Impact of cultivars, bio-products and chemical salts on managing apple scab in Egypt

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Abstract — In Egypt, apple scab caused by *Spilocaea pomi* (the conidial state of *Venturia inaequalis* (Cke.) Wint.), is one of the most constrain of it's production. Isolation trials from scabbed apple leaves yield many fungal isolates. The isolated fungi were purified and identified as : *Alternaria alternata, Fusarium solani, Penicillium* sp., *Spilocaea pomi, and Stemphylium* sp. Pathogenicity test of the isolated fungi revealed that only *S.pomi* was the responsible for causing apple scab. The reaction of three apple cultivars, *i.e.* Anna, Ein Shemer and Balady cvs. of 10 years old to the natural infection by the disease was assessed during 2011 growing season at Nobaria district, El-Behera governorate. Results showed that Balady cv. was the most susceptible one to the incidence and severity of the disease followed by Anna cv. Meanwhile, Ein Shemer cv. was the lowest affected one. Three bio-products, *i.e.* AQ10 (*Ampelomyces quisqualis*), Bio-ARC (*Bacillus megaterium*) and Bio-Zeid (*Trichoderma album*) as well as five chemical salts, *i.e.* calcium chloride, calcium bicarbonate, potassium bicarbonate, potassium mono-phosphate and sodium bicarbonate were evaluated, under summer pruning practices, for their effect on the severity of the disease on (Balady cv.) compared with the fungicide Topas and untreated control . All the tested treatments resulted in significant reduction to the disease with significant increase to the produced fruit yield compared with control treatment. Results revealed that the alternative treatment between potassium bicarbonate and the fungicide Topas was the most effective treatment in this regard followed by Topas alone then the alternation between potassium bicarbonate and the bio-product AQ10. On the other hand, in case of untreated control but with summer pruning practices reduced the severity of the disease and increased the produced fruit yield compared with untreated and un-pruned control.

Key words —Apple scab, bio-products, chemical salts , disease incidence, disease severity , pruning , Topas, varietal reaction,.

1 Introduction

Apple (*Malus domesticsa* L.) is belonging to deciduous trees and pomes fruit, fam. Rosaceae. It is one of the most important fruit trees in the world ,which at least 55 million ton has been the yield of apple in the world; USA are the first in production, 7.5% of the world apple production.

Turkey, France , Italia and Iran are the most important apple production exporter countries in the world, (Marku *et al.,* 2014).

In Egypt, the cultivated area reached about 79383 feddan, which produced about 623625 ton with an average of 10.2 ton per feddan (Anonymous, 2014).

Apple scab is a serious disease of apples , resulting in great losses due to severe surface blemishing of fruit. It is most severe in coastal and foothill areas, where spring and early summer weather is cool and moist. However, it can be a problem wherever apples grow when conditions are favorable for pathogen development.

Apple trees are liable to infection by many bacterial , fungal , viral and virus-like diseases in addition to physiological disorder and nematode (Jones and Aldwinkle ,1990 and Ohlendorf, 1999). However, scab is the most constrain one (Jones and Aldwinkle, 1990; Creemer *et al.*,2008 ; Slatnar *et al.*, 2012 and Marku *et al.,* 2014). Its primary effect is reduction of the quality of the infected fruits, scab also reduces fruit size or causing premature fruit drop, defoliation and poor fruit bud development of the next year, and it reduces the length of time to infected fruit can be kept in storage (Marku *et al.,* 2014). It survives the winter in the infected leaves and fruits fallen from the trees, in wet and cool spring weather. The causal fungus infected young leaves, flower parts, fruits and succulent twigs. Specific management practices very important to improve plant health to resistant the fungus disease and control apple scab, specially pruning by removing dry shoots and crowded proper thinning of the crown this technique will improve air circulation to reduce the humidity relative inside the tree and leaves to reduce the fungal infection , also rake and dispose of fallen leaves and fruits because they are source of infection for next year. (Forest health care 2001).

Scab can destroy an apple crop, which young infected flowers or fruits can drop, or the fruit can become malformed, cracked, and unsightly, rendering it unusable. Defoliation follows severe, early leaf infection.

