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Biofungicide Trichodex WP

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Abstract**—**Grey mold on grape is caused by the fungus *Botrytis cinerea* Pers. Trichodex WP, a new biofungicide, that contains fungal spores of *Trichoderma harzianum* Rifai, was used for biological control of Grey mold on grape. The efficacy of Trichodex WP has been reported from many experiments. Experiments were carried out in the locality – Banatski Karlovac, on grapevine species – talijanski rizling. The trials were set according to instructions of methods PP1/152(2) and PP1/17(3) , according to a fully randomized block design. Phytotoxicity was estimated by PP methods 1/135(2), the intensity of infection according to Towsend Heuberger , the efficiency by Abbott, the analysis of variance with Duncan test and PP/181(2). Application of Trichodex WP is limited to the first two treatments. Other treatments are performed with the fungicides based on a.i. procymidone, vinclozoline and iprodione.

Keywords**—**Biofungicides, efficacy, grey mold, Trichodex WP.

I. Introduction

DURING the growing period grapevine is affected by varios parasites and pests. One of the most serious among them is *Botrytis cinerea* Pers., the agent of gray mold. The pathogen *Botrytis cinerea* infects leaves, stems, flowers and fruits, causing grey mould, and is responsible for severe losses in many fruit, vegetable and ornamental crops [1].

Botrytis bunch rot caused by fungus *Botryotinia fuckeliana* may be very harmful during humid seasons or in high humidity locations. Botrytis bunch rot is a greater problem for grapevine cultivars with tight clustered, white varieties such as Chardonnay, Riesling, Sauvignon Blanc, Semillon, and red varieties such as Pinot Noir and Baco Noir. A good viticulture practice is the most important for the suppression of *Botrytis* bunch rot in vineyards for organic production, particularly in relation to varieties with a small berry and thin skin, e.g., Pinot Blanc, Pinot Gris or Gewürztraminer.

Effective disease managment usually requires sanitation and other cultural practices to avoid introducing the pathogen, manipulation of environmental conditions to discourage disease development, and fungicide applications to prevent or limit disease spread. Therefore, fungicides traditionally have played an important role in managing leaf, stem, and flower blights in grape production. However, resistance in population of *B. cinerea* to the most commonly used classes of fungicides, the benzimidazoles and dicarboximides, has compromised their efficacy.

Chemical control remains the most commonly employed method to control the disease. Therefore, chemicals alone cannot be relied on to give control of gray mould. It is difficult to spray all surfaces of the plant where infection may occur. The fungicides currently registered are protective types and do not have a systemic action of control. Fungicides only provide a protective barrier to the outside part of the plant that discourages fungal spore development. They do not cure the disease once it has developed. Chemicals used are iprodione, procimidone, pyrimentanil, ciprodinil + fludioxonil. It would not be necessary to exceed 1 or 2 treatments per year, trying to change the products and intervening in presence of initial hotbeds. However, chemical control has undesirable environmental side effects, and may negatively affect pollination, seed set, and fruit formation [2]. Furthermore, fungal pathogen populations may develop resistance, rendering chemical control ineffective [3]-[4]-[5]-[6]-[7]-[8]-[9]-[10].

There has been a strong research and development thoughts in the area of the biological control of plant pathogens [11]-[12]-[13]. Many products and microrganisms have been discovered that discourage the growth and survival of plant pathogens, and these antagonists are now arriving in the marketplace. The organisms tested for effectiveness, are now mass-produced and are processed to ensure shelf life.

Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism, production of inhibitory compounds, light spectral quality [14]-[15]-[16].

