

MANAGEMENT OF SHOT-HOLE DISEASE OF STONE FRUIT TREES CAUSED BY *Stigmina carpophila*

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ABSTRACT

Shot-hole on leaves of stone fruit, caused by *Stigmina carpophila* (Lev.) M.B. Ellis was serious on peach, apricot and plum trees in Nobariya area, 6th October and Menofiya governorates in 2007-2008 seasons. Isolation carried out from different cultivars revealed no variation among isolates from samples collected from different localities. Calcium and potassium salts (chloride and hydroxide) were more effective than (nitrate and phosphate) in reduction of *S. carpophila* in the *In-vitro* and open filed, Higher efficacy of salts to control shot hole disease was recorded for the higher salt concentration as shown in trees sprayed with 1800 ppm. Antagonism studies done on the pathogen and bioagents i.e. *Trichoderma viride*, *T. harzainum*, B.light stop (*T. harzainum*), and *Bacillus subtilis* either in *In-vitro* or in greenhouse reduced disease severity in both 2007 and 2008 seasons. Fungicides tested i.e. (Punch, Sumi-8, Sythane-24, Topas-100, Flint, and Cam Zen) in the *In-vitro* and in open field gave the best control of shot hole disease.

Keywords: Stone fruit trees, shot- hole, *Stigmina carpophila*, disease management.

INTRODUCTION

Shot-hole disease of stone fruits, caused by *S. carpophila* (Lev.) (Adaskaveg *et al.*, 1990) is a serious disease of *Prunus* species in many temperates to semi-arid regions of the world. *S. carpophila* survives winter months in association with dormant buds of stone fruits and disperse from twig cankers by splashing water (Shaw *et al.*, 1990). Biological control of shot-hole disease on apricot was reported by Esitken *et al.*, (2002) and Butin (2003). Two bacterial strains of *Bacillus spp.* were reported to have great potential of antagonistic activity against *S. carpophila* on apricot. However, the disease is routinely controlled using fungicides (Shaw *et al.*, 1990). The current study aimed to monitor fungal isolates associated with shot-hole disease on different stone fruits in different localities, investigating the favorable environmental conditions, and studying the efficacy of certain chemical and biological agents to suppress disease development in the open field.

MATERIALS AND METHODS

Isolation and identification of the causal organism:

Diseased samples were subjected to culturing on PDA. Conidia were produced by culturing of the fungus *S. carpophila* on PDA and incubated under dark conditions on 20–22°C for 7 days (Shaw *et al.*, 1990). The fungus was purified using the single spore technique, and was identified according to Barnett and Hunter (1972) and Ellis (1976).

Survey of the disease:

The disease was surveyed at Nobariya area, 6th October and Menofiya governorates in the seasons 2007 and 2008. One-hundred trees were randomly chosen for each of Kanino variety of apricot; Florida prince variety of peach and Beauty variety of plum cultivated at each locality were examined to determine disease severity by estimating the healthy and infected area in leaves in all of examined tree according to disease scale of the researcher :- No spots, disease intensity=0 grade=0; 1-5 spots from 1-20% grade =1; 6-10 from 20-40% grade 2; 11-15 spots from 41-60% grade 3; 16-25 spots from 61-80% grade 4; >25 spots >80% grade 5

Disease severity was computed using formula:
$$D.S. = \frac{\text{Sum } (n * k)}{N * K} \times 100,$$

Where: n = number of infected leaves

k= disease rate

N= total number of examined leaves

K= maximum disease rate

Pathogenicity tests:

Stone fruits seedlings inoculation and incubation in greenhouse:

Five replicates of potted 2 – years old seedlings of Kanino variety of apricot; Florida prince variety of peach and Beauty variety of plum, in April 2008 in the greenhouse, were used in this study. Inoculation with *S. carpophila* (2.5×10^6 conidia/ml) was made by spraying 10 ml/seedling. Five seedlings sprayed with water served as check control and all treatments were fertilized and irrigated as recommended by Korra (1989). Pathogenic potentials of isolates were determined after two weeks in terms of percentage of infection. The inoculated fungus was re-isolated for verification of pathogenicity.

**Effect of potassium and calcium salts on inhibition of *S. carpophila* :
In-vitro studies:**

Different concentrations of potassium and calcium elements in the form of (chloride, hydroxide, nitrate, and phosphate salts) were individually dissolved in water, sterilized and added to warm (40°C) PD broth flasks to give concentrations of 0, 100, 200, 300, 400, 500 and 600 ppm. The flasks were inoculated with *S. carpophila* isolate and incubated at 25°C for 2 weeks, where 5 flasks were used for each treatment as replicates. Dry weight of the fungus was determined as routinely employed.

***In-vivo* studies effect of potassium and calcium sprays on disease severity:**

Different concentrations of potassium and calcium elements Table (4, 5, and 6) were sprayed on stone fruit trees for disease control in two growing seasons, 2007 and 2008. The trees considered were 7-year-old field raised of Kanino variety of apricot; Florida prince variety of peach and Beauty variety of plum. Fertilization, irrigation were adopted as recommended. Five trees from each cultivar were sprayed after full bloom with each salt concentration. Untreated trees were used as control. Disease severity was recorded after two weeks.

