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FOLIAR APPLICATION OF FUNGICIDES AND SYNERGISTS OF POPULIN EXTRACT FOR CONTROL APPLE SCAB VENTURIA INAEQUALIS

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ABSTRACT

In the field control strategy of six spray of fungicides vis. (Goldazim, Champion, Punch, Piristine, Champion and Punch) was remarkable in reducing scab incidence of apple leaves to 51% and fruits 43.73% and their efficacy have been achieved by 40% and 57.7%. Reduction of scab severity enquired on leaves and fruits was 18.59 and 10.2%, thus, it was more efficient in preventing disease attack by 62% and 72.5%, respectively.

KEYWORDS: Apple scab, *Venturia inaequalis*, Foliar Application, Plant Extract, Fungicides.
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INTRODUCTION

Apple scab that caused by the fungus *Venturia inaequalis* (Cooke) Winter (anamorph: *Spilocaea pomi* Fr. Previously = *Fusicladium dentricum* (Wallruth) Fuckel) is the most common important disease of apple in the world (MacHardy, 1996), particularly on susceptible commercial cultivars, since its control is become difficult, and losses caused about 70% loss if no controlled (Demeyere and De turck, 2002).

The disease is more severe in areas with cool, moist spring and summer (Agrios, 2005) hence, the fungus colonizes most succulent apple tissues of the sepals, leaves, fruits, petioles, blossoms and even young twigs of the tree, with though diagnostic symptoms commonly evidenced on the leaves and fruits. Scab infection leads to premature leaf and fruit fall, with deformation in shape and size, and increase predisposed of tree to injuries of temperature extremes (MacHardy, 1996).

In Integrated Scab Management systems, is currently controlled by up to 7-10 applications of protective and curative fungicides during the growing season, regardless of the presence of ascospores in the orchards (Demeyere and De turck, 2002). Prediction systems have been developed for apple scab and used successfully to assist in timing fungicide applications (MacHardy, 1996; Trapman and Polfliet, 1997; Jamar and Lateur, 2005). The pathogen become resistant to some fungicides such as dodine, benomyl and sterol demethylation inhibitors (DMI) fungicides according to (Carisse and Pelletier, 1994; Kunz *et al.*, 1997).

The severity of apple scab infestation on commercial apple cultivars can be significantly reduced by using 1% populin plant extract, a less expensive alternative and a highly available product that will reduce conidia germination and further infection by asexual spores. Populin had the most noxious effect on scab infestation on Golden leaves and fruits, and it significantly reduced scab severity on Jonathan fruit in the first year of treatment (in dry weather conditions). Populin had a similar effect as synthetic fungicides on both Golden and Jonathan in the second year (under wet climate conditions) (Heijne *et al.*, 2006; Balint *et al.*, 2014).

In Duhok province, Kurdistan region, apple scab caused the most considerable damage, since the poor practices, susceptible cultivars, and restricted chemical control applied (Cooperation Program between the General Directorate of Agriculture and University of Duhok, 2017). The current work aimed to Reducing of asexual reproduction of apple scab using of chemical sprays and plant extract of (populin).

MATERIALS AND METHODS

An experiment design was carried out in the Tazhika,Sarsnk (N37.02˚ , E43.28˚), 50 km North of Duhok . Plots of apple original seedling without grafting on any rootstock Planted in 2000 at spacing of 3 × 3 meters , three trees per plot replicated three times used in factorial RCBD . The trees were grown in heavy clay soil (3-5 % organic matter, pH 7.8). Weeds were controlled during the experiment, fertilization and irrigation were applied as needed.

For the field trail, the factor A consisted of standard strategy with six alternativeness sprays (Petal fall, 1cm, 2 cm, 3 cm, 4 cm and 6 cm fruits) to prevent the occurrence of the pathogen's resistant (Percival, 2008), from the petal fall until to pre-harvest according to (Gadoury *et al.*, 1989). Four curative and protective fungicides were used, trade name, formulation, active ingredient are shown in (Table 1). Another strategy including only three sprays at (Petal fall, 2 cm fruit , 6cm fruit or pre-harvest) using the fungicides of (Goldazim , punch and pristine) respectively. Control treatments included water spray with no fungicide.

