



PROTECTIVE AND CURATIVE ACTIVITY OF AZOXYSTROBIN AND ITS MIXTURES AGAINST POWDERY MILDEW ON SQUASH

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ABSTRACT

Squash is one of the important crops of the cucurbits family and powdery mildew causes less yield every year, Strobilurin group are common fungicides used to control powdery mildew disease. In addition, Strobilurin is more safe for humans and the environment because it is derived from a natural fungicidal source (the production of the active ingredients are achieved by wood-rotting of *Strobilurus tenacellus*). Strobilurin contains a group of active ingredients that control various diseases such as powdery and downy mildew on cucumber, grapes, and watermelon, early blight and late blight on tomatoes and potatoes, scab on apple and others. This study focuses on the effectiveness of protective and curative applications of Azoxystrobin as one of the active ingredients of Strobilurin group alone and mixtures against powdery mildew on squash. The induction of resistance of plants against powdery mildew by using KH_2PO_4 and K_2HPO_4 , protective and curative applications. The tested compounds against powdery mildew on squash at protective method were Azoxystrobin mixture with Difenconazole 83.3%, Azoxystrobin mixture with Tebuconazole 76.6%, Azoxystrobin alone 70.7%, Difenconazole 62.3%, Tebuconazole 55.9%, K_2HPO_4 16.7% and KH_2PO_4 10.8%. The curative effectiveness of the tested products against powdery mildew on squash was as follows Azoxystrobin mixture with Difenconazole 69.3% > Azoxystrobin mixture with Tebuconazole 61.2% > Azoxystrobin alone 57.6% > Difenconazole 44.2% > Tebuconazole 42.7% > K_2HPO_4 12.1% > KH_2PO_4 7.4%. Accordingly, Azoxystrobin alone and its mixtures were most effective against powdery mildew on squash compared to Difen-

conazole, Tebuconazole, K_2HPO_4 and KH_2PO_4 alone in both protective and curative applications. However the protective method was more effective compared to the curative method and the mixture of Azoxystrobin with Difenconazole was more effective compared to all tested treatments followed by the mixture of Azoxystrobin with Tebuconazole and both treatments were slightly better or comparable to Azoxystrobin alone. In addition, the foliar application by KH_2PO_4 and K_2HPO_4 was inferior in activity to resistant powdery mildew infection under the field conditions.

Keywords: Azoxystrobin, Strobilurin, Powdery mildew, Curative, Protective, Activity, Cucurbits

INTRODUCTION

Azoxystrobin is one of the most important active ingredient of the fungicide Strobilurin group. It is leading the worldwide fungicides possessing broad spectrum systemic activity against the four major disease classes of pathogenic fungi. The strobilurin group of fungicides was detected by derivation of b-methoxyacrylic acid from natural fungicidal group and the production of it by wood-rotting of *Strobilurus tenacellus*. The mode of action of the strobilurins against fungi is their ability of inhibit mitochondrial respiration by binding these-called Qo site of cytochrome b. located in cytochrome bc1 complex and that part of the inner of mitochondrial membrane of fungi. This inhibition blocks the electrons transfer between cytochrome b and cytochrome c1 that causes disruption of the fungus energy cycle, within halting the production of ATP (Bartlett et al 2002). *Cucurbitaceae* is an important family, which supply humans with a lot of

edible products and useful fibers. Plants of cucurbits family are very similar in vegetative development, but they have a high genetic diversity for fruit shape and characteristics, resulting in a variety range of uses (**Bisognin, 2002**). Powdery mildew disease causes devastation in cucurbits and yield can decline with increase of disease severity (**Lamsa, 2011**). However the plants may resist the disease if they have good vegetative state by using some non-chemical products like KH_2PO_4 and K_2HPO_4 . The foliar fertilizers of phosphates and potassium salts are convenient to use with a potential useful impact on control the disease (**Reuveni et al 1995**). Powdery mildew caused by *Erysiphe cichoracearum* (**Bardin, 1999**) and *Podosphaera fusca* (synonyms: *P. xanthii*, *Sphaerotheca fulginea* and *S. fusca*), (**Janousek et al 2009, McGrath and Thomas 1996, Pérez-García et al 2009, Alejandro, 2009**). Squash is a member of cucurbits family and the powdery mildew causes less yield. The objective of the study is to measure the effectiveness of Azoxystrobin and its mixtures against powdery mildew on squash and determine the induction of resistance of plants against powdery mildew by using KH_2PO_4 and K_2HPO_4 .