The need for a tool that will replace the current fungicides has led to the introduction of biofungicide which are effective in the control of *B. cinerea*. Biocontrol of *Botrytis*-incited diseases has been extensively investigated over the last 50 years. The key microbial genera that have shown greatest potential for *Botrytis* disease control include the filamentous fungi *Trichoderma*, *Gliocladium* and *Ulocladium*, the bacteria *Bacillus* and *Pseudomonas* and the yeasts *Pichia* and *Candida*. Commercial success has been achieved in glasshouse and post-harvest environments where stable environmental conditions allow greater control over the application of the biocontrol agent and expression of its biological activity. Considerable progress has also been made in achieving more consistent biocontrol under field conditions, particularly in vineyards, but

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the complexities of the plant, microbe, environment interaction and its inherent variability will always pose a severe challenge to achieving effective and consistent field biocontrol. In recognising this, current research aims to define more clearly the biological and economic barriers that limit biocontrol efficacy and future research should focus on the strategic integration of biocontrol systems with other cultural, chemical and genetic methods to provide more sustainable disease control.

Fungus *Trichoderma harzianum* is a cosmopolitan species that may be found in the ground. As an antagonist, this fungus suppresses grey mould on the grapevine when applied at the beginning of vegetation. This fungus is not harmful for humans, different mammals and birds. Apart from grapevine, this active substance is applied also for suppressing *Botrytis cinerea* on vegetables and greenhouses [17]. Isolates of *Trichoderma spp*. are known for their ability to control plant pathogens [18]. The first biocontrol agent to be commercialized, registered and used in greenhouse crops and vineyards was isolate T39 of *T. harzianum* (TRICHODEX), which effectively controlled diseases caused by *B. cinerea, Sclerotinia sclerotiorum* and *Cladosporium fulvum* in greenhouse grown tomato and cucumber and in vineyards [17]. On strawberry, *Trichoderma* isolates, and *T. harzianum* T39 in particular, have effectively controlled *B. cinerea* under laboratory and greenhouse conditions [19]-[20]. However, frequent application is necessary. More frequent applications of the T39 isolate, every 2 days, resulted in better control than less frequent applications of every 7 or 10 days [19]. However, frequent application of a biocontrol agent, especially in the field, is costly and labour-intensive, and frequent entry with sprayers may cause mechanical damage to the foliage and fruit. An efficient and inexpensive solution for continuous dissemination of biocontrol agents in greenhouses and in the field is needed.

The aim of this paper is to examine the possibility of application biofungicide Trichodex the control of *B. cinerea* in grapevine.

II. Material and Methods

Trichodex WP (Makhteshim Chemical Works, LTD) is a commercial product containing a natural isolate of *T. harzianum* with fungal mycelium and conidia, minimum 1x109 per gram CFU (Colony Forming Units) of T-39 isolate. It is biofungicide, which is formulated in the form of suspension, and the active component are the conidia and mycelium of fungi *Trichoderma harzianum* (isolate no. T39), whose mechanism of action is based on the antagonistic effect on other fungi education antibiotics.

It is designed to control pathogen that cause gray mold of grape (*Botrytis cinerea*) in combination with products vinklozin, iprodione, procymidone, folpet and alternately: two treatments with Trichodex WP, and two with some of these fungicides.

The product is compatible with active substances on the basis of captan, folpet and TMTD. With treatment begins in full bloom at a dose 2.0 - 4.0 kg / ha or in a concentration between 0.2 to 0.4%. In Table I we can see fungicides which were used in experiments.

TABLE I
FUNGICIDES AND BIOFUNGICIDE

No	Fungicide	Active Substances	Produsers
1	Iprodion (kidan)	260 g/l	"Rhone Poulenc", Lyon
2	Dihlofuanid (euparen)	50%	"Bayer", Leverkusen
3	Tebukonazol (folicur)	250 g/l	"Bayer", Leverkusen
4	Trichodex	conidia, mycelium	"Makhteshim" – Agan, Israel
5	Prosimidon (sumilex)	250 g/l	"Sumithomo", Osaka
6	Tiram (TMTD Župa S-80)	80%	"Župa", Kruševac
7	Vinklozolin (ronilan)	50%	"BASF", Ludwigshafen/RH
8	Folpet (folpet)	50%	"Zorka-ATH and acides", Subotica

The appearance and development of grey mold is followed by the initial appearance and development of the disease on the control variation, as well as through accomplishment of a clear difference between the control and other variations on which biofungicide were applied.