The factor B consisted of sprays 1% of poplar leaf extract (populin) and control (non-treated). Populin applied until run-off immediately or within 2hrs of the conventional treatments using 16 L sprayer (with an amount of 1 L poplar in 100L water). The treatments were repeated at the 10 to 20 days interval. Scab Assessment (incidence and severity) was undertaken during August and October.

Assessment Of Apple Scab Incidence And Severity

For estimating leaf scab incidence, 10% of trees examined for each area, randomized 12 shoots per tree selected from top, bottom, inside and outside of the tree in mid-June, 2015. For leaf severity, ten leaves selected for each shoot scored according to the European and Mediterranean Plant Protection Organization (EPPO) protocol PP1/5(3) , 2004 derived from 0-4 scores of disease intensity described by Croxall *et al*., (1953) and MacHardy (1998) as follows : 0 = Disease free , 1= < 10% leaf area diseased , 2=11-20% leaf area diseased, 3= 21-40% leaf area diseased , 4= > 40% leaf area diseased .

Table (1): Trade name, Formulation, Type, Active ingredient and rate of application fungicides:

Trade name	Formul-aion	Type	Active ingredient (a:i)	Application Rate	Origin
Goldazim	SC	Protective	Carbendazim 50%	0.5mL/1L	Agriphar, Belgium
Champion	WP	Protective &curative	77% Copper Hydroxide	2.5gm /1L	Nufarm , Austeria
Punch	EC	Protective &curative	Fluzilazole 40%	0.18 mL/1L	Agropesticides, France
Pristine	WP	Protective &curative	Pyraclostrobin12.8% Boscalid 25.2%	0.75gm/1L	Basf, Germany

The disease severity (DS) on the leaves was evaluated using the equation described by Townsen and Heuberger (1943).

$$\% \, TH_{vmax} = \frac{\sum(n \times v)}{V_{max} \times N} \times 100$$

When (TH_{vmax} = disease severity (%), **v =** infection scores (0, 1, 2, 3, 4), V_{max} = highest infection score, **n** = amount of leaves/fruits in each score, **N** = total amount of evaluated leaves/fruits).

Disease assessment on fruit was made on harvested fruits from 15 to 31 October. **Fruit incidence (FI)** was calculated as the proportion of infected fruits with at least one scab lesion. Scab severity on fruits was based on a scale of 1 to 6 following the standard diagram method reported by Croxall *et al.*, (1952).

where: 1 = 0 (no scab), 2 = 1–2%; 3= 2.1 – 5 %, 4= 5.1 – 20 %, 5= 20.1 – 50 %, 6= ≥ 50 %, fruit surface covered by scab.

Fruit severity (FS) was defined as the mean proportion of the fruit surface covered by scab, and it was calculated using the following equation:

$$\mathbf{FS} = \frac{(\mathbf{n1} \times \mathbf{0}) + (\mathbf{n2} \times \mathbf{0.5}) + (\mathbf{n3} \times \mathbf{3}) + (\mathbf{n4} \times \mathbf{12.5}) + (\mathbf{n5} \times \mathbf{35}) + (\mathbf{n6} \times \mathbf{75})}{\mathrm{Nt}}$$

where n1 to n6 represent the number of fruits in each category; Nt represents the total number of fruits; and the coefficients 0, 0.5, 3, 12.5, 35 and 75 represent the median of the lower and upper boundaries of classes 1 to 6, respectively. For more understanding, models of scab severity on both apple leaves and fruits were exhibited in **(Fig. 1 & 2).**

