

Efficacy of Sterol-Inhibiting Fungicides on the Control of Scab Disease (*Venturia Inaequalis*) in Apple Tree

Edlira Shahinasi and Ferdi Brahushi

Abstract—Apple scab, caused by *Venturia Inaequalis* is one of the most problematic diseases of apples in Korça region. The control of this disease requires different applications of fungicides. The aim of the study was the estimation of efficacy of sterol-inhibiting fungicides such as myclobutanil and penconazole on the control of scab disease. The effectiveness of fungicides was evaluated for two cultivars Golden Delicious and Starking and the minimum and the maximum recommended doses were applied. The obtained results showed that scab prevalence in leave ranges from 79.2% to 85.6% respectively in untreated trees of Golden Delicious and Starking cultivars. The scab prevalence of leaves in apples treated with pesticides ranges from 18.1% in Golden Delicious cultivar to 22.2% in Starking cultivar, meanwhile the scab prevalence in fruit varies from 4.2% in Starking cultivar to 6.1% in Golden Delicious cultivar. The disease index of leave in untreated control varies from 38.4% to 40.1% respectively for Golden Delicious cultivar and Starking cultivar, while the severity of fruit in untreated trees varies from 45.6% in Starking cultivar to 49.0% in Golden cultivar. Therefore, the data showed that the uses of sterol-inhibiting fungicides were effective in the control of scab disease.

Index Terms—Efficacy, Apple Scab, Prevalence, Sterol-Inhibitor, Severity.

I. INTRODUCTION

Apple scab caused by *Venturia Inaequalis* is a devastating disease of apples in the world, and the poor control of it can lead to reduction of quality and quantity of the fruit. In some circumstances, the losses from apple scab can be 70% or more of the total fruit. Over the years, fungicides have become the sole means to control apple scab and there has been little effort to commercialize alternative strategies [1-3]. Most apple cultivars are susceptible to scab, and not only in the commercial treatments are needed frequent fungicide applications (up to 15-20 different types of fungicides annually), but even in Integrated Pest Management systems [1, 4].

Among many classes of fungicides, sterol demethylation inhibitors (DMI) are the most effective. DMI They serve as backbone of apple disease management programs with the main focus on the control of apple scab [5].

According to Fungicide Resistant Action Committee (FRAC) this class of fungicides belongs to Code Group 3 and represents single-site inhibitor [6]. They bind to the cytochrome P450 a monooxygenase enzyme, thereby inhibit

the biosynthesis of ergosterol which is responsible for the fluidity and stability of the membrane [7, 8].

The current study estimates the effectiveness of sterol-inhibiting fungicides such as myclobutanil and penconazole on the control of apple scab.

II. MATERIALS AND METHODS

A. Experimental field

The experiment was conducted in an apple orchard in the region of Korça during the growing seasons of 2015 and 2016. The orchard was divided in blocks with three replications. Each replicate consisted of five trees. Two apple cultivars Golden Delicious and Starking were tested during the experiment, as the most affected cultivars by the scab disease. The control of apple scab disease was investigated by application of two fungicides myclobutanil and penconazole, with two levels of concentrations. Fungicide concentrations were: (1) myclobutanil (Brik 24; 24g/liter myclobutanil) at 15 mg/liter and 25 mg/liter, and (2) penconazole (PEN 10 EC; 10g/liter) at 25 mg/liter and 40mg/liter. The applications of the fungicides were applied by a tractor in which two sprays were mounted.

B. Scab incidence and severity

Disease incidence and severity were assessed after the last application. Thus, 10 leaves/fruits were taken randomly from each tree. The leaves and fruits were chosen from different sides of the tree, then were transferred to the laboratory for the analyses. The incidence and severity were estimated visually based on the analyses of leaves and fruits.

C. Disease incidence

The incidence was calculated using formula (1)

$$P = \frac{n}{N} \times 100 \quad (1)$$

where,

n - number of infected leaves/fruits screened

N – the total number of leaves/fruits screened

D. Disease index (severity)

Disease assessment was calculated using a scale developed by Tomerlin and Jones. The scale consisted at five categories: (0) no visible lesions; (1) < 5% leave/fruit surface infected; (2) 5-15% leave/fruit surface infected; (3) 15-25% leave/fruit surface infected; (4) 25- 50 leave/fruit surface infected, (5) >50% leave/fruit surface infected. Leave and fruit severity was calculated using Townsend-Huberger's formula (2):

Published on June 20, 2019.