Biological control trials:

Bacterial and fungal antagonists examinations:

B. subtilis (BS1 and BS2) provided by Dr Gomaa, A. Bacterial Diseases Department, Plant Pathology Res. Institute, Agric. Res. Center.; *T. harzianum* and *T. viride* from diseased leaves of Florida prince variety of peach, isolated and identified by the authors and further verified by dr Hafez, T. in the Central *In-vitro* of Organic Agriculture, were used., Bacterial strains were grown on nutrient agar. Bacterial suspension was prepared in 500 ml flasks containing nutrient broth (NB), on a rotating shaker (150 rpm) overnight at 25°C (Schaad, 1988). The resulting bacterial suspensions were used for spraying fruit trees.

Field trials:

In 2007 and 2008, field experiments were conducted on an infected 7 years-old stone fruit trees at 6th October governorate (Gharb El-Dreesa village) on Kanino variety of apricot; Florida prince variety of peach and Beauty variety of plum. Spraying with 5 liter / tree by bio-agents; bacterial suspension, 10⁹ CFU ml⁻¹, of bacterial isolates; *B. subtilis* (Bs1, Bs2 and the commercial product Rhizo-N (*Bacillus sp.*)), 5 x 10⁶ spores/ml of *T. viride*, *T. harzianum* and Bilight stop (*T. harzianum*) produced by the Central *In-vitro* of Organic Agriculture, (Howell, 2003). Each application was made after full bloom. Untreated trees were used as control. Disease severity on trees was determined after two weeks as previously shown.

Fungicides tests:

Effect of fungicides *in – vitro* :

Punch (flusilazole), Sumi-8 (diniconazole), Sythane-24 (myclobutanil), Topas-100 (penconazole), Cam-zen 50%WP (Carbendazim), Flint (trifloxystrobin), Cupprio-top 38% WG (Pyraclostrobin + Metiram) and Copper acrobat (copper hydroxide)), were used *in vitro* to evaluate fungicidal effect on linear growth of *S. carpophila*. Different concentrations from 25 to 1000 ppm, based on the active ingredient of each fungicide, were prepared as standard volume of PDA medium before pouring plates. PDA plates were inoculated with 5 mm-fungal discs from 7- days-old cultures of the pathogen , incubated on 25°C for 7days, where the fungus linear growth was measured.

Effect of fungicides *in – vivo* :

Field trials were made in two growing seasons, 2007 and 2008, in the three locations selected in this work. Fertilization, irrigation, and other cultural practices were practiced as recommended. Application of the fungicides in concern was made after bloom stage as replicated plots of five trees. Each compound was sprayed three times, after full bloom and at 2 weeks intervals. Untreated trees were used as control. Disease data were recorded after two weeks as disease severity rates of the tested fungicides in controlling the disease was calculated according to the following formula: disease severity estimating by compute the healthy and infected area in leaves in all of tree according to disease scale of the researcher No spots, disease intensity=0 grade=0; 1-5 spots from 1-20% grade =1;6-10 from 20-40% grade 2;11-15 spots from 41-60% grade 3; 16-25 spots from 61-80% grade 4; >25 spots >80% grade 5

Disease severity was computed using) formula:
$$D.S. = \frac{\text{Sum (n * k)}}{N * K} \times 100,$$

$$\% \text{ Efficiency} = \frac{\text{Disease severity in control \%} - \text{Disease severity \% in treatment}}{\text{Disease severity \% in control}} \times 100$$

Statistical design and analysis:

A split split plot design was used for this experiment and obtained data were analyzed using analysis of variance (ANOVA) according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Isolation and identification of the causal organism:

Isolation of the pathogen was made from the foliage of an infected apricot; Florida prince variety of peach and Beauty variety of plum. at 6th October, Nobariya and Menofiya governorates. The fungus was isolated and purified. The isolates were identified by the authors as *S. carpophila* Fig (1): shot hole in leaves of Florida prince cv. of peach. FIG (2): shot hole in fruits of Florida prince cv. of peach. FIG (3): spores of *S. carpophila*. FIG (4): shot hole in leaves of Florida prince cv. of peach. FIG (5): shot hole in leaves of kanino cv. of apricot.

Survey of the shot hole of stone fruit trees disease:

Data in Table (1) show that Disease severity (%) of one-hundred stone fruit trees of Kanino variety of apricot; Florida prince variety of peach and Beauty variety of plum, trees chosen at random at 6th October, Nobariya and Menofiya governorates in 2007 and 2008, in cv. Kanino of apricot reached 87.36%. 85% and 80.34 % in Menofiya, Nobariya and 6th October governorates, respectively. Florida Prince peach recorded 88%, 86.7 % and 83 % Infection at Menofiya ,Nobariya and 6th October governorates, respectively. Meanwhile, Beauty plum recorded 70% in Nobariya, 6th October and Menofiya governorates. Statistical analysis of disease severity (%) on cultivars and different localities revealed in- significant differences between the three localities.



Fig. (1): Shot hole in leaves of Florida prince cv. of peach.

Fig. (2): shot hole in fruits of Florida prince cv. of peach.

Fig. (3): spores of *S. carpophila*.

Fig. (4): shot hole in leaves of Florida prince cv. of peach.

Fig. (5): shot hole in leaves of kanino cv. of aprico

Table (1) : Disease severity % on shot-hole of stone fruit trees .

Localities	Stone fruits	Disease severity(%)		Mean
		2007	2008	
06-Oct	peach	78.6	83.3	80.9
	plum	80.3	70.0	76.7
	apricot	80.3	80.3	80.3
Nobariya	peach	78.0	86.7	82.3
	plum	76.0	70.0	73.3
	apricot	82.6	85.0	83.8
El-Menofiya	peach	73.3	88.0	80.6
	plum	80.3	70.0	76.7
	apricot	87.3	87.3	87.3
Mean	peach	76.7	86.7	81.7
	plum	78.9	70.0	74.5
	apricot	79.3	85.2	82.2

LSD 0.05: Location(L) = 0.54; Stone fruits (S) = 0.31; Year (Y) =1.88, L x S = 0.17, Y*S=0.75, S*L*Y=1.89.