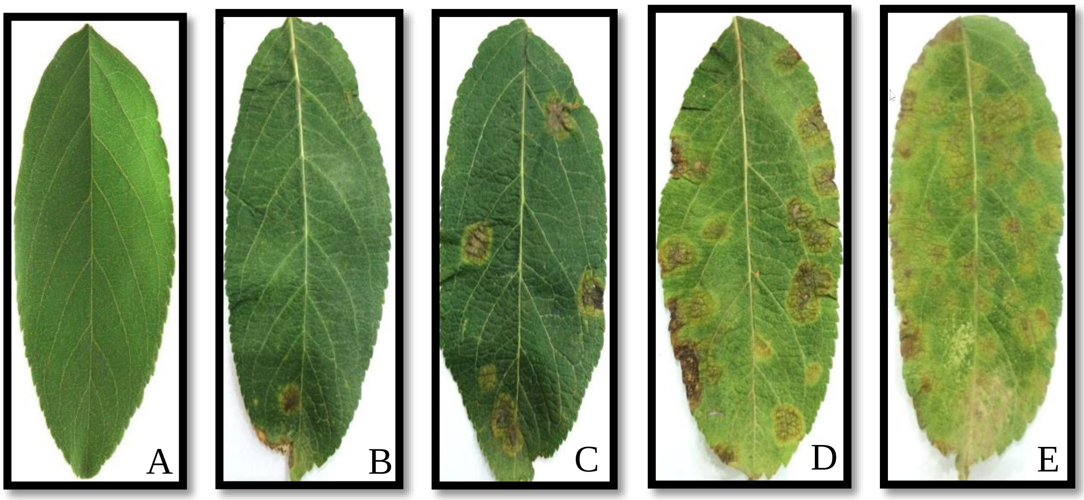


Fig (1): Scores of apple leaves (A) 0=healthy leaves, (B) 1= <10% infected leaf area, (C) 2= 10.1 -20% infected leaf area (D) 3= 20.1- 40% infected leaf area (E) 4= >40% infected leaf area (Croxall, *et al.*,1953).

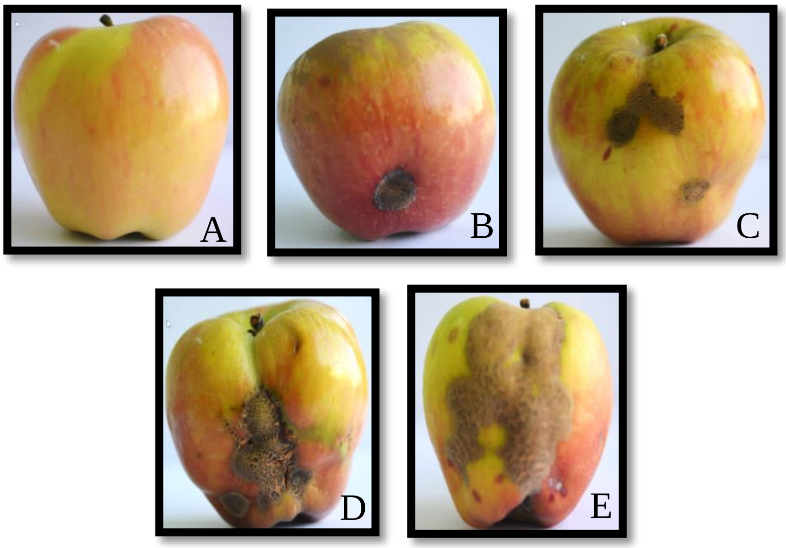


Fig (2): Scores of Fruit (A) 1= 0 (Healthy) (B) 2= 1-2 % infected fruit area (C) 3= 2.1-5% infected fruit area (D) 4= 5.1-20% infected fruit area (E) 5= 20.1-50% infected fruit area. (F) 6= >50% infected fruit area. (Croxall, *et al.*, 1952).

RESULTS AND DISCUSSION

SCAB INCIDENCE AND SEVERITY

The data presented in the (Table 2) showed that incidence and severity of scabbed leaves and apple fruits (Starking Delicious cv.) grown in Tazhika, Duhok , were considerably reduced using conventional sprays and populin (poplar extract) treatments particularly when applied strategy of six fungicide sprays viz. (Goldazim , Champion , Punch , Piristin , Champion , Punch) respectively, with and/or without populin, throughout the season at petal fall and repeated every 15 – 18 days. Strategy of three sprays included (Goldazim , Punch and Pristin) applied at the same time. Alternative of Champion protective and curative sprays and other curative fungicides applied may be prevented the occurrence of resistant pathogen's strains as described by (Gao *et al.,* 2009).