Pruning , including summer pruning of apple can have a positive impact on disease management in two basic ways ; through removal of dead tissues and incoulum, and through alternation of the canopy microclimate.However summer pruning is used exclusively as a horticultural tool to improve fruit quantity and quality. (Cooley and Autio, 2011).

Bicarbonate salts such as potassium bicarbonate, sodium bicarbonate and calcium chloride were screened by Masoud (2014) for their antifungal activity . Potassium bicarbonate (PBC) and sodium bicarbonate (SBC) are effective against apple scab, and not cause any phytotoxicity at given application doses. External fruit quality parameters were comparable with fruit produced with fungicide treatment, these products are not toxic to human health, PBC or SBC can potentially present a perspective protection in the apple orchards, especially for the control of apple scab in organic fruit growing (Slatnar *et al.,* 2012). The use of potassium bicarbonate, potassium phosphate and potassium mono phosphate was evaluated as an anti- resistance strategy, which looked upon salts spraying in rotation with chemical fungicides. Also potassium bicarbonate can be considered as an ideal product for scab control in organic orchards at moments of low infection risk (Creemers *et al.,*2008) . Potassium bicarbonate , potassium mono hydrogen phosphate, calcium chloride at the rate of 20 mM , and bioagents ; *Trichoderma harzianum, T. veredi* and *Bacillus subtilis* were evaluated to their ability to reduce foliar diseases of vegetables. The recorded foliar diseases were significantly reduced at all treatments compared with untreated plants (Abdel-Kader *et al*., 2012).

Bioagents reduce spore production of the apple scab causal pathogen *Venturia inaequalis* (Telomorph of *Spilocaea pomi*) when applied from August to November time, which considered the major weakness time in the life cycle of the causal pathogen (Sutton *et.al*., 2000 and Carisse and Rolland, 2004).

This work aimed to evaluate three apple cvs. to the natural infection be scab and evaluating some commercial bio-products and chemical salts on the incidence and severity of the disease as well as the produced fruit yield under field conditions.

2 Materials and Methods

Isolation, purification and identification of the causal pathogen

Leaf samples of Balady apple cv. showing typical symptoms of scab disease were collected from Nobaria district, El-Behera governorate during 2011 growing season. The collected samples washed thoroughly with tap water , cut into small pieces , surface sterilized by immersing in 2.0% sodium hypochlorite for two minutes then rinsed three times in sterilized water and dried by filter paper. The sterilized pieces placed on PDA medium containing 1000 ppm. of streptomycin sulfate and incubated at 18-23 ^{o}C for one week with daily observation.

The emerged fungi were picked up, purified using either single spore or hyphal tip techniques as mentioned by Dingra and Sinclair (1985) and identified based on their morphological shape and cultural characters (Sivanesan,1977 and 1984). Identification was confirmed by Mycological Center, Fac. of Sci., Assuit Univ. Assuit, Egypt. Pure culture stocks of the isolated fungi were kept on 5^{o}C for further studies.

Pathogenicity test :

Healthy potted apple transplants (Balady cv.) of one year old , kept in a greenhouse were artificially inoculated by spraying the leaves with the spore suspension of any of the tested fungi 10^5 cfu/ ml water, which wounded before spraying. The inoculated plants covered with plastic bags two days for increasing humidity in order to encourage the infection process. Control treatment sprayed only with water. Five replicates were used for each isolated fungus as a treatment and single transplant for each replicate. The occurrence of infection of the inoculated transplants was recorded (Kunz *et al.,* 2008).

Field experiments :

An orchard of apple (10 years old) located at Nobaria district, El-Behera governorate cultivated Anna, Ein Shemer and Balady cvs. was chosen to carry out field experiments during 2011/2012 and 2012/2013 growing seasons . Pruning, including summer pruning through removal dead tissues as well as collecting pruning debris that play an inoculum source of the causal pathogen was carried out each season according to Cooley and Autio (2011) . In addition, all agricultural practices of irrigation, fertilization , weed and other bests control for apple trees were carried out as recommended by Min. of Agric., and Land Reclamation.

Varietal reaction :

The three cvs., *i.e.* Anna, Ein Shemer and Balady were evaluated to the natural infection by the disease. single apple tree was carefully examined during 2011 as a replicate for each cv. and five randomized replicates were used for each cv. Ten shoots per tree were examined, where 10 older leaves for every shoot were counted and evaluated for disease assessment. The reaction to the incidence and severity of scab was assessed as mentioned under disease assessment three times in the growing season (April , July and September) and the average was recorded.