Pathogenicity test:

Table (2) shows that all *S. carpophila* isolates were pathogenic to peach, apricot and plum seedlings. Statistical analysis of pathogenicity of isolates from different cultivars revealed significant differences between isolates from stone fruits species and in- significant differences of isolates from different localities. Shot- hole disease on leaves of stone fruit trees caused by *S. carpophila* in many of temperate to semi arid regions of the world and is a serious disease on *Prunus* species in Egypt. Obtained isolates were pathogenic to all checked stone fruits without any significant differences in their virulence. These findings are in harmony with those reported by Ashour and Allam (1954); Aldrich *et al* (1974); Adaskaveg *et al.* (1990); Shaw *et al.* (1990); Ogawa and English, (1991); Sahzad, and Mir, (1996); and Butin (2003).

Table (2): Pathogenicity of *S. carpophila* isolates on stone fruit trees

	Disease severity (%)				Grand Mean
	Peach	Apricot	Plum	Mean	
Peach th 6-october isolate	50.0	50.0	47.0	49.0	49.0
Peach Nobariya isolate	50.0	50.0	47.0	49.0	
Peach Menofiya isolate	50.0	50.0	47.0	49.0	
Apricot th 6-october isolate	47.0	47.0	46.0	46.6	46.6
Apricot Nobariya isolate	47.0	47.0	46.0	46.6	
Apricot Menofiya isolate	47.0	47.0	46.0	46.6	
Plum th 6-october isolate	46.0	46.0	46.0	46.0	46.0
Plum Nobariya isolate	46.0	46.0	46.0	46.0	
Plum Menofiya isolate	46.0	46.0	46.0	46.0	
Mean of Peach isolates	50.0	50.0	47.0	49.0	47.2
Mean of Apricot isolates	47.0	47.0	46.0	46.6	
Mean of Plum isolates	46.0	46.0	46.0	46.0	
Grand Mean	47.6	47.6	46.3	47.2	

LSD 0.05: Isolates (I) = 0.54; Stone fruits (S) = 0.31; I x S = 0.17.

Surveying of shot hole of stone fruit trees disease during 2007 and 2008 growing seasons in the inspected governorates, i.e. Menofiya, Nobariya, 6th October of Kanino variety of apricot; Florida prince variety of peach and Beauty variety of plum, was carried. Statistical analysis of Infection (%) on Kanino apricot trees; Florida Prince peach Beauty plum on cultivars and different localities revealed significant differences.

Effect of calcium and potassium salts on dry weight of *S. carpophila*:

The inhibitory effects of potassium and calcium salts i.e. (chloride, hydroxide, nitrate and phosphate) on dry weight of *Stigmia carpophila* is shown in Table (3), where K and Ca hydroxide and chloride decreased the dry weight of the tested fungus at all tested concentrations. On the other hand, potassium and calcium, either in the form of nitrate or phosphate was showed less effective on dry weight of *S. carpophila*. The investigated effect of seven concentrations of 8 potassium and calcium salts on dry weight of *S. carpophila* indicated that Ca and K (chloride and hydroxide/ each) decreased the dry weight of the tested fungus at all concentrations. However, nitrate and phosphate salts showed lower inhibitory effects. This finding referred to the varied effect of anion radicals of tested salts which in turn may affect their efficiency when applied *in vivo*. Data was demonstrated rated the general toxicity of calcium and potassium salts to growth of the pathogenic fungus. The toxic effect varied according calcium and potassium salts as well as pathogen, these results agree with those reported by Biggs *et al.* (1997) and El-Baz, Sahar *et al.* (2007).

Table (3): Calcium and Potassium salts effect on *S. carpophila* dry weight in *In-vitro*.

Salt	Fungus dry weight (g) at salt concentration (ppm)					
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm
Potassium hydroxide	0.80	0.80	0.80	0.60	0.60	0.60
Potassium nitrate	1.30	1.20	1.10	1.10	1.10	1.10
Potassium chloride	1.00	0.90	0.90	0.80	0.80	0.70
Potassium phosphate	1.20	1.20	1.10	1.10	1.10	1.10
Calcium hydroxide	0.90	0.90	0.80	0.60	0.60	0.60
Calcium nitrate	1.20	1.10	1.10	1.10	1.10	1.00
Calcium chloride	1.10	1.00	0.90	0.90	0.80	0.80
Calcium phosphate	1.30	1.20	1.20	1.10	1.10	1.10
Control	2.30	2.30	2.30	2.30	2.30	2.30

Evaluation of calcium and potassium salts for disease control:

Data in Tables (4, 5 and 6) showed that spraying of stone fruit trees with calcium chloride or hydroxide decreased disease severity. Similar effect was observed for potassium chloride and hydroxide. However, nitrate and phosphate forms were showed less effective. Higher efficacy of salts to control shot hole disease was recorded for the higher salt concentration as shown in trees sprayed with 1800 ppm followed by 1200 ppm and then 600 ppm in 2007 and 2008, in Menofiya, Nobariya and 6th October governorates, respectively.

Table (4): Effect of calcium and potassium salts on disease severity of shot-hole in peach.