The results also demonstrated a significant (≤ 0.05) reduction of leaf and fruit scab, the lowest infected leaves were 50.92 % when applied six sprays plus populin extract, this strategy without populin was prevented pathogen's attack on leaves when noticed by 51.10 % with severity 18.63 % , compared to 84.9 % and 49.1 in control . Three sprays was also sufficient for reducing leaf scab to a range (55.22 to 62.40 %) with severity (22.54 to 26.75 %). Therefore, the results emphasized the worth efficient of six sprays with 39.87 % to 40.08 % and fruit scab with 42.26 % to 49.15% in reducing incidence of leaf scab though non-significantly with three sprays.

The schedule of alternating sprays with systemic and protectant fungicides used was very necessary to prevent pathogen's resistance, since the systemic specific action chemical carries most of the weight in controlling the disease, whereas the protectant or non-specific chemical reduces the possibility of the survival any strains of the pathogen that may develop resistance to the systemic chemical (Mc-Grath, 2001). The difficulty control of out break apple scab attributed to wind-blown conidia that may dispersed randomly through growing season , though chemical sprays application. Recently, literatures suggested the importance of conidia as a source of primary inoculum that consider a crucial factor for apple scab dissemination as well as numerous of discharged ascospores (Xu *et al.,* 2013). Therefore, relatively the moderate infection percentage after sprays may be referred to that scab lesions comprised of subcuticular mycelium and thousands of conidia responsible for disease build-up during summer. Subsequently, several secondary cycles can occur during June – October, this result was also confirmed in the American project performed by (Jones and Aldwinckle, 1990).

In contrast, though kindly reducing of fruit scab incidence to 40.9 % for trees sprayed six times without populin from that of control, this result was non-comparable with other investigated spraying treatments, when the scabbed fruits occurred between 46.5 to 53.7 %.

Despite the recommendation reported by Balint *et al.,* (2014) about significant reduction of conidial germination and further asexual spores using 1% populin extract. Our results under field conditions revealed its worthless for preventing fruit scab severity and occurred with 2.9 .

Table (2): Effect and efficiency of standard strategy of fungicide sprays supported with (populin) extract on the apple scab (Starking Delicious).

Sprays Programme	Populin Extract 1%	% Scab Incidence		% ABB Efficiency of Sprays [w]		Scab Severity		% ABB Efficiency of Sprays	
		Leaf Scab	Fruit Scab	Leaf Scab	Fruit Scab	% Leaf Scab	Scores of Fruit Scab	Leaf Scab	Fruit Scab
Control	Control (without populin)	84.99 a[α] ±3.24[b]	80.56 a ±0.63	-	-	49.14 a ±3.04	3.73 a ±0.23	-	-
	Populin Extract	72.46 b ±1.06	69.39 a ±2.58	14.74 c ±2.54	13.86 b ±1.39	34.02 b ±1.53	2.90 b ±0.09	30.76 c ±2.23	22.17 c ±3.48
Three Sprays[d]	Control (without populin)	55.22 cd ±0.36	53.75 b ±1.32	35.03 a ±2.14	33.27 a ±2.11	22.54 cd ±0.32	2.01 c ±0.26	54.12 ab ±2.55	45.90 b ±2.03
	Populin Extract	62.40 c ±3.90	51.38 b ±2.06	26.57 b ±3.69	36.22 a ±3.05	26.75 c ±1.98	1.21 d ±0.08	45.55 b ±2.53	67.52 a ±2.76
Six Sprays[e]	Control (without populin)	51.10 d ±2.42	40.96 b ±2.28	39.87 a ±3.91	49.15 a ±3.16	18.63 d ±0.77	1.01 d ±0.06	62.08 a ±2.03	72.81a ±0.73
	Populin Extract	50.92 d ±1.04	46.51 b ±2.31	40.08 a ±3.48	42.26 a ±3.02	18.56 d ±0.93	1.04 d ±0.12	62.22 a ±2.40	72.13 a ±1.89