MATERIALS AND METHODS

This study was carried out during two growing seasons 2015/2016 and 2017/2018 under open field conditions at Katta, Qalyubia Governorate, Egypt.

1. Squash cultivars used

Local variety of squash "Eskandrany" was sown in autumn from July to August. (**Agricultural Research and Extension Center**)

2. Plantation techniques

Seeds were directly sown in soil (3 kg/ acre), at 40 cm intervals between plants, 1 m row space between rows and the maintenance application against Whitefly and Aphid were applied by Actara 25% WG and Vertemic 1.8% EC according to the recommendation of the Ministry of Agriculture.

3. Plot size and replicates number

A randomized block design with three replicates was adopted for two seasons. Each replicate contained three plots, the plot size was 15 m². Each plot consisted of three rows, each row was five meters length and one meter width and plants distance was 40 cm.

4. Applications and Type of soil

The first season was in sandy soil at Katta Governorate, curative application after infestation was applied in October/ November of 2015 and the second season was in clay soil at Qalyubia Governorate, protective application before infestation was applied in October of 2018. The number of applications was three times according to local practice. The application interval was ten days, depending on the disease development in the field.

5. Application equipment

The applications were achieved by using Back sprayer, hollow nozzle, size 1.5 and the pressure of spray was 1.5 – 2 bars.

6. Treatments

Seven treatments were applied: Azoxystrobin + Difenoconazole 325 SC (**1**), Azoxystrobin + Tebuconazole 320 SC (**2**), Azoxystrobin 250 SC (**3**), Difenoconazole 250 SC (**4**), Tebuconazole 430 SC (**5**), K_2HPO_4 (**6**) and KH_2PO_4 (**7**), Azoxystrobin (purity > 97%), Difenoconazole (purity > 99%). These products alone and mixture were purchased from Syngenta Ago Co, Azoxystrobin + Tebuconazole purchased from AAKO BV Co. and Tebuconazole produced from Jiangsu Rotam Chemistry Co., Ltd (purity > 95.7%).

7. Evaluations

Disease severities were evaluated from 2 to 3 leaves taken from plants grown in middle row selected randomly. The evaluations were made each 5 days and before applications for all plots. Disease severity was recorded 20 days after the last application with the following scale: (0) = no symptoms, (1) = 0% to 5%, (3) = 6% to 10%, (5) = 11% to 20%, (7) = 21% to 40%, and (9) = more than 40% from the natural infected surface area of the leaf covered with mildew, produced from 25 leaves per plot (75 leaves per treatment). The disease index for each treatment was calculated using the following formula:

$$DI = \frac{\sum_{i=0}^9 N_i \times i}{\sum_{i=0}^9 N_i \times 9} \times 100$$

Where (*i*) is the disease severity (0 to 9) and (*N_i*) indicates the number of leaves with the severity of (*i*).

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Disease control was calculated as follows (Qingrong Peng et al 2017):

$$DC (\%) = \left(\frac{DI_{UTC0} - DI_{CT1}}{DI_{UTC0}} \right) \times 100$$

Where (UTC0) indicates the disease severity of the untreated control before the first treatment and (CT1) stands for the disease severity of any treatment after the last treatment.

Table 1. Treatments list of commercial products which used for the field trials and their rates

Treatment number	Treatments	Formulation	Rates (g or ml/ hl)	Active ingredients/ hl*
1	Azoxystrobin (AZ) + Difenoconazole (DFZ)	325 SC	50	10 AZ + 6.25 DFZ
2	Azoxystrobin (AZ) + Tebuconazole (TBZ)	320 SC	100	12 AZ + 20 TBZ
3	Azoxystrobin	250 SC	50	12.5
4	Difenoconazole	250 SC	50	12.5
5	Tebuconazole	430 SC	35	81.3
6	K ₂ HPO ₄	-	100	-
7	KH ₂ PO ₄	-	100	-

*Hectoliter (hl) = 100 liter of water

RESULTS AND DISCUSSION

1. Effectiveness of Azoxystrobin alone and in mixtures compared to other treatments against powdery mildew on squash

Azoxystrobin alone and in mixture were effective against powdery mildew in both protective and curative applications and they were better in activity compared to Difenoconazole, Tebuconazole, K₂HPO₄ and KH₂PO₄. However the mixture of Azoxystrobin with Difenoconazole was superior compared to all tested treatments and the mixture of Azoxystrobin with Tebuconazole was slightly better in activity compared to Azoxystrobin alone (Tables 2 & 3).