Ca. &K. salts	Dose /ppm	Disease severity (%)							
		6- th october		Nobareya		El-Menofeya		Grand Mean	
		2008	2007	2008	2007	2008	2007	2008	2007
K. hydroxide	1800	30.0	28.00	30.0	28.0	30.0	28.0	30.0	28.0
	1200	65.0	60.0	65.0	57.5	70.0	52.5	66.6	56.6
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
K. nitrate	1800	67.5	62.5	67.5	62.5	67.5	62.5	67.5	62.5
	1200	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
K. chloride	1800	22.5	25.0	25.0	25.0	25.0	25.0	24.1	25.0
	1200	37.5	40.0	50.0	40.0	52.5	40.0	46.6	40.0
	600	57.5	57.5	55.0	57.5	67.5	57.5	60.3	57.5
K. phosphate	1800	72.5	70.0	75.0	70.0	82.5	70.0	76.6	70.0
	1200	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Ca. hydroxide	1800	35.0	30.0	35.0	25.0	40.0	25.0	36.6	26.6
	1200	72.5	62.5	72.5	62.5	75.0	62.5	73.3	62.5
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Ca. nitrate	1800	65.0	60.0	65.0	67.5	67.5	62.5	65.8	63.3
	1200	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Ca. chloride	1800	25.0	25.0	25.0	25.0	30.0	25.0	26.6	25.0
	1200	72.5	60.0	70.0	55.0	75.0	55.0	72.5	58.3
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Ca. phosphate	1800	75.0	70.0	80.0	70.0	70.0	70.0	75.0	70.0
	1200	87.5	82.5	87.5	82.5	87.5	85.0	87.5	83.3
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Control check	1800	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
	1200	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
	600	92.5	92.5	92.5	92.5	92.5	92.5	92.0	92.5
Grand Mean	1800	53.8	51.4	55.0	51.7	56.1	51.1	55.0	51.4
	1200	78.3	75.0	80.0	74.1	81.0	73.8	79.7	74.3
	600	88.6	88.6	88.3	88.6	89.7	88.6	88.8	88.6

L.S.D 0.05: Salt (S) = 3.03, Conc. (C) = 1.75, Location (L)= 1.75, Year (Y) = 2.75, S*C = 5.3, S*L = 5.3

S x Y = 5.75, L x Y = 1.33, C*L = 3.03, C x Y = 2.05, C*L*S = 9.1, Y* C*L*S = 8.1 .

Infection on CV. Florida Prince of peach, CV. Beauty of plum and CV. Kanino of apricot trees was reduced by spraying the trees with Ca and K (chloride and hydroxide / each) at 1800 ppm and Ca and K (nitrate and phosphate/ each) recorded moderate effect at the three localities in 2007 and 2008. These finding *in vitro* studies for salt efficiency confirm that these salts may affect directly the fungus growth *in- vivo* and the disease development. Data show a significant reduction in disease severity with increasing calcium and potassium compounds concentrations, the highest effect on disease severity for all the tested salts was recorded at the concentration 1800 ppm. The increase in calcium and potassium availability may increase the resistance of cell walls to the penetration of the pathogen. At least 60% of

the calcium present in the cells is located in the cell wall (Tobias *et al.*, 1993) and (El- Baz, Sahar *et al.*, 2007).

Table (5): Effect of calcium and potassium salts on disease severity of shot- hole in plum.

Ca. &K. salts	Dose /ppm	Disease severity (%)							
		6- th october		Nobareya		El-Menofeya		Grand Mean	
		2008	2007	2008	2007	2008	2007	2008	2007
K. hydroxide	1800.00	35.0	30.0	35.0	30.0	35.0	30.0	35.0	30.0
	1200.00	65.0	60.0	65.0	60.0	65.0	60.0	65.0	60.0
	600.00	85.0	82.5	85.0	82.5	85.0	82.5	85.0	82.5
K. nitrate	1800.00	65.0	70.0	65.0	70.0	70.0	70.0	66.6	70.0
	1200.00	90.0	87.5	90.0	87.5	100.0	87.5	93.3	87.5
	600.00	97.5	92.5	97.5	92.5	97.5	92.5	97.5	92.5
K. chloride	1800.00	20.0	20.0	25.0	25.0	25.0	25.0	23.3	23.3
	1200.00	45.0	40.0	45.0	40.0	55.0	40.0	48.3	40.0
	600.00	57.5	55.0	62.5	55.0	67.5	55.0	62.5	55.0
K. phosphate	1800.00	82.5	77.5	82.5	82.5	92.5	80.0	85.8	80.0
	1200.00	92.5	92.5	85.0	85.0	95.0	85.0	90.8	85.0
	600.00	100.0	95.0	95.0	95.0	97.5	95.0	97.5	95.0
Ca. hydroxide	1800.00	33.0	30.0	33.0	30.0	33.0	30.0	33.0	30.0
	1200.00	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0
	600.00	65.0	60.0	65.0	60.0	65.0	60.0	65.0	60.0
Ca. nitrate	1800.00	62.5	60.0	62.5	60.0	70.0	60.0	65.0	60.0
	1200.00	87.5	85.0	87.5	85.0	87.5	85.0	87.5	85.0
	600.00	100.0	97.5	100.0	92.5	100.0	92.5	100.0	94.1
Ca. chloride	1800.00	32.5	30.0	25.0	30.0	32.5	30.0	30.0	30.0
	1200.00	70.0	57.5	65.0	57.5	75.0	57.5	70.0	57.5
	600.00	100.0	95.0	100.0	92.5	100.0	92.5	100.0	94.1
Ca. phosphate	1800.00	70.0	65.0	90.0	65.0	100.0	65.0	90.0	65.0
	1200.00	87.5	75.0	100.0	92.5	100.0	92.5	95.1	94.1
	600.00	100.0	95.0	100.0	92.5	100.0	92.5	100.0	94.1
Control check	1800.00	100.0	97.5	100.0	92.5	100.0	92.5	100.0	94.1
	1200.00	100.0	97.5	100.0	92.5	100.0	92.5	100.0	94.1
	600.00	100.0	97.5	100.0	92.5	100.0	92.5	100.0	94.1
Grand Mean	1800.00	55.6	53.3	57.5	53.8	62.0	80.2	58.7	53.6
	1200.00	70.8	71.1	75.8	71.6	80.2	71.6	77.2	72.0
	600.00	89.4	85.5	89.4	83.8	90.2	83.8	82.7	84.6

