificial infection with 2 days. But in case of spraying this fungicides after artificial infection with 3 days, Filint, Topas and Punch C. were the most effective fungicides. Spray fungicides after artificial infection with 7 days, Filint was the best one in controlling *Phomopsis viticola*. In case of spraying the fungicides after the artificial infection with 15 and 35 days, it found that all fungicides were not effective of, but Filint recorded the least degree of disease. Generally, Filint, Topas and Punch C., respectively were the most effective fungicides in controlling *Phonopsis viticola*. These results confirmed results obtained by Politi (1998), Saber (1998), Nita *et al.* (2006), Nita *et al.* (2007) and Armeng (2009).

Table (4). Control of dead-arm disease of grapevine genotype Superior seedless with antioxidant by spraying under greenhouse condition.

Antioxidant	Disease severity % on grapevine seedlings				
	Days before inoculation	Days after inoculation			
	2	3	7	15	35
Boric acid	2.25[a]*	2.5[a]	2.75[a]	3.75[a]	3.00[a]
Citric acid	0.75[a]	2.75[a]	3.5[a]	3.25[a]	2.75[a]
Oxalic acid	2.00[a]	4.00[a]	2.25[a]	2.50[a]	3.00[a]
Ascorbic acid	1.75[a]	2.00[a]	2.25[a]	3.50[a]	2.00[a]
Sodium benzout	2.75[a]	3.00[a]	2.5[a]	2.75[a]	2.50[a]
Control (untreated)	2.75[a]•	2.75[a]	2.75[a]	2.75[a]	2.75[a]
LSD at 0.05	2.16	2.02	2.00	2.92	2.89

The means followed by the same letters are not significantly different according to LSD at 5%.
* Mean of 3 replicates, data were recorded 45 days after inoculation.
• Mean of 4 replicates, data were recorded 45 days after inoculation.

Table (5). Chemical control by fungicides.

Fungicides	Disease severity % on grapevine seedlings				
	Days before inoculation	Days after inoculation			
	2	3	7	15	35
Flowable sulfer	0.50^{b*}	1.00^{b}	2.50^{a}	1.50^{ab}	2.25^{ab}
Champion	0.25^{b}	0.75^{b}	1.75^{ab}	2.00^{ab}	2.50^{ab}
Filint	0.00^{b}	0.00^{b}	0.00^{b}	0.25^{b}	0.75^{ab}
Topas	0.00^{b}	0.00^{b}	0.25^{b}	0.75^{b}	1.75^{ab}
Punch C	0.00^{b}	0.00^{b}	1.00^{b}	1.00^{ab}	2.00^{ab}
Pleals	0.00^{b}	0.25^{b}	1.50^{b}	1.50^{ab}	1.75^{ab}
Ridomil gold	0.00^{b}	0.00^{b}	1.75^{b}	1.75^{ab}	2.25^{ab}
Somieat	0.00^{b}	0.50^{b}	1.25^{b}	1.25^{ab}	1.50^{ab}
Kosied 101	0.00^{b}	0.00^{b}	1.75^{b}	1.75^{ab}	2.25^{ab}
Rubigan	0.00^{b}	0.00^{b}	0.75^{b}	0.75^{ab}	1.25^{ab}
Control (untreated)	2.75$^{a\bullet}$	2.75^{a}	2.75^{a}	2.75^{a}	2.75^{a}
LSD at 0.05	1.71	1.74	1.85	1.94	1.94

The means followed by the same letters are not significantly different according to LSD at 5%.
* Mean of 3 replicates, data were recorded 45 days after inoculation.
• Mean of 4 replicates, data were recorded 45 days after inoculation.

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مقاومة مرض تبقع الأوراق وقصبات الفوموبسس فى العنب

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الملخص العربى :

تم دراسة رد فعل أصناف العنب المختلفة لمرض تبقع الأوراق وقصبات الفوموبسس فى العنب (الذراع الميت) المتسبب عن فطر *Phomopsis viticola* وأوضحت النتائج أن جميع أصناف العنب تحت الدراسة أظهرت حساسيتها للإصابة بالمرض ، وكان أعلى الأصناف حساسية هو صنف الإيرلى سويت وأقلها حساسية هو صنف البرايم . أما بالنسبة لعدوى الأصول فقد أظهرت جميعها حساسيتها للإصابة بمرض الذراع الميت ، وكان أقلها حساسية هو أصل الرمزى.

كما تم عمل مقاومة بعدة طرق لمقاومة مرض الذراع الميت فى العنب ، وقد أوضحت النتائج أنه فى حالة استخدام أملاح الكالسيوم كانت أفضل مركبات الكالسيوم هى ملح فوسفات الكالسيوم وذلك عند استخدامه فى رش شتلات العنب صنف السوبريور بتركيز ٤٠٠ جزء فى المليون قبل عمل العدوى بيومين . وكذلك أوضحت النتائج فى حالة استخدام مضادات الأكسدة فى مقاومة الذراع الميت فى العنب كان أفضل هذه المركبات هو حمض الأسكوربيك عند استخدامه فى رش شتلات العنب صنف السوبريور بتركيز ٢٠٠ جزء فى المليون وذلك قبل عمل العدوى الصناعية بيومين . وأوضحت النتائج أنه عند استخدام المبيدات الكيماوية كان أفضل هذه المبيدات وأكثرها تأثيراً هو مركب فيلنت وكان يليه مركب التوباس ثم البانش سى وذلك فى حالة استخدامهم فى رش شتلات العنب صنف سوبريور بالتركيز الموصى به من قبل الشركة المنتجة .