The trials were set in accordance with methods [21]- [22], and the treatment plan was made according to a fully randomized block design. The experiment was conducted in four repetitions on basic plots consisting of 8 trees (1 x 3 m apart), 25 m^2 in total.

For each plot, the number of diseased plants was registered. The symptom severity was estimated for each plant and all the plants were grouped into infection classes, calculating the frequencies. The tomato gray mould severity was rated according the following evaluation scale: 0 = no symptoms, healthy plants; 1 = less 10% of infected stems and leaves with lesions for no more 10% of shoot length; 2 = less 20% of infected stems and leaves with lesions for no more 20% of shoot length; 3 = less 40% of infected stems and leaves with lesions for no more 50% of shoot length; 4 = less 80% of infected stems and leaves with lesions for no more 80% of shoot length; 5 = infected areas covering whole the stems and leaves causing wilting and death of plants.

Regarding the method of application and amount of water per unit surface, the fungicides were applied using the backstroke sprayer “Solo”; with a consumption of 1000 L/ha of water.

The intensity of disease was assessed by the method of EPPO: Guideline for the efficacy evaluation of fungicides – *Botrytis cinerea*, no. [23]. Phytotoxicity was estimated according to instructions of methods [21]. Severity and diffusion of infection were obtained by resorting to the

McKinney index [24]. The McKinney index (1) was obtained by using the following equations:

$$I=\Sigma\,(f\,v):N x X \quad x\,100 \qquad (1)$$

where:
f = infection class frequencies
v = number of plants of each class
N = total of observed plants
X = highest value of the evaluation scale.

Data processing was performed using standard statistical methods (intensity of infection according to Towsend-Heuberger [25], the efficiency according to Abbott [26], analysis of variance according to Duncan test [27] and methods [28]. The differences of the disease intensity were evaluated by the analysis of variance and LSD-test.

III. Results

A. Results of Physical and Chemical Testing

Active ingredient: spores and mycelial Trichoderma harzianum. Declared content : 10^{10} spores/kg. Appearance: gray-green powder. Loss on drying at 105^{0} C: 2.32% Receipt of moisture in the air relative humidity 95%: 9.53%.The content of particles larger than 0044 mm: 3.1% . Wettability with stirring: 13 seconds. Suspensibility particles in standard hard water: 67.00%

B. Biological Testings

Parasite: *Botrytis cinerea*
Locality: Banatski Karlovac
Grape variety: talijanski rizling
The way of breeding: goatherd modified system <3,2 x 1,5 (0,5)>
Plot size: 8 vines or 22.4 m2
No of repetition: 3

Date of treatment:		Growth stages of development:
I	03.07.2012.	the end of flowering
II	12.07.2012.	bunch closure
III	22.08.2012.	occurrence hinge
IV	09.09.2012.	21 before harvest

Treatment was done with atomizer with water consumption 900 l / ha. For protection against Plasmopara viticola Champion was applied in concentrations 0.4% and of Uncinula necator was used Tilt 250 EC concentration of 0.015%.

The experiment estimation: First estimation: 21.08.2012. (Table II) Second estimation: 01.10.2012.(Table III).

TABLE II
First Estimation of Fungicides and Biofungicide Efficacy on Grape

No	Fungicide	Doses (kg-l/ha)	Infection (%)	Efficacy (%)	Standard (Sumilex 25)
1	Kidan	2.0	1.91 ab	78.00	85.28
2	Kidan	3.0	0.48 a	94.46	103.28
3	Euparen	4.0	3.10 abc	64.24	70.24
4	Folicur EC 250	0.04	3.56 abc	58.96	64.47
5	Folicur EC 250 + Euparen WP 50	0.025+0.025	7.04 bcd	18.73	20.48
6	Folicur EC 250 + Euparen WP 50	0.04+0.25	5.89 abc	32.04	35.03
7	Trichodex WP	2.0	5.85 abcd	32.46	35.49
8	Trichodex WP	4.0	2.30 ab	73.50	80.36
9	Sumilex FL (I) + TMTD (II)	2.0+4.0	1.78 ab	79.50	86.92
10	Sumilex 25 FL	2.0	0.74 a	91.46	100.00
11	Untreated	-	8.67 cd	0.00	0.00
	LSD005=			**4.96**	