L.S.D 0.05: Salt (S)= 2.87, Conc. (C) = 1.66, Location (L)= 1.66,Year (Y) = 2.55, S*C = 4.98
 S x Y = 5.75, L x Y = 1.33, C*Y = 1.89, S*L = 4.98 ,C*L = 2.87, C*L*S = 8.6, Y*C*L*S = 8.2 .

Table (6): Effect of calcium and potassium salts on disease severity of Shot- hole in Apricot

Ca. &K. salts	Dose /ppm	Disease severity (%)							
		6- th october		Ca. &K. salts		El-Menofeya		Grand Mean	
		2008	2007	2008	2007	2008	2007	2008	2007
K. hydroxide	1800	35.0	32.0	35.0	32.0	40.0	35.0	36.6	33.0
	1200	70.0	65.0	70.0	65.0	70.0	65.0	70.0	65.0
	600	97.5	92.5	97.5	92.5	97.5	92.5	97.5	92.5
K. nitrate	1800	65.0	62.5	65.0	62.5	75.0	62.5	68.3	62.5
	1200	97.5	92.5	97.5	92.5	100.0	92.5	98.3	92.5
	600	97.5	92.5	97.5	92.5	100.0	92.5	98.3	92.5
K. chloride	1800	15.0	15.0	15.0	15.0	25.0	25.0	18.3	18.3
	1200	40.0	40.0	45.0	40.0	45.0	40.0	43.3	40.0
	600	57.5	62.5	62.5	62.5	67.5	62.5	62.5	62.5
K. phosphate	1800	75.0	75.0	75.0	75.0	85.0	75.0	78.3	75.0
	1200	97.5	92.5	97.5	92.5	97.5	92.5	97.5	92.5
	600	100.0	92.5	97.5	92.5	97.5	92.5	98.3	92.5
Ca. hydroxide	1800	35.0	30.0	35.0	30.0	40.0	30.0	36.6	30.0
	1200	65.0	62.5	70.0	62.5	75.0	62.5	70.0	62.5
	600	95.0	92.0	95.0	92.5	95.0	92.5	95.0	92.5
Ca. nitrate	1800	65.0	62.5	70.0	62.5	75.0	62.5	70.0	62.5
	1200	95.0	92.5	95.0	92.5	100.0	92.5	96.6	92.5
	600	100.0	92.5	100.0	92.5	100.0	92.5	100.0	92.5
Ca. chloride	1800	25.0	25.0	25.0	25.0	30.0	25.0	26.6	25.0
	1200	65.0	62.5	70.0	62.5	75.0	62.5	70.0	62.5
	600	100.0	92.5	100.0	92.5	100.0	92.5	100.0	92.5
Ca. phosphate	1800	75.0	75.0	80.0	75.0	90.0	75.0	81.6	75.0
	1200	100.0	92.5	100.0	92.5	100.0	92.5	100.0	92.5
	600	100.0	92.5	100.0	92.5	100.0	92.5	100.0	92.5
Control check	1800	100.0	92.5	100.0	92.5	100.0	92.5	100.0	92.5
	1200	100.0	92.5	100.0	92.5	100.0	92.5	100.0	92.5
	600	100.0	92.5	100.0	92.5	100.0	92.5	100.0	92.5
Grand Mean	1800	54.4	52.1	55.5	52.1	62.2	53.6	57.4	52.6
	1200	76.9	76.9	82.7	76.9	84.7	76.9	82.8	76.9
	600	83.6	89.1	61.3	89.1	95.2	89.1	94.6	89.1

L.S.D 0.05: Salt (S)= 3.13, Conc. (C) = 1.81, Location (L)= 1.81, ,Year (Y) = 2.65, S*C = 5.44. S x Y = 5.85, L x Y =1.42, C*Y= 1.90, S*L = 5.44 ,C*L =3.13, C*L*S =9.42 Y*C*L*S= 8.2.

Biological control:

***In - vitro* tests:**

Data in Table (7) show that *T. viride* and *T. harzianum* caused the highest reduction in linear growth of *S. carpophila* (Nobariya, 6th october and Menofiya isolates), respectively. Meanwhile, *B. subtilis* isolates (BS1 and BS2) caused low reduction of the linear growth of *S. carpophila*.

Table (7): Effect of antagonistic bioagents on *S. carpophila* causing (shot- hole) *in vitro*

Bioagents	Reduction in linear growth (in mm)			
	Nobariya iso.	6 october iso.	Menofiya iso.	Mean
<i>T. harzainum</i>	54.4	54.7	54.4	54.5
<i>T. viride</i>	61.5	62.3	61.5	61.8
<i>B. subtilis 1</i>	40.3	41.1	40.3	40.6
<i>B. subtilis 2</i>	43.3	43.9	43.3	43.5
Mean	49.9	50.5	49.9	50.1

LSD 0.05: Bioagents =3.34; Fungus isolates = 2.6; B x F = ns. ; Ns= non significant.