E. Shahinasi is with the Department of Chemistry, Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania.

F. Brahushi is with the Department of Agroenvironment and Ecology, Agricultural University of Tirana, Albania.

$$I = \frac{\sum n_i k_i}{N \times K} \times 100 \quad (2)$$

where,

I – Index of disease

n_i – the number of leaves/fruits per category

k_i – degree of infection according to the scale

N – total number of leaves/fruits taken for analysis

K – the highest degree of category

E. Estimation of fungicides' effectiveness

The effectiveness of myclobutanil and penconazole was calculated using equation (3) [9]

$$\text{Efficacy (\%)} = \frac{X-Y}{X} \times 100 \quad (3)$$

where, X – disease incidence/severity in untreated trees Y – disease incidence/severity in treated tree

F. Statistical analysis

Statistical analyses were done using the analysis of variance, two ways ANOVA. To identify significant treatment effects, the mean values were separated using Fisher's least significant difference (LSD) at 95% confidence level (P<0.05)

III. RESULTS AND DISCUSSION

Obtained data are presented in the Table I. These data indicated that disease prevalence in leaves and fruits of untreated trees in Golden Delicious cultivar was very high. Thus, the prevalence in leaves and fruits in untreated tree was 79.2% and 95.0%, respectively. The similar situation was observed even in Starking cultivar. The prevalence in leaves and fruits in untreated trees of this cultivar was 85.6% and 97.8%, respectively. The scab incidence of leaves in apples treated with pesticides ranges from 18.1% in Golden Delicious cultivar to 22.2% in Starking cultivar, meanwhile the scab incidence in fruit varies from 4.2% in Starking cultivar to 6.1% in Golden Delicious cultivar (Table I). The efficacy of sterol-inhibiting fungicides in leaves varies from 74.1% to 77.1%, respectively for Starking and Golden Delicious cultivar, meanwhile the efficacy in the fruits ranges from 94.4% to 95.7% for Golden Delicious and Starking cultivar, respectively.

The statistical analysis of disease incidence data in leaves and fruits showed no significance differences between minimum and maximum doses of applied fungicides, whereas a great significance difference was observed between application of fungicides in apple trees and untreated apple trees.

TABLE I: PROGRESS OF DISEASE INCIDENCE IN LEAVES (L) AND FRUITS (F) OF GOLDEN DELICIOUS AND STARKING CULTIVARS

Cultivar	Treatment	Disease prevalence (%)				Mean*		Efficacy (%)	
		2015		2016					
		L	F	L	F	L	F	L	F
Golden	Untreated tree	74.5	97.0	83.9	93.0	79.2 ^b	95.0 ^b		
	Maximum	17.2	5.0	18.9	6.5	18.1 ^a	5.3 ^a	77.1	94.4
	Minimum	17.2	5.6	18.9	6.7	18.1 ^a	6.1 ^a	77.1	93.6
Starking	Untreated tree	77.2	98.0	77.2	98.0	85.6 ^b	97.8 ^b		
	Maximum	17.2	3.9	19.4	4.5	18.3 ^a	4.2 ^a	78.6	95.7
	Minimum	19.5	4.5	25.0	5.2	22.2 ^a	4.9 ^a	74.1	94.9
LSD						6.49	2.89		

*Means within columns with different letters are significantly (P<0.05) different according to Fisher's least significant difference

The presented data in the Table II showed that the severity as well as the prevalence was very high in the untreated trees. The severity of leave in untreated trees varies from 38.4% to 40.1% respectively for Golden Delicious and Starking cultivar, while the disease index of fruit in untreated trees varies from 45.6% in Starking cultivar to 49.0% in Golden cultivar.

The disease index of leaves in treated apples tree with pesticides ranges from 8.9% in Golden Delicious cultivar to 13.5% in Starking cultivar, meanwhile the disease index in

fruit varies from 1.2% in Starking cultivar to 2.2% in Golden Delicious cultivar.