Effect of bio-products and chemical salts on the severity of apple scab and fruit yield:

Three commercial bio-products, *i.e.* AQ 10 (*Ampelomyces quisqualis*), Bio-ARC (*Bacillus megaterium*) and Bio-Zeid (*Trichoderma album*) and five chemical salts, *i.e.* calcium chloride, calcium bicarbonate, potassium bicarbonate, potassium mono phosphate and sodium bicarbonate were evaluated under open field conditions at El Nobaria district , El–Behera governorate during 2011/2012 and 2012/2013 growing seasons to their efficiency on reduction the severity of the disease on Balady cv. (the most susceptible cv.), compared with Topas fungicide and untreated control as shown in Table (1). All the tested bio-products and chemical salts were sprayed each alone twice ; The first one was carried out after summer pruning (after harvest) and the second was done in the beginning of each season. Adding Trition (B 1956) at the rate of 25 ml/ 100 l water.

In case of alternation between AQ 10 or Topas with potassium bicarbonate . AQ 10 and Topas, each alone , was sprayed on apple trees after summer pruning one week after spraying potassium bicarbonate. The second treatment was done in the beginning of each season by spaying AQ 10 and/ or Topas, each alone, one week after spraying potassium bicarbonate. Trition (B 1956) was added to each sprayed treatment at the rate of 25 ml/ 100 l water.

Five replicates for each treatment were used and single tree was used as a replicate.

Ten shoots per tree were examined, where 10 older leaves for every shoot were counted and evaluated for disease assessment as mentioned under disease assessment. Also, the produced fruit yield was weighed after each harvest and the average weight of fruits (kg.) / tree was recorded.

Table (1) : The tested bio –product and chemical salts .

Trade name	Active ingredient or chemical structure.	Dose/ 100 L water
AQ 10	*Ampelomyces quisqualis*	5 gram
Bio-ARC	*Bacillus megaterium*	250 g
Bio-Zeid	*Trichoderma album*	250 g
Calcium chloride	$Ca\ Cl_2$	20 g /L water
Calcium bicarbonate	$Ca\ HCo_3$	500 g
Potassium bicarbonate	$K\ H\ Co_3$	500 g
Potassium mono phosphate	$K_2\ H\ Po_4$	435g
Sodium bicarbonate	$Na\ HCo_3$	500 g
Topas	Penconazole	25 ml

Disease assessment :

Observation were made on 10 randomized shoots per every tree , 10 older leaves for every shoot counted and evaluated for leaves scab (Kunz, 2008) to estimate disease incidence and severity using the following formula:

$$\%\ \text{Disease incidence} = \frac{\text{Number of scabbed shoots}}{\text{Total number of tested shoots}} \times 100$$

Disease severity depending on the modified scale (0-5) using the following formula :

$$\%\ \text{Disease severity} = \frac{\sum (n \times v)}{5N} \times 100$$

Where:

n = Number of leaves in each class
v = Number of each class.
N = Total number of the tested leaves
5 = The highest value of the scale.

Efficiency of sprayed treatments was estimated using the following formula :

$$\%\ \text{Efficiency} = \frac{\text{Infection in control} - \text{infection in treatment}}{\text{Infection in control}} \times 100$$

Statistical analysis :

Data were statistically analyzed and treatments were determined according to Duncan's multiple range test (Duncan ,1955).

3 RESULTS

Disease symptoms :

Disease symptoms as a small brown - green spot, later can darken nearly to black, which enlarge and turn brown and corky. Infected leaves became distorted in shape, turn yellow and drop early in the summer. Fig. (1) .

Fig. (1). Typical symptoms of apple scab on the leaves of Balady cv. (a) infected leaves and (b) healthy leaves .

a- Infected leaves b - Healthy leaves

Isolation , purification and identification of the associated fungi:

Isolation trials from naturally infected leaves of Balady apple cv. Fig. (1) collected from Nobaria district, El-Behera governorate during growing season 2011 yielded many fungal isolates . Isolation trials from scabbed apple leaves yielded many fungal isolates. The isolated fungi were purified and identified as : *Alternaria alternata, Fusarium solani, Penicillium* sp.*, Spilocaea pomi, and Stemphylium* sp. Identification was confirmed by Mycological Center, Fac. of Sci., Assuit Univ., Assuit, Egypt.