[α] Means within the same column followed by the same letter aren't significant different ($p\leq 0.05$)
[b] ± Values of standard error. [d] **Three Sprays :** Strategy including three alternativeness sprays of Goldazim at Petal fall , punch at 2 cm fruit , and pristine at pre-harvest, 6cm fruit [e] **Six Sprays :** Standard strategy with six alternativeness sprays of (Goldazim , Champion , punch , Pristin , Champion , Punch) respectively, from the petal fall until to pre-harvest**.** [w] ABB: Efficiency of chemical and extract sprays according to Abbott formula.

However, six sprays strategy was restricted scab severity to 1.01 and 1.04 without and with populin, respectively from that 3.73 and 2.9 in control. Conversely, the remarkable effect of populin on the reduction of fruit scab was also apparent on fruits treated with three sprays, which perceived with 1.21 comparable to 3.73 in control. However, in spite of lowest efficiency of plant extract on reducing scab incidence and severity can be considering low costs and more environmentally friendly, so can be take in consideration in IPM program for apple scab (Holb, 2009).

Discriminating efficiency of leaf scab severity after six sprays strategy was realized to (62.22 % and 62.08 %) with and without populin. However, application of three sprays without populin also gave resemble result. Both strategies of six and three spray (with populin) were continued their efficacy to fruiting stage and decreasing of scab severity prorate more than 72 % and 67.5 % , respectively compared to without populin sprays 45.9 %. Sutton *et al.,* (2000) reported that populin had similar effect as synthetic fungicides under wet climate conditions, and this non predominate in Duhok which featuring with moderate to warm climate at the time of spraying fungicides and relatively less moist. However, populin extract reduced leaf and fruit scab severity to 34.02 % and scores of 2.9, respectively. Incidence of leaf scab with an extract reduced to 72.46 % compared to 84.9 % in control, its effect on fruit scab was non significant.

The effective control of scab also depends on the availability of efficient and specific fungicides, but in Iraq that depends greatly on the imported fungicides, and a few non-expensive and selective fungicides might be ready for growers, throughout growing season. Thus, the continuous monitoring and assessing spray times and development of fungal resistance or sensitivity to most common fungicides are very essential in any scab management programme (Gao *et al.,* 2009).

In spite of relatively, a positive and remarkable effects of chemicals to disease control, differences of the curative and preventive actions of investigated fungicides on the scab incidence without populin were rather convergent and ranged (40.96 % to 53.75 %) on fruit , (51.1% to 55.2 %) on leaf for both spray programme.

According to the results of several authors, concerning the best timing recommended for effective scab control in commercial orchards, trees should be sprayed before, during or immediately after a rain from the time of bud opening until the ascospore discharging from pseudothecia and this required repeated spray between 9-15 times (Biggs and Warner, 1990; Hellmann and Sessler, 1995). MacHardy (1996) and Holb *et al.,(*2005a) recommended that only two sprays were applied in summer scab observed on harvested fruits might arisen from secondary infection , especially where partially controlled primary infection.

Practically, under local climate fluctuation during disease development, the poor management have been performed via some growers to suppression overwintering propagules of a pathogen such as inattention removing of apple leaf litter, the greatest hibernating dormant inoculum of pseudothecia that enable ascospores to become established and discharged intensively in the early spring and then progress along summer months. With this approach subsequent sprays for control conidial infection becomes essential as reported by (Köller *et al.,* 2005a ; Köller *et al.,* 2005b ; Rosenberger and Cox ,2010).

Worthily, great efforts have been made to reduce the number of fungicide sprays, and this due to our knowledge that symptomatic scab occurrence delayed to mid-May, i.e. shortening of conidial dispersal duration in addition susceptibility of apple blossoms and other succulent tissues to fungicides action that mostly coincide with relatively high temperature, and may lead to downfall and blossom's blight.