The efficacy of sterol-inhibiting fungicides in leaves varies from 66.3% to 76.8% respectively for Starking and of Golden Delicious cultivar, meanwhile the efficacy in the fruits ranges from 95.5% to 97.4% for Golden Delicious and Starking cultivar, respectively. Despite the fact that some studies have reported resistance of *Venturia inaequalis* to sterol-inhibiting fungicides [10, 11] they still provide excellent control of apple scab.

TABLE II: PROGRESS OF DISEASE SEVERITY IN LEAVES (L) AND FRUITS (F) OF GOLDEN DELICIOUS AND STARKING CULTIVARS

Cultivar	Treatment	Disease severity (%)				Mean		Efficacy (%)	
		2015		2016					
		L	F	L	F	L	F	L	F
Golden	Untreated tree	36.9	47.0	39.8	51.0	38.4 ^c	49.0 ^c		
	Maximum	8.7	1.6	9.1	1.8	8.9 ^a	1.7 ^a	76.8	96.5
	Minimum	9.0	2.1	9.7	2.3	9.3 ^a	2.2 ^a	75.8	95.5
Starking	Untreated tree	38.5	43.7	41.7	47.5	40.1 ^d	45.6 ^b		
	Maximum	12.2	1.0	12.5	1.4	12.4 ^b	1.2 ^a	69.1	97.4
	Minimum	13.2	1.3	13.9	1.7	13.5 ^b	1.5 ^a	66.3	96.7
LSD						1.28	1.48		

*Means within columns with different letters are significantly (P<0.05) different according to Fisher's least significant difference

The statistical analysis of disease severity data in leaves and fruits showed no significance differences between

minimum and maximum doses of applied fungicides, whereas a great significance difference was observed

between treated and untreated apple trees. Thus, the obtained data demonstrated that apple scab disease can be controlled successfully by application of sterol-inhibiting fungicides as myclobutanil and penconazole.

Based on statistical analyses, using maximum of doses of fungicides has not significant effect on apple scab efficacy compared to minimum fungicide concentration and its application can only contribute on the residues in apple fruits and environmental pollution.

IV. CONCLUSIONS

The results of the study indicate that the application of sterol-inhibiting fungicides reduce significantly incidence and severity of apple scab in leaves and fruits as well. The data related to the effectiveness of scab control provided by these fungicides demonstrate that they were very effective for the control of the scab disease in apple trees. The efficacy of these fungicides ranges from 66.3% to 76.8% in leaves and from 95.5% to 97.4% in fruits. The statistical analyses showed that between two treatments (minimum and maximum doses) had no significant differences therefore, minimum recommended doses of fungicides myclobutanil and penconazole can be applied to control the apple scab disease, as they were very effective and can reduce possible pesticides residues in apple fruits and environment.

REFERENCES

- [1] Misba Majeed, Nazir A. Bhat, Zaffar A. Badri, Vaseem Yousuf, Tanveer A. Wani, Mudassir Hassan, Md. Saleem, Stanzin Dorjey, Shazia Paswal. Non-Chemical Management of Apple Scab- A Global Perspective. *International Journal of Environment, Agriculture and Biotechnology (IJEAB)*, vol. 2, no. 2, pp. 912-921, Mar-Apr 2017
- [2] Laurent Jamar, Marc Cavelier, Marc Lateur. Primary scab control using a "during-infection" spray timing and the effect on fruit quality and yield in organic apple production. *Biotechnol. Agron. Soc. Environ.* vol 14 no.3, pp. 423-439, February 2010.
- [3] Laurent Jamar B. Lefrancq & M. Lateur. Control of apple scab (*Venturia Inaequalis*) with bicarbonate salts under controlled environment. *Journal of Plant Diseases and Protection*, vol. 114, no.5, pp. 221-227, October 2007.
- [4] S. Kunz, G. Mögel, M. Hinze, F. Volk. Control of apple scab by curative applications of biocontrol curative <http://www.researchgate.net/publication/44191814>
- [5] Emily E. Pfeufer and Henry K. Ngugi. Orchard Factors Associated with Resistance and Cross Resistance to Sterol Demethylation Inhibitor fungicides in Populations of *Venturia Inaequalis* from Pennsylvania. *Phytopathology* vol. 102, no 3, pp 272-82, March 2012.
- [6] Fungicide Resistance Action Committee (2019). FRAC code list 2019: Fungal control agent sorted by cross resistance pattern and mode of action (including FRAC code numbering). <http://www.frac.info/publications>
- [7] Eugene O. Erikson & Wayne F. Wilcox. Distributions of Sensitivities to Three Sterol Demethylation Inhibitor Fungicides among Populations *Ucinula nectar* Sensitive and Resistant to Triadimefon. *Phytopathology* vol. 87, no.8, pp 784-791, August 1997.
- [8] Chao-Xi Luo & Guido Schnabel. The Cytochrome P450 Lanosterol 14 α -Demethylase Gene is a Demethylation Inhibitor Fungicide Resistance Determined in *Monilinia fructicola* Field Isolates from