Pathogenicity test :

Pathogenic capability of the isolated fungi was evaluated under greenhouse conditions on apple Balady cv. Data presented in Table (2) indicate that *Spilocaea pomi*, only was able to infect apple leaves causing typical symptoms of apple scab and no symptoms were occurred by the other fungi and control treatment.

Table (2) : Pathogenicity test of the isolated fungi on apple cv. Balady, under greenhouse conditions.

Tested fungi	Infection
Alternaria alternata	-
Fusarium solani	-
Penicillium sp.	-
Spilocaea pomi	+
Stemphylium sp.	-
Control *	-
Control **	-

- : No infection + : Occurring infection
* : Wounded leaves and inoculated with distilled water.
** : Un wounded leaves and inoculated with distilled water.

Varietal suscetibility under open field conditions:

Susceptibility of three apple cultivars, *i.e.* Anna, Ein shemer and Balady cvs. (Naturally infected), were evaluated to the infection by scab disease during 2011 growing season.

Data presented in Table (3) indicate that all the tested cvs. were susceptible to the natural infection of apple scab disease. On the other hand, Balady cv. was the most susceptible one being, 52.0% disease incidence and 33.6%

disease severity followed by Anna cv. being, 34.0% disease incidence and 26.0% disease severity. Meanwhile, Ein Shemer was the lowest susceptible one, being 6.0% disease incidence and 5.4% disease severity.

Table(3): Susceptibility of three apple cultivars to the natural infection by scab during 2011growing seasons.

Cultivars	%, Disease incidence	%, Disease severity
Anna	34.0 ^{b}	26.0 ^{b}
Ein shemer	6.0 ^{c}	5.4 ^{b}
Balady	52.0 ^{a}	33.6 ^{a}

Duncan multiple range significant at Alpha (0.05). Means with the same letter are not significantly different. a,b,c., values in the same column with different superscripts differed significantly.

Effect of bio-products and chemical salts on the severity of apple scab and fruit yield.

Data presented in Tables (4 and 5) show the effect of foliar spraying of three bio-products, *i.e.* AQ10 (*Ampelomyces quisqualis*), Bio-ARC (*Bacillus megaterium*) and Bio-Zeid (*Trichoderma album*) and five chemical salts, *i.e.* calcium chloride , calcium bicarbonate potassium bicarbonate, potassium mono phosphate and sodium bicarbonate compared with the spraying Topas fungicide and untreated control. This effect were evaluated under pruning of the treated trees Balady apple cv. In the open field during 2011/2012and 2012/2013 growing seasons.

Data shown in Table (4) indicate that spraying any of the tested treatments significantly reduced disease severity and increased the fruit yield under pruning practices even with untreated control but with pruning, being 28.4% for disease severity and 16.4 kg. fruit per tree compared with untreated control , being 33.6% disease severity and 12.4 kg. fruit per tree. The alternation between Topas and potassium bicarbonate was the most effective treatment in this regard , being 94.0% for efficiency in reduction disease severity with 45.0 kg. fruit/ tree followed by the fungicide Topas alone, being 90.5% for efficiency , 40.8 kg. fruit per tree then the alternation between the bio-product AQ10 and potassium bicarbonate, being 81.6% efficiency and 36.4 kg. fruit per tree . On the other hand, potassium bicarbonate was the most effective salt, being 73.8% efficiency and 34.2 kg. fruit/ tree followed by potassium mono phosphate and calcium bicarbonate, being 70.8 and 70.2% efficiency and 32.0 and 31.0 kg. fruit/tree , respectively. Meanwhile sodium bicarbonate and calcium chloride were the lowest effective salts, being 67.3 and 68.5% for efficiency and 26.4 and 28.2 kg. fruit per tree. However, AQ10 was the most effective bio-product in reducing the disease and increasing the produced fruit yield than the other two tested bio-products, being 69.6% efficiency and 28.8 Kg. fruit per tree.

Data presented in Table (5) indicate that all the treatments in the second growing season of 2012/2013 showed the same trend in results with the obtained data in the first season of 2011 / 2012.