Furthermore, most growers disregarded apple orchards that restrict on a single spray of fungicide and frequently with delay practices required for reducing inoculum potential of the pathogen particularly for the leaf litter and weed control results weakened and predisposed trees to different pathogens and other abiotic stress.

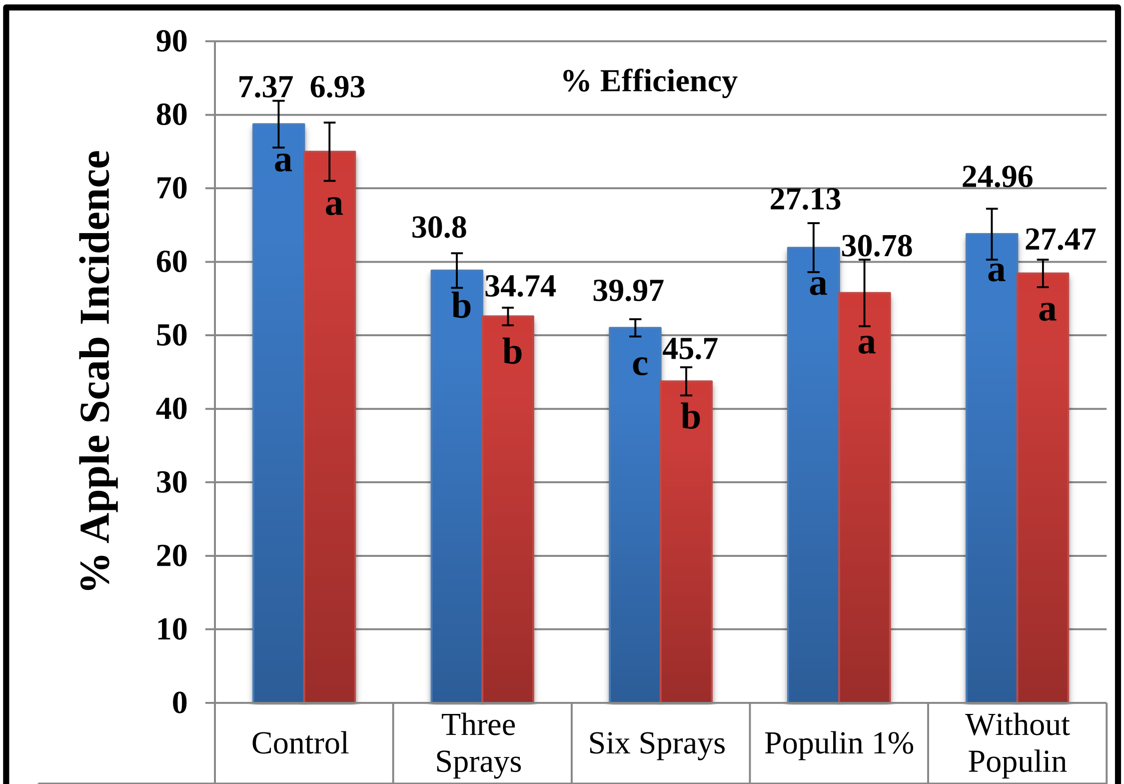
Efficacy Of Fungicides And Populin On Scab Incidence

Data represented in the (Fig. 3) illustrated that six spray of fungicides was more effective and remarkable in decreasing of scab incidence on apple leaves to 51 % and fruits to 43.73 % i.e. its efficacy amounted to 40 % and 45.7 %, respectively. In contrast three sprays gave convergent and non significant efficacy on fruit scab.

Sprays with and without populin were not comparable in reducing scab incidence that ranged between 55.76 % and 63.77% on leaves and fruits.