***In vivo* tests:**

Data in Table (8) show that the bioagent B.light Stop (*T. harzianum*) and *T. viride* (6th October isolate) have significantly reduced the causal organism of shot-hole *S. carpophila* in stone fruit trees in seasons 2007 and 2008. However, Rhizo-N and *T. harzianum*, *B. subtilis* 1 and 2 showed the lowest reduction in shot hole on stone fruit trees comparing with the control check.

Table (8): Effect of bioagents against *S. carpophila* in stone fruit seedling in greenhouse.

Bioagents	Disease severity % 2007			Disease severity % 2008			Grand Mean		
	peach	plum	apricot	peach	plum	apricot	peach	plum	apricot
<i>T. harzainum</i>	73.0	78.0	73.0	70.0	73.0	70.0	71.5	75.5	71.5
<i>T. viride</i>	36.0	42.0	41.0	35.0	40.0	40.0	35.5	41.0	40.5
<i>B. subtilis 1</i>	64.0	64.0	64.0	74.0	77.0	66.0	69.0	70.5	65.0
<i>B. subtilis 2</i>	87.0	87.00	87.0	90.0	90.0	90.0	88.5	88.5	88.5
Rizo-in	43.0	49.0	48.0	42.0	47.0	46.0	42.5	45.0	47.0
B.light stop	35.0	46.0	40.0	38.0	42.5	40.0	36.5	44.2	40.0
Control	96.5	91.0	98.5	96.0	90.0	99.000	98.750	90.500	96.250

LSD 0.05: Bio-agent (B) = 2.76; Stone fruit (S) = 2.13 ; Year (Y) = 0.14 ; B x S = 4.78; B x Y = 1.53 ; S x Y = ns ; B x S x Y = ns .

Bio-agents tests in *In-vitro* with *B. subtilis* 1, and 2, recorded the highest reduction in linear growth of *S. carpophila*. Meanwhile, *T. viride* and *T. harzianum* recorded the lowest reduction of the linear growth of *S. carpophila*. B.light stop preparation of (*T. harzianum*) and *T. viride* could reduce the incidence of pathogen of shot hole in stone fruit trees in seasons 2008 and 2009, respectively. Meanwhile, Rhizo-N (*B. subtilis* local produce) recorded moderate effect and *T. harzianum* (isolated from the infected leaves of stone fruit trees), *B. subtilis* 1 and 2 showed the lowest reduction in disease severity. These results were almost similar to those obtained *in vitro* study and agreed with the findings of Esitken *et al.* (2002) who found that *T. harzianum* and *T. viride* were the best bioagents against *S. carpophila* because it grow more than the fungus and cover it.

Chemical control:

***In- vitro* tests:**

Table (9) Topas-100, Punch, Sythane-24 and Sumi-8 resulted in complete inhibition of fungal growth of the lowers concentration tested (25 ppm). Limitation effect was observed with Flint at 1000 ppm, lower growth was experienced at lower concentration. Other tested fungicides were low effect.

Table (9): Effect of Fungicides on *S. carpophila* in the *In-vitro* experiment.

Fungicides	Linear growth per mm / fungicides conc. (per ppm)					
	25	50	100	200	500	1000
Copper acrobat (copper hydroxide)	44.0	36.0	34.0	31.0	29.0	25.0
Flint (trifloxystrobin)	11.0	10.0	10.0	7.0	7.0	0.0
Cam-zen (Carbendazim)	44.0	43.0	33.0	21.0	16.0	10.0
Copprio-Top(Pyraclostrobin+ Metiram)	22.0	18.0	17.0	14.0	13.0	13.0
Topas-100 (penconazole)	0.0	0.0	0.0	0.0	0.0	0.0
Punch (flusilazole)	0.0	0.0	0.0	0.0	0.0	0.0
Sythane-24 (myclobutanil)	0.0	0.0	0.0	0.0	0.0	0.0
Sumi-8 (diniconazole)	0.0	0.0	0.0	0.0	0.0	0.0
Control	90.0	90.0	90.0	90.0	90.0	90.0

LSD 0.05: Fungicides = 1.12; Dose = 0.91; F x D = 2.74.

***In- vivo* evaluation of certain fungicide efficiency against shot-hole disease:**

Tables (10, 11 and 12) in three localities in seasons, 2007 and 2008. showed that the shot- hole disease on peach, apricot and plum trees were completely inhibited after spraying the trees with Sumi-8, Sythane, Punch, Topas and Flint at 1000 ppm. Also, the concentration of 500 ppm of these fungicides showed greatly reduce of fungal growth compared with the other fungicides and the control, where the least effectiveness was obtained by Copprio-Top and Copper acrobat. It was also noticed a positive correlation between the fungicide concentrations and disease suppression. The fungicides, Sumi-8, Sythane, Punch, Topas-100 and Flint completely inhibited the linear growth of *S. carpophila* in *In-vitro* test, at concentrations from 25-1000 ppm. However, Cam-zen and copprio-top at 50-500 ppm showed moderate suppressive effects, The lowest inhibition was obtained by Copprio-top. These results are in harmony with the findings of Shaw *et al.* (1990). Disease severity (%) on peach, apricot and plum trees was highly reduced after spraying in the fields with Sumi-8, Sythane, Punch, Topas and Flint followed by Cam-Zen and Copprio-Top at 1000 ppm in the tested localities. Meanwhile, at 500 ppm Sumi-8, Punch and Topas inhibited the infection followed by Flint, Cam-Zen and Sythane and Copprio-Top. The higher effect of tested fungicides was obtained with higher fungicide concentration; 1000 ppm followed by 500 ppm and at the least was 250 ppm. These results agree with the findings of Shaw *et al.* (1990) and Shahzad and Mir (1996). Who they found that the disease is routinely controlled by using similar types of these groups of fungicides. However, this study showed that several

