ISSN 0494-3244

Тутун/Tobacco,Vol. 65, N^0 1-6, 23-29, 2015

UDC: 633.71-248.843:632.937
632.937:633.71-248.843
Original Scientific paper

SOME ECOLOGICALLY FRIENDLY METHODS FOR CONTROL OF PATHOGENIC FUNGUS *ALTERNARIA ALTERNATA* ON TOBACCO

Biljana Gveroska

Scientific Tobacco Institute-Prilep, R. Macedonia

e-mail: gveroska@t-home.mk

ABSTRACT

Contemporary trends in plant protection are directed to use ecological sustainable methods that will make possible limited use of chemical pesticides.
The influence of *Trichoderma harzianum* (pure culture) and a bioproduct Trilogy 70 EK (1%) were investigated vs. the contact fungicide Antracol WP-70 (0.25%).
Some variants were involved: the treatment several times during the vegatation, 48 hours before inoculation and immediately after inoculation, while the fungicide, only just after inoculation.
The lowest intensity of the disease was observed in the variant with treatment of plants with *T. harzianum* several times during vegetation. These results are better than those of fungicides Antracol WP-70.
A bioproduct Trilogy 70 EK (1%) has shown more weakly results, but the same as Antracol WP-70.
Biological method is a good alternative to chemical method of control the parasitic fungus *Alternaria alternata*, the causing agent of the brown spot on tobacco

Key words: *Alternaria alternata***,** biocontrol, biocontrol agent, biopreparate

НЕКОИ ЕКОЛОШКИ ПРИФАТЛИВИ МЕТОДИ ЗА СУЗБИВАЊЕ НА ПАТОГЕНАТА ГАБА *ALTERNARIA ALTERNATA* НА ТУТУНОТ

Современите трендови во заштитата на растенијата се насочени кон примена на еколошки прифатливи методи кои ќе овозможат намалување на стандардните хемиски средства.
Испитувано беше влијанието на *Trichoderma harzianum* (чиста култура) и биопрепаратот Trilogy 70 EK (1%), наспроти контактниот фунгицид Antracol WP-70 (0,25%).
Вклучени беа неколку варијанти: третирање неколку пати во текот на вегатацијата, 48 часа пред инокулацијата и непосредно по инокулацијата, додека кај фунгицидот- само по инокулација.
Најмал интензитет на напад од болеста беше констатиран кај варијантата каде се вршеше третирање на растенијата со *T. harzianum* неколку пати во текот на вегетацијата. Овие резултати се подобри во споредба со тие кај фунгицидот Antracol WP-70.
Биопрепаратот Trilogy 70 EK (1%) даде нешто послаби резлтати, но исти како и Antracol WP-70.
Биолошкиот метод претставува добра алтернатива за хемискиот начин на сузбивање на паразитната габа *Alternaria alternata*, предизвикувач на кафената дамкавост кај тутунот

Клучни зборови: *Alternaria alternata***,** биолошка борба, биоконтролен агенс, биопрепарат

INTRODUCTION

Contemporary trends in plant protection are focused on application of environmentally safe methods that would allow reduce standard chemical pesticides. Integral Protection (IPM), despite preventive measures include biological control as an effective, modern method of protection that ensures a healthy and clean environment.

Biological control of pathogens is based not only on application of commercial products and bioagents, but also on their multiplication. Lately, there has been increasing interest in biochemical-based products and antagonistic relations among microorganisms.

Considering the fact that biological control does not offer general solution, it develops separately from crop to crop and from pathogen to pathogen.

The causing agent of the brown spot disease is a parasitic fungus *Alternaria alternata*. There are effective chemical agents for its control. But, efforts to reduce the number of treatments, in periods with increased possibility of its occurrence, implies the application of certain ecological methods of preventing the disease.

Fungi of the genus *Trichoderma* are the most powerful biocontrol agents. There are numerous mechanisms involved in biocontrol against plant pathogens (Harman et al., 2004; Harman, 2006). Biological control of *Trichoderma* is confirmed in more pathogens on tobacco (Gveroska, 2013 a). This is the strongest in soil pathogens. Hence, these agents can be used in control of the the damping off disease on tobacco seedling (Gveroska, 2013 b). However, their application in control of foliar pathogens also give excellent results. *T. harzianum* is a biocontrol agent used in control of *A. alternata* (Monte, 2001; Roco and Perez, 2001; Sempere and Santamarina, 2007).

Sustainable Agricultural Production strives to apply other bioproducts, based on plant extracts or other biochemical components.

There are many essential oils that show insecticidal and fungicidal activity to suppress important plant pathogens (Isman, 2000). Their potential for crop protection and positive impact on healthy environment ensure a commercialization of these biopesticides.

There are proven antifungal components in Neem oil (*Azadirachta indica*) - it is a mixture of tetranortriterpenoids that the antifungal activity express in the highest degree as mixture, which suggess on additional / synergistic effect (Govindachari et al., 1998). Azadirachtin is the most powerful tetranotriterpenoid of the Neem tree and by toxicological tests, use as a biopesticide, dose of 500, 1000 and 1500 mg / kg / day for 90 days caused no sign of toxicity, mortality, changes in tissue weight or pathological changes in blood parameters. The highest dose (1500 mg / kg) can be taken as the basal dose to calculate the safe limits (Raizada et al., 2001).

Data presented defined the aim of our research - to examine some alternative, ecologically friendly methods of preventing the tobacco from the brown spot disease. That is, to examine the effect of *Trichoderma harzianum* and bioproduct Trilogy 70 EK on *Alternaria alternata* comparing them with chemical control - contact chemical fungicide Antracol WP-70.