Table (4) : Effect of spraying bio-products and chemical salts on fruit yield and scab disease severity of apple trees cv. Balady, field experiment during 2011/2012 growing season.

Treatments	%,Disease severity	% Efficiency	Average Fruit yield Kg./tree
AQ 10	10.2 ^{d}	69.6	28.8 ^{e}
Bio-ARC	12.4 ^{c}	63.1	26.8 ^{f}
Bio-Zeid	11.8 ^{c}	64.9	24.4 ^{f}
Calcium chloride	10.6 ^{d}	68.5	28.2 ^{e}
Calcium bicarbonate	10.0 ^{d}	70.2	31.0 ^{d}
Potassium bicarbonate	8.8 ^{d}	73.8	34.2 ^{c}
Potassium mono phosphate	9.8 ^{d}	70.8	32.0 ^{d}
Sodium bicarbonate	11.0 ^{c}	67.3	26.4 ^{f}
Topas	3.2 ^{f}	90.5	40.8 ^{b}
Alternation between AQ10 and potassium bicarbonate	6.2 ^{e}	81.6	36.4 ^{c}
Alternation between Topas and potassium bicarbonate	2.0 ^{f}	94.0	45.0 ^{a}
Control *	28.4 ^{b}	-	16.4 ^{g}
Control**	33.6 ^{a}	-	12.4 ^{g}

Duncan multiple range significant at Alpha (0.05). Means with the same letter are not significantly different. a,b,c., values in the same column with different superscripts differed significantly.

* Untreated with any of the tested materials but pruned .

** Untreated with any of the tested materials as well as un-pruned .

Table (5) : Effective of spraying bio-products and chemical salts on fruit yield and scab disease severity of apple trees cv. Balady , field experiment during 2012/2013 growing season.

Treatments	%,Disease severity	%, Efficiency	Average Fruit yield Kg./tree
AQ 10	10.6 ^{d}	71.9	33.2 ^{d}
Bio-ARC	13.0 ^{c}	65.6	31.0 ^{d}
Bio-Zeid	12.6 ^{cd}	66.7	30.6 ^{de}
Calcium chloride	11.2 ^{d}	70.4	32.8 ^{d}
Calcium bicarbonate	10.2 ^{d}	73.0	33.8 ^{d}
Potassium bicarbonate	9.2 ^{e}	75.7	37.2 ^{c}
Potassium mono. Phosphate	10.2 ^{de}	73.0	35.4 ^{c}
Sodium	12.0 ^{c}	68.3	31.4 ^{d}

bicarbonate			
Topas	3.6 g	90.5	44.2 b
Alternation between AQ10 and potassium bicarbonate	6.4 f	83.1	42.2 b
Alternation between Topas and potassium bicarbonate	2.2 g	94.2	47.2 a
Control *	28.8 b	-	18.4 f
Control**	37.8 a	-	9.8g

Duncan multiple range significant at Alpha (0.05). Means with the same letter are not significantly different.a,b,c., values in the same column with different superscripts differed significantly.

* Untreated with any of the tested materials but pruned and .

** Untreated with any of the tested materials as well as un-pruned.

4 Discussion

Although the development of the concept of sustainable agriculture may lead to greater interest in environmentally friendly methods of crop disease and pest control, agriculture is currently still a big consumer of pesticides. Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., is the main disease targeted by an average of 70% of plant protection product treatments in conventional fruit growing (Mac Hardy, 1996). Among the means of protecting plants against bio-aggressors, chemical control using synthetic active substances remains the most effective solution. It is relatively easy to use and appears to be the most cost-effective in the medium term. However, it is associated with various environmental issues, risks of residues and risks of resistant pathogens developing (Deguine *et al*., 2009).