alternatives to fungicides could be adopted to suppress the infection with shot-hole disease on stone fruits including spraying of chloride or hydroxide of potassium and calcium salts or spraying *Trichoderma* spp. formulated as blight stop as well as *B. subtilis* bacterium formulated as Rhizo-N. These application including fungicides or alternatives should be adopted when environmental conditions favor disease incidence and severity represented by 20-25°C under high humidity.

Table (10): Effect of fungicides on infection % with shot- hole on peach trees .

Fungicides	Dose/ ppm	Disease severity %							
		El-Menofiya		Nobariya		6- th october		Grand Mean	
		2008	2007	2008	2007	2008	2007	2008	2007
Copprio-Top (Pyraclostrobin Metiram)	1000	31.0	35.0	28.0	35.0	28.0	35.0	29.0	35.0
	500	41.0	55.0	37.5	55.0	37.5	55.0	38.7	55.0
	250	90.0	92.5	54.5	92.5	78.0	92.5	74.2	92.5
Sythane-24 (myclobutanil)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	35.0	28.0	35.0	28.0	35.0	30.3	35.0
Flint (trifloxystrobin)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	28.0	33.0	30.0	33.0	28.0	33.0	28.7	33.0
	250	37.5	40.0	37.5	40.0	37.5	40.0	37.5	40.0
Cam-Zen (Carbendazim),	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	33.0	30.0	32.0	30.0	32.0	30.0	32.3	30.0
	250	40.0	50.0	37.5	50.0	37.5	50.0	38.3	50.0
Sumi-8 (diniconazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	25.0	28.0	22.5	28.5	20.0	30.5	22.5
Punch(flusilazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	25.0	28.0	22.5	28.0	20.0	30.3	22.5
copper acrobat (copper hydroxide)	1000	47.5	47.5	45.0	47.5	44.0	47.5	45.5	47.5
	500	89.0	75.0	74.0	75.0	70.0	75.0	77.7	75.0
	250	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Topas-100 (penconazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	34.0	25.0	28.0	25.0	32.5	25.0	31.5	25.0
Control	1000	92.5	92.5	90.5	92.5	88.5	92.5	90.5	92.5
	500	92.5	92.5	90.5	92.5	88.5	92.5	90.5	92.5
	250	92.5	92.5	90.5	92.5	89.0	92.5	90.7	92.5
Grand Mean	1000	17.1	19.4	16.3	19.4	16.0	19.4	16.5	19.4
	500	28.3	31.7	26.4	31.7	27.8	31.7	27.5	31.7
	250	49.1	53.0	42.6	52.5	45.1	51.9	45.6	52.5

L.S.D 0.05: Fungicides (F)= 2.95, Conc. (C) = 1.70, Location (L)= 1.70, Year(Y) = 2.15,

F*Y= 2.55, L*Y=1.35

C*Y =4.34, F*C = 5.0, F*L = 5.0 ,C*L =2.95, C*L*F =8.90, F*C*L*Y=5.25.

Table (11): Effect of fungicides on infection % with shot- hole on plum trees

Fungicides	Dose/ppm	Disease severity %							
		El-Menofiya		Nobariya		6- th october		Grand Mean	
		2008	2007	2008	2007	2008	2007	2008	2007
Copprio-Top (Pyraclostrobin + Metiram)	1000	31.0	30.0	28.0	35.0	28.0	35.0	29.0	33.3
	500	41.0	40.0	37.5	45.0	37.5	47.0	38.7	44.0
	250	90.0	87.5	54.5	82.5	78.0	80.0	74.2	83.3
Sythane-24 (myclobutanil)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	34.0	30.0	30.0	30.0	30.0	30.0	31.3	30.0
Flint (trifloxystrobin)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	30.0	30.0	30.0	33.0	30.0	33.0	30.0	32.0
	250	45.0	55.0	45.0	45.0	45.0	50.0	45.0	50.0
Cam-Zen (Carbendazim)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	35.0	33.0	35.0	33.0	35.0	33.0	35.0	33.0
	250	45.0	50.0	45.0	50.0	45.0	50.0	45.0	50.0
Sumi-8 (diniconazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	25.0	28.0	25.0	28.5	25.0	30.5	25.0
Punch (flusilazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	25.0	28.0	25.0	28.0	25.0	30.3	25.0
Copper acrobat (copper hydroxide)	1000	47.5	45.0	45.0	47.5	44.0	47.5	45.5	46.7
	500	89.0	75.0	74.0	75.0	70.0	75.0	77.7	75.0
	250	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Topas-100 (penconazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	25.0	28.0	25.0	28.0	25.0	30.3	25.0
Control	1000	92.5	92.5	88.5	92.5	82.5	92.5	87.8	92.5
	500	92.5	92.5	92.5	92.5	88.5	92.5	91.2	92.5
	250	92.5	92.5	92.5	92.5	89.0	92.5	91.3	92.5
Grand Mean	1000	17.10	18.61	16.15	19.44	15.45	19.44	16.23	19.17
	500	28.75	30.06	26.90	30.94	26.10	31.17	27.25	30.72
	250	50.40	53.61	44.35	51.94	46.40	52.22	47.05	52.59

L.S.D 0.05 : Fungicides (F) = 2.92, Conc. (C) = 1.65, Location (L)= 1.65, Year(Y) = 2.05, F*Y= 2.45,C*Y =4.44, L*Y=1.35, F*C = 5.0,F*L = 5.0,C*L =2.92, C*L*F =8.75, F*C*L*Y=5.17.