Efficacy Of Fungicides And Populin On Scab Severity

Results from (Fig. 4) demonstrated that six sprays programme decreased severity of scabbed leaves and fruits to 18.59 % and 10.2 %, and therefore efficiency was augmented to 62 % and 72.5 % , respectively. Three sprays strategy were less effective on lessening of scab severity to 24.65 % and 16.2 % on leaves and fruits. Using of populin (plant extract) spray show better results in reducing the leaf and fruit scab severity (26.44 % and 17.1 %) with efficiency (46 % and 54%) than control (without populin) 30.1 % and 22.5 with efficiency 39 % and 39.5, respectively. Thiesz *et al.*, (2007) found that using of plant extract (populin) under field and laboratory conditions significantly effect compare to control and high mortality of ascospores .



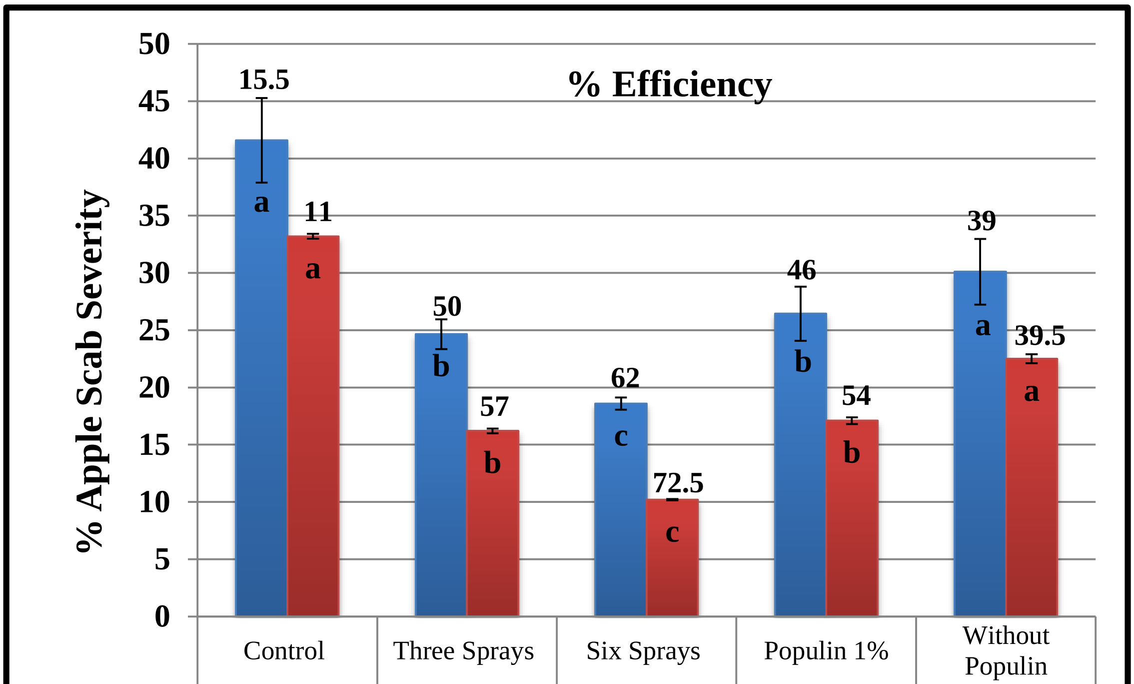
	Control	Three Sprays	Six Sprays	Populin 1%	Without Populin
Leaf Scab	78.72 a	58.81b	51.01 c	61.92 a	63.77a
Fruit Scab	74.97 a	52.56 b	43.73 b	55.76 a	58.42a

Fig (3): Effect and efficiency of standard Strategy of Fungicides and populin sprays on leaf and fruit scab incidence

The significant and efficient of six sprays programme began in mid- April and repeated at 15-18 days intervals may be contributed as a major factor to the hottest summer from June to mid-July with average of daytime temperatures above 40 ˚C for several consecutive days caused a decrease in the conidia density, this reduction was exceeded when combined with the kind of eradicant scab activity of such DMIs fungicide as Punch (Flusilazole 40%) which also concluded by (Siebels and Mendgen ,1994; Beresford *et al.*, 2013). This eradicative and curative fungicide inhibited sterole biosynthesis in cell membrane (FRAC, 2015), that increased permeability of pathogen cell membrane to the chemical.