Isolation trials from scabbed apple leaves yield many fungal isolates. The isolated fungi were purified and identified as : *Alternaria alternata, Fusarium solani, Penicillium* sp., *Spilocaea pomi* (conidial state of *Venturia inaequalis*) , and *Stemphylium* sp. Identification was confirmed by Mycological Center, Fac. of Sci., Assuit Univ., Assuit, Egypt. Pathogenicity test of the isolated fungi revealed that only *S.pomi* was the responsible for causing apple scab. The obtained data are in harmony with those reported by (Mac Hardy,1996 and Carisse and Rolland ,2004)

The three apple cultivars, *i.e.* Anna, Balady and Ein Shemer cvs. were susceptible for the natural infection by the disease under field condition. In addition, Balady cv. was the most one followed by Anna cv. and Ein Shemer was the lowest susceptible one. The resistant cvs. of apple trees are a main target for management of apple scab (Jones and Aldwinkle, 1990 and Rosenberger, 2003). However, It is important to remember that resistance is not immunity. Even highly resistant varieties can succumb to any disease under extreme environmental conditions and stress.

It has been found that the tested bio-products ,*i.e.* AQ10 (*Ampelomyces quisqualis*) , Bio-ARC (*Bacillus megaterium*) , Bio-Zeid (*Trichoderma album*) and some chemical salts, *i.e.* calcium chloride, calcium bicarbonate, potassium bicarbonate , potassium mono phosphate and sodium bicarbonate significantly reduced the disease with significant increase to the produced fruit yield compared with untreated control during 2011/2012 and 2012/2013 growing seasons on Balady apple cv. under field conditions. AQ10 was the most effective bioagent against the disease than Bio-Zeid and Bio-ARC. The use of bioagents in management of apple scab was frequently used by many researchers (Jones and Aldwinkle , 1990 ;Young and Andrews,1990 ; Whipps,1992 and Sirca,2001). Moreover, the alternation treatment between Topas and potassium bicarbonate was the most effective and superior treatment in reducing disease severity and increasing the production of fruit yield followed by Topas fungicide alone then by the alternation treatment between AQ10 and potassium bicarbonate. Potassium bicarbonate was the most effective salt followed by Potassium mono phosphate and calcium bicarbonate. Meanwhile , sodium bicarbonate was the lowest effective salt.

Ilhan *et al*. (2009) reported that in two orchard experiments to investigate the efficacy of sodium bicarbonate (SBC) alone or in combination with a reduced dose of tebuconazole in inhibiting apple scab, applications of 1% SBC to trees at 10-d intervals significantly reduced disease incidence and severity on leaves and fruit compared to the water-treated control. In the first experiment, by the last assessment, the 1% SBC treatment had reduced the disease incidence on leaves to 29.6% compared with 62.6% in the water-treated control. The efficacy of 1% SBC was comparable with that of the label dose of tebuconazole on leaves and fruit. Combining 1% SBC with a reduced dose (10% of label dose) of tebuconazole did not improve the efficacy of 1% SBC alone. Treatments of 2% SBC were phytotoxic to leaves, but 1% SBC was neither phytotoxic to leaves nor did it adversely affect quality parameters of harvested fruit. Both inorganic fungicides such as potassium bicarbonate (PBC) and sodium bicarbonate (SBC) are effective against apple scab and not cause any phytotoxicity with no significant differences between fungicide and PBC or SBC treatments were observed in the content of analyzed phenolic compounds. Jamar *et al.*(2007) controlled apple scab (*Venturia inequalis*) with bicarbonate salts, and they found that potassium bicarbonate acts as a contact fungicide and is not likely to be systemic or curative . The content of phenolic compounds in leaves, were comparable to those in fungicide treated trees, this only indicates the activity of defense mechanisms in apple leaves, also use of PBC positively affected the potassium accumulation in leaves through the growing season (Jamar *et al.*,2007 and Slatnar *et al*., 2012). In addition, bicarbonate salts specially potassium bicarbonate is recommended by American researchers at Cornell University as an agricultural fungicide and it developed across Europe as bio-fungicide. It is significantly reduced apple scab severity on leaves and fruits compared with untreated control (Marku *et al*., 2014).

In conclusion, to increase the efficiency of the tested bio-products and chemical salts sanitation can be effective (picking up and disposing of fallen leaves in the autumn) but is usually not practical for large orchard operations.

Also, applications of urea to trees just before leaf drop or to leaves on the ground in the autumn, tilling the fallen leaves into the soil, or chopping them into small pieces, are all used to speed up leaf decomposition thereby decreasing the amount of ascospore inoculum in the following season. Moreover, trees should be pruned regularly to enhance air movement and to allow sunlight to penetrate, which speeds up drying of leaves and fruit.

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