Table (12): Effect of fungicides on infection% with shot- hole on apricot trees .

Fungicides	Dose/ ppm	Disease severity %							
		El-Menofiya		Nobariya		6- th october		Grand Mean	
		2008	2007	2008	2007	2008	2007	2008	2007
Copprio-Top (Pyraclostrobin+ Metiram)	1000	31.0	27.0	28.0	27.0	28.0	30.0	29.0	28.0
	500	41.0	37.5	37.5	37.5	37.5	37.5	38.7	37.5
	250	90.0	75.0	54.5	75.0	78.0	75.0	74.2	75.0
Sythane-24 (myclobutanil)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	34.0	28.0	30.0	28.0	30.0	31.0	31.3	29.0
	250	41.0	37.5	37.5	37.5	37.5	37.5	38.7	37.5
Flint (trifloxystrobin)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	30.0	25.0	30.0	25.0	30.0	25.0	30.0	25.0
	250	37.5	28.0	37.5	28.0	37.5	28.0	37.5	28.0
Cam-Zen (Carbendazim)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	33.0	30.0	32.0	30.0	30.0	30.0	31.7	30.0
	250	40.0	40.0	37.5	40.0	37.5	40.0	38.3	40.0
Sumi-8 (diniconazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	30.0	28.0	30.0	28.5	27.0	30.5	29.0
Punch (flusilazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	30.0	28.0	30.0	28.0	27.0	30.3	29.0
copper acrobat (copper hydroxide)	1000	47.5	45.0	45.0	45.0	44.0	45.0	45.5	45.0
	500	89.0	75.0	74.0	75.0	70.0	75.0	77.7	75.0
	250	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5
Topas-100 (penconazole)	1000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	250	35.0	30.0	28.0	30.0	28.0	27.0	30.3	29.0
Control	1000	92.5	92.5	92.5	92.5	88.5	92.5	91.2	92.5
	500	92.5	92.5	92.5	92.5	88.5	92.5	91.2	92.5
	250	92.5	92.5	92.5	92.5	89.0	92.5	91.3	92.5
Grand Mean	1000	17.1	18.3	16.6	18.3	16.1	18.6	16.6	18.4
	500	32.0	32.0	29.6	32.0	28.6	32.3	30.1	29.3
	250	49.9	50.6	43.6	50.6	45.7	49.6	46.4	50.3

L.S.D 0.05 : Fungicides (F)= 3.17, Conc . (C) = 1.85, Location (L)= 1.85, Year(Y) = 2.35,
F*Y= 2.25,C*Y =4.24,

L*Y=1.30, F*C = 5.1,F*L = 5.1 ,C*L =3.17, C*L *F =9.4, F*C*L*Y=5.07. .

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مكافحة مرض التثقب فى اشجار الحلويات المتسبب عن الفطر *Stigmia carpophila*

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تثقب الاوراق فى الفاكهة ذات النواة الحجرية والمتسبب عن الفطر (*S. carpophila* (Lev.) M.B. Ellis. من الامراض الخطيرة فى اشجار الفاكهة ذات النواة الحجرية وخاصة الاصناف الحديثة فى الخوخ (الفلوريدا برنس) والمشمش (الكا نينو) والبرقوق (البيوتى)، وكان المرض يظهر سابقا فى نهاية الموسم بعد جمع المحصول ولكن ظهر حديثا على الاصناف الحديثة فى بداية موسم النمو (مارس) من كل عام فى محافظات 6 اكتوبر والمنوفية ومنطقة النوبارية فى عامى 2007-2008 . ولاتوجد اختلافات فى مزارع الفطر المتحصل عليها من مختلف اجزاء النبات او انواع الفاكهة ذات النواة الحجرية او مختلف المسافات والمناطق، واعلى شدة اصابة بالمرض تكون فى الخوخ و المشمش . درس تأثير املاح الكالسيوم والبوتاسيوم على فطر ستيجمينا كاروفيليا فى المعمل والحقل فوجد أن أملاح (الكلوريد والهيدروكسيد) كان لها تأثير اكبر من أملاح (النترات والفوسفات) على الفطر على مستوى المعمل والحقل , وكانت أعلى فاعليه لاعلى تركيز (1800 جزء فى المليون) فى كل الاملاح للحد من المرض . اعطت المقاومة البيولوجية (الحيوية) نتائج مشجعة باستخدام كل من الكائنات الحيويه (ترايكودرما فيردى – ترايكودرما هاريزيانم – باسلاس ساتلس 1,2 – بلايت ستوب –رايزوان) حيث قللت من شدة الاصابة واعطوا نتيجة جيدة فى مكافحة مرض التثقب فى الحلويات .

واختبرت مكافحة الكيماوية فى المعمل وفى الحقل بواسطة مبيدات البانث و السومى-8 و النوباز-100 والسبسين-24 والفنت و الكام-زين والكيريوتوب و اكروبات النحاس فى تلك الفترة فى محافظات 6 اكتوبر والمنوفية ومنطقة النوبارية فى عامى 2007-2008، واعطت ال-7 مبيدات الاولى احسن النتائج على الترتيب.

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