Pristine (active ingredient of Boscalid & Pyraclostrobin) is a single fungicide subjected to single-change resistance with the target gene and hence are at high risk for resistance that may appear abruptly (Fontaine *et al.*, 2009; Quello *et al.*, 2010). Boscalid is anew broad-spectrum fungicide, class carboxamides (succinate dehydrogenase inhibitors). Stammler *et al.*, (2007) Poplished that it's effective against spore germination and germ-tube elongation , appressoria formation and mycelial growth through impairing respiration process (FRAC, 2015) .

Chemical group of pyraclostrobin is Quinone outside inhibitor (QOIs) act as inhibitor of *V.inaequalis* mitochondrial respiration by binding to mitochondrial cytochrome complex and disrupting electron transport (Ypema and Gold, 1999). Therefore, they are recommended in a fungicide alternating programmes as a strategy to slow down fungicide resistance development (de Waard *et al.*, 2006 ; Jobin and Carisse , 2007).



	Control	Three Sprays	Six Sprays	Populin 1%	Without Populin
Leaf Scab	41.58 a	24.65 b	18.59 c	26.44 a	30.1 a
Fruit Scab	33.2 a	16.2 b	10.2 c	17.1 a	22.5 a

Fig. (4): Effect and efficiency of standard Strategy of Fungicides and populin sprays on leaf and fruit scab severity.

The active component of champion fungicide investigated by Heitefuss (2000) are copper salts that dissolve in water and copper ions , this ions can be able to penetrate *Venturia inaequalis* spores and denature proteins with various inhibition of enzyme in the cell. This hypothesis supposes that water solubility of the copper compound is important. On the other hand, *V.inaequalis* spore exudates interact with copper insoluble compounds resulting more toxic complexes to the pathogen than dissolved ions in cell (Montag *et al.,* 2005). Goldazim (Carbendazime 50%) is a systematic fungicide , works by inhibiting the development of *V. inaequalis* probably by interfering with spindle formation at mitosis (cell division) and also inhibits the development of germ tubes , formation of appressoria and growth of mycelia (Carisse *et al.,* 2010).

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بكارئينانا دەرمانێن كەرويان لسەر كەسكاتيا دارێ دگەل گيراوێ (Populin) ژبوو كونترول كرنا گوريبونا سێڤێ (Venturia inaequalis)

پوختە

د ڤەكولينا شەش رەشاندنا لسەر دارا ب دەرمانێن كەرويان ئەوژى (Goldazim, Champion Punch, Piristine, Champion and Punch) دياربوو كارتێكرنەكا بەرچاڤ هەبوو لسەر دياربوونا نەخوشيا كولبوونا بەلگى ب رێژەيا 51 % و كولبوونا بەرهەمى (سێڤا) ب رێژەيا 43.73 % و شيانێن وان گەهشتنە 40 % و 57.7 %. هەروەسا نەخوشيا بەلگان و بەرهەمى كێمكريە بو رێژا 18.59 % و 10.02 % دياربوو كو شيانێن وێ پتربوون لسەر نەهێلانا هێرشا ئەگەرێ نەخوشيێ ب رێژا 62 % و 72.5 % ديڤ ئێك دا.

استخدام المبيدات الفطرية رشاً على المجموع الخضري مع المؤازر مستخلص ال (Populin) لمقاومة جرب التفاح *Venturia inaequalis*

الخلاصة

اظهرت ستة رشات بالمبيدات الفطرية الاتية فى الحقل (Goldazim, Champion Punch, Piristine, Champion and Punch) تاثيراً اختزالياً معنوياً في ظهورالمرض على الاوراق بنسبة 51 % و 43.7 % على الثمار و بكفاءة بلغت 40 % و 57.7 %, كما اختزلت شدة اصابة بالجرب على الاوراق والثمار بنسبة 18.59 % و 10.2 % على التوالى و بذلك فانها اظهرت كفاءة في منع هجوم المرض و بكفاءة بلغت 62 % و 72.5 % على التوالي.