TABLE III
Second Estimation of Fungicides and Biofungicide Efficacy on Grape

No	Fungicide	Doses (kg-l/ha)	Infection (%)	Efficacy (%)	Standard (Sumilex 25)
1	Kidan	2.0	4.47 ab	89.28	103.05
2	Kidan	3.0	2.03 a	95.12	109.79
3	Euparen	4.0	14.97 bc	64.08	73.96
4	Folicur EC 250	0.04	20.63 cd	50.48	58.26
5	Folicur EC 250 + Euparen WP 50	0.025+0.025	11.83 abc	71.60	82.64
6	Folicur EC 250 + Euparen WP 50	0.04+0.25	12.67 abc	69.60	80.33
7	Trichodex WP	2.0	27.43 d	34.16	39.43
8	Trichodex WP	4.0	21.93 cd	47.36	54.66
9	Trichodex WP (I,II) + Sumilex FL (III,IV)	2.0+2.0	21.60 cd	48.16	55.59
10	Trichodex WP (I,II) + Sumilex FL (III,IV)	4.0+2.0	4.00 ab	90.40	104.34
11	Sumilex FL (I,IV) + TMTD (II,III)	2.0+4.0	3.90 ab	90.64	104.62
12	Sumilex 25 FL	2.0	5.57 ab	86.64	100.00
13	Untreated	-	41.67e	0.00	0.00
	LSD005=			**11.50**	

Biological evaluation of fungicides were made in Banatski Karlovac, the vine varieties Talijanski Rizlng. During tests were carried out by two estimation of experiments. After the first two treatments (03.07., And 12.07.), due to a previously realized infection, made the first estimation 21.08.2012, when the grape were infected of 6.6%. In such circumstances, higher doses of Trichodex WP 4.0 kg / ha, compared to a lower dose of 2.0 kg / ha, was also effective and were at the similar level of a standard fungicide. After four treatments during infection of 41.6%, efficiency of Trichodex was low (43.1% at the lower dose of 2.0 kg / ha and 47.3% at the higher dose application of 4.0 kg / ha).

When combined application, the first two treatments (growth stages the end of flowering and closing cluster) at a dose of Trichodex WP 2.0 kg / ha, and the next two (single cluster of ripe berries and before to harvest) Sumilex FL 25 at a dose 2.0 l / ha it was obtained an efficiency of 47.3%. It was at the level of efficiency of the Trichodexa WP 4.0 kg / ha in the first two treatments and the normal dose of Sumilex FL 25 for the other two treatments,and it was high efficiency of 90.4%. This efficiency is at the same level of the efficiency combinations Sumilex 25 FL (I and IV treatment) and TMDT (II and III treatment), but better than we used Sumilex 25 FL alone in program protection with a four-dose treatment of 2.0 l / ha.

IV. Conclusion

Taking into account the results of tests, can be reliably determined, that the combined application of higher doses of Trichodexa WP (4.0 kg / ha) for the first two treatments (end of flowering and closing the cluster) and normal doses of the Sumlex 25FL (at an appearance before the hinge and harvesting), can provide high efficiency, even higher than in the application of the Sumlex 25 FL with four treatment. The practice of such fungicides is justified, because it is a biological product whose introduction into practice to reduce the number of treatments with chemical preparations and reduces the risk of occurrence of resistant parasites.

Application of Trichodex WP is limited to the first two treatments. Four of them is commonly used to protect grapes from this disease at the stage of the end of fowering and closing the cluster. Other treatments are performed with the fungicides based on a.i. procymidone (product Sumilex), vinclozoline (product Ronilan) and iprodione (products Kidan and Rovral).

Application dose of Trichodex WP are 4.0 kg/ha or in a concentration 0.4%, or 40 g/100m2 or 40 g/10 l of water. Trichodex WP is compatible with fungicides based on a.i. Faltan, Captan and TMDT.

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