Georgia. *Applied and Environmental Microbiology*. vol. 74, no 2, pp 359-366, January 2008.

- [9] Milan Stevic, Biljana Pavlovic and Brankica Tanovic. Efficacy of fungicides with different mode of action in raspberry spur blight (*Didymella applanata*) control. *Pestic. Phytomed.* Belgrade. vol. 32, no. 1, pp 25-32, January 2017.
- [10] Köller W, Wilcox WF, Barnard J, Jones AL, Brown PG. Determination and Quantification of Resistance of *Venturia inaequalis* Populations to Sterol Demethylation Inhibitors. *Pathology* vol. 87, no. 2, pp184-190, February 1997.
- [11] R.M. Beresford, P.J. Wright, P.N. Wood and N.M. Park. Sensitivity of *Venturia inaequalis* to myclobutanil, penconazole and dodine in relation to fungicide use in Hawke's Bay apple orchards. *New Zealand Plant Protection* vol 65 pp 106-113, January 2012.

ACKNOWLEDGEMENTS

We wish to thank Mr. Admir Sulo, who allowed designing the experiment field on his orchard during the growing seasons of 2015 and 2016 and for his useful contribution on pesticides' application process.



Edlira Shahinasi was born in Bilisht (Devoll), Albania on the 14th of April 1979. She has been graduated in 2002 as Chemical Engineer (Industrial Chemist) at the Faculty of Natural Sciences; University of Tirana. In 2006 she got Master Degree in the environmental issues, at the Faculty of Natural Sciences, and in 2009 she attended a Postgraduate Specialization Program in "Food Quality and Chemistry of Natural Products" in Crete, Greece. In 2015 she has completed an internship period at the

University of Jaen, Spain where she has acquired specific expertise in the evaluation of sensorial analysis of olive oil. Currently, she is working at Department of Chemistry, Faculty of Biotechnology and Food, Agricultural University of Tirana. She is PhD candidate.

She has experience in teaching and is familiar with the laboratory work. During her academic career she has been participated in International Conferences and has published several research articles in national and international journals. As a result of her interdisciplinary studies environment and food her main work is focused on both areas, especially in the pesticide residues in food and environmental fate of pesticides.

MSc. Shahinasi has been member of several national and international research projects in the field of food and environment.



Ferdi Brahushi is currently Professor at Department of Agroenvironment and Ecology, Agricultural University of Tirana. He has born in Berat, Albania on August 11, 1965. He has graduated as Engineer agronomist and got PhD in the field of agricultural sciences at Agricultural University of Tirana.

He has a long experience in teaching, research and expertise in agriculture and environment. During his academic and research career has published many peer review research articles, in national and international journals as well several communications and reports, which are related to environmental pollution and remediation, pesticides residues and fate in environment, soil science, land management and evaluation, natural resources and the economics of inputs on agriculture and rural development. The main research interest is the pesticides residues in food and feed, ecotoxicology and environmental fate of pesticides.

Prof. Brahushi has been coordinator or member of several national and international research and education project in the field of environment, agriculture and rural development. He is member of some international and national scientific organization and associations and Editor in chief of Albanian Journal of Agriculture Science (AJAS).