Table 2. Mean % effectiveness of Azoxystrobin alone and in mixtures as curative application compared to other treatments against powdery mildew on squash.

Treatments	% Efficacy
Azoxystrobin + Difenoconazole	69.3 ^a ±3.2
Azoxystrobin + Tebuconazole	61.2 ^b ±3.2
Azoxystrobin	57.6 ^b ±3.3
Difenoconazole	44.2 ^c ±3.0
Tebuconazole	42.7 ^c ±3.3
K ₂ HPO ₄	12.1 ^d ±2.1
KH ₂ PO ₄	7.4 ^d ±1.9
F value	438.33**
LSD	5.5408

The results of the curative method in **Table (2)** showed that the effectiveness of Azoxystrobin mixture with Difenoconazole was 69.3% against powdery mildew on squash. However, Azoxystrobin mixture with Tebuconazole was 61.2% effective and both treatments were better in activity compared to Azoxystrobin alone 57.6% efficacy. In addition Azoxystrobin alone and in mixture showed better in effectiveness compared to the Triazols Difenoconazole 44.2% and Tebuconazole 42.7%. K₂HPO₄ 12.1% efficacy, KH₂PO₄ 7.4% and they were efficacy were inferior in their control against powdery mildew on squash compared to all tested treatments.

Table 3. Mean % effectiveness of Azoxystrobin alone and mixture as protective application compared to other treatments against powdery mildew on squash.

Treatments	% Effectiveness
Azoxystrobin + Difenoconazole	83.3 ^a ±3.2
Azoxystrobin + Tebuconazole	76.6 ^{ab} ±3.2
Azoxystrobin	70.7 ^b ±3.3
Difenoconazole	62.3 ^c ±3.0
Tebuconazole	55.9 ^c ±3.3
K ₂ HPO ₄	16.7 ^d ±2.1
KH ₂ PO ₄	10.8 ^d ±1.9
F value	313.31**
LSD	7.8575

The result of protective method in **Table (3)** showed that the effectiveness of Azoxystrobin mixture with Difenconazole was 83.3% against powdery mildew on squash. However, Azoxystrobin mixture with Tebuconazole was 76.6% effective and both treatments were better compared to Azoxystrobin alone 70.7%. In addition Azoxystrobin alone and mixture was more effective compared to the Triazoles Difenconazole 62.3% and Tebuconazole 55.9%. K_2HPO_4 16.7% effective and KH_2PO_4 10.8% and were least effective against powdery mildew on squash compared to all tested treatments.

Several authors evaluated the effectiveness of Azoxystrobin alone and in mixtures against powdery mildew on cucurbits. For example Azoxystrobin (Amistar 25 SC) showed successfully arrest of disease progression compared to Mancozeb at 1 kg/ha and Carbendazim at 500 g/ha against downy mildew and powdery mildew on cucumber (**Anand et al 2008**). Azoxystrobin 25 SC at 0.1 %, four applications at seven days interval was better in activity, followed by four applications of Carbendazim 50 WP at 0.1 per cent, followed by Azoxystrobin 25 SC at 0.08 % and Penconazole 10 EC at 0.05 % against powdery mildew (*Erysiphe cichoracearum*) of cucurbits from initial onset of infection (**Ilhe et al 2007**). Also the mixture of Azoxystrobin showed more effectiveness compared to Azoxystrobin alone. Other authors showed similar results of Azoxystrobin mixture against powdery mildews of sugar beet. The mixtures treatments of Azoxystrobin and Pyraclostrobin with Difenconazole or Cyproconazole showed better control compared to single applications for each mixture of them against powdery

mildew on sugar beet (**Karaoglanidisa and Karadimosb 2006**). The mixture of Azoxystrobin and Difenconazole was more potent compared to the mixtures of each of them with Sulphur against *Erysiphe betae* on sugar beet because Azoxystrobin was more effective than that of Difenconazole and both of them were more potent compared to sulphur treatment by protective and curative applications (**Anesiadis et al 2003**). Good control against Conidial germination of *Podosphaera pannosa* pathogen causing powdery mildew in a Petri plate was recorded in Difenconazole (85.96 %) followed by Trifloxystrobin + Tebuconazole (83.60%), Tebuconazole (82.07%), Hexaconazole (78.99%), Propiconazole (77.20%) and Dinocap (71.97%) (**Kumar and Chandel (2018)**).

2. Protective treatment effectiveness of Azoxystrobin alone and in mixture compared to curative treatment against powdery mildew on squash plants

All treatments of protective application were highly active against powdery mildew compared to the same treatments in the curative application (**Table 4**). Several authors evaluated treatments of protective application compared to curative application and the results showed that curative applications of Azoxystrobin, Myclobutanil, and Pyraclostrobin against powdery mildew of watermelon were less effective compared to preventative applications (**Anthony and Virginia 2004**). Effective control of strobilurin against *Cercospora beticola*, was obtained when applied at 24 h after inoculation, but they were less effective when applied at 96 h after inoculation (**Karadimos et al 2005**).

Table 4. Mean % effectiveness of protective application treatments compared to the same curative of application treatments against powdery mildew on squash

Treatments	% Effectiveness		F value	LSD
	Curative app.	Protective app.		
Azoxystrobin + Difenconazole	69.3 ^a ±3.2	83.3 ^b ±3.2	73.41*	4.5354
Azoxystrobin + Tebuconazole	61.2 ^a ±3.2	76.6 ^b ±3.2	58.62*	5.5734
Azoxystrobin	57.6 ^a ±3.3	70.7 ^b ±3.3	127.08**	3.2245
Difenconazole	44.2 ^a ±3.0	62.3 ^b ±3.0	96.34**	5.1233
Tebuconazole	42.7 ^a ±3.3	55.9 ^b ±3.3	44.99*	5.4427
K_2HPO_4	12.1 ^a ±2.1	16.7 ^a ±2.1	3.23	5.3633
KH_2PO_4	7.4 ^a ±1.9	10.8 ^a ±1.9	2.47	8.2003

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الفاعلية الوقائية والعلاجية لأزوكسيستروبين ومخاليطه ضد البياض الدقيقي على الكوسة

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على الكوسة مقارنة بباقي المعاملات الاخرى. المعاملة بالازوكسيستروبين مخلوط مع التيبوكونازول اظهرت فاعلية جيدة لمقاومة البياض الدقيقي على الكوسة وهي المعاملة التي تلي في فاعليتها معاملة الازوكسيستروبين المخلوط مع الداى فينوكونازول. اظهرت معاملات الازوكسيستروبين مخلوطا بالتريازول فاعلية افضل من المعاملة به منفردا. اظهرت جميع المعاملات الوقائية فاعلية افضل على البياض الدقيقي مقارنة بمثلتها من المعاملات العلاجية. اظهرت معاملات فوسفات الاحادي والثنائي البوتاسيوم فاعلية ضعيفة لحد النبات علي مقاومة المرض. لم تظهر اي مظاهر للسمية النباتية علي النبات نتيجة استخدام المركبات المختبرة وقائيا او علاجيا.

الكلمات الدالة: أزوكسيستروبين، ستروبورين، بياض دقيقي، وقائي، علاجي، فاعلية، قرعيات

الموجز

يهدف هذا البحث الى تقدير الفاعلية الوقائية والعلاجية للازوكسيستروبين كأحد اهم المواد الفعالة لمجموعة الستروبورين الفطرية سوء باستخدامها بشكل فردي او مخلوطاً بمركبات التريازول لمواجهة البياض الدقيقي علي القرعيات. تم اجراء هذه الدراسة على نبات الكوسة في موسمين مختلفين الموسم الاول سنة 2015 في التربة الصفراء في منطقة ابو غالب والموسم الثاني سنة 2018 في التربة الطينية في منطقة قها محافظة القليوبية. قد تم معاملة نباتات الكوسة في الموسم الاول بشكل علاجي بعد اصابة البياض الدقيقي للنباتات اما الموسم الثاني تم معاملة النباتات قبل الاصابة بالبياض الدقيقي. المعاملة بالازوكسيستروبين المخلوط مع الداى فينوكونازول كانت الافضل في فاعليتها لمقاومة البياض الدقيقي