MATERIAL AND METHODS

Investigations were made with variety P 23, grown in usual way in biological laboratory. Twenty plants per each variant and the check were planted .

Fresh tobacco leaves with expressed symptoms of Brown spot disease were used for inoculation.

Inoculation with the pathogen was

performed at the end of the growing season, as the most suitable period for infection and disease occurrence.

Trichoderma harzianum was used as **biocontrol agent** because of its remarkable reducing effect on most tobacco pathogens, including *A. alternata*.

Suspension of pure culture was used for plant treatment (spraying of plants) -2 Petry dishes in 250 ml distiled water.

Bioproduct Trilogy 70 EK is an extract of Neem oil, obtained from the Neem tree (Azadirachta indica) which grows in India. It was applied in the recommended rate of 1%.

Several variants were included in the biocontrol agent and in bioproduct:

- 3 treatments during the growing season
- 1 treatment 48 hours prior to inoculation
- 1 treatment immediately after inoculation

According the aim of investigations, the **contact fungicide** Antracol WP-70 (propineb 70%) in the rate of 0.25% was also included for chemical treatment, immediately after inoculation.

The test plants were sprayed only with water and treated in the same way as other plants. Plants were covered with polyethilene bags and kept 10 days in noncontrolled conditions in biological laboratory. Estimation was made on detached tobacco leaves, categorized on a scale from 0 to 5:

0 - no symptoms of disease
1 - 1 spot on the leaf
2 - 2-5 spots
3 - 6 -10 spots
4 - 11 - 25 spots
5 - over 50% of leaf surface infected

Disease intensity was calculated using the formula of *McKinney:*

$$i = \frac{\sum n \cdot k}{N \cdot K} x100$$

Where:

n - leaf number in corresponding category
k - category
N - total number of analyzed leaves
K - total number of categories

RESULTS AND DISCUSSION

Brown spot disease is manifested by the appearance of brown spots on the leaf surface, by which it was named (Fig. 1, 2). Its appearance depends on weather conditions and the proper agrotechnical operations, primarily timely harvest.

It appears first on the lower and spreads to the upper leaves and attacks the flower buds and shoots, too (Fig. 3).

The causing agent of the disease is pathogenic fungus *A. alternata*. In artificial inoculation with the pathogen we can get similar symptoms (Ph. 4). Artificial inoculation is a good way of testing the intensity of attack by the disease. After a period of incubation and categorizing the leaves in the corresponding category of the scale, intensity of attack for each variant is calculated (Table 1 and 2).

Ph. 1 Brown spot disease in tobacco

Ph. 2 Brown spot disease in oriental tobacco leaves

Ph. 3 Spreading of disease from the lower to upper leaves

Ph. 4 Symptoms of disease- artificial inoculation

The contact chemical fungicide Antracol WP-70 showed weak results in control of the pathogen *A. alternata*.

Amomg variants with the biocontrol agent, the lowest intensity of attack by the disease (in both years of testing) is performed in the variant were plants were treated with *T. harzianum* several times during vegetation (19.31 and 21.09%). What is important is that these results are better than those of the contact fungicide Antracol WP-70 (Table 1 and 2).

According to Quarles (2000), the beneficial microorganisms are effective only at high relative humidity (often 60 to 80%) and their effect is limited in biolaboratories or areas with low relative humidity during the vegetation season. The bacteria are less sensitive to moisture and therefore they have a wide range of application. These data justify multiple application of the fungus *T. harzianum*.

The multiple application of this biocontrol agent enables its rapid multiplication. On the other hand, it is possible constantly acting and development of the three main mechanisms of biocontrol - competition, antibiosis, mycoparasitism etc. Also, development of other biocontrol mechanisms that act synergistically, which certainly influences on the such little intensity of attack by disease.

Most biofungicides are applying as a preventive measure and act as a barrier between the pathogenic fungus and plant tissues and it is necessary to apply before the emergence of new leaves or sensitive part of the plant, at the first signs of the disease or in the presence of favorable climatic conditions for the emergence of the disease (Quarles, 2000). In the constant presence of the biocontrol agent with multiple treatment, there is a covering of all moments in preventing the disease.

Treating plants with T. harzianum 48 hours before and immediately after inoculation gave significantly poorer results (Table 1 and 2). The average values of disease intensity in these two variants are - 44.16 and 42,55% (Graph 1). In this case there are favorable conditions for the growth of the pathogen and the host plant and the time of 48 hours is very little to release certain substances that limit the growth of the pathogen. Similar findings were presented by the BPIA (2014). Fungi require specific environmental conditions to proliferate and their means of affecting the target organisms are diverse.

Table 1. The influence of biological and chemical way for control of *Alternaria alternata* (I st year)

Variant	Total number of leaves	% of infecte leaves	Intensity of attack (%)
Check Ø	676	98,52	58,48
Antracol WP-70 + *A. alternata*	210	65,71	31,43
T. harzianum (several times)+ *A. alternata*	359	58,50	19,31
T. harzianum (before 48 hours) + *A. alternata*	537	81,19	42,61
T. harzianum.+ *A. alternata*	520	78,65	40,51
Trilogy 70 EK (several times) + *A. alternata*	360	85,56	46,94
Trilogy 70 EK (before 48 hours) + *A. alternata*	333	75,08	42,54
Trilogy 70 EK + *A. alternata*	417	71,94	30,77

Neem oil has an expressive fungicidal properties and can be used to effective control of the leaf pathogens that cause spots. Among the most fungi that act, there is *A. tenuis* (Govindachari et al., 1998). The content of Azadirachtin affects the fungus and its spreading among susceptible plants. For effective control of the brown spot disease, it needs spraying once in 10 days (Teaser, 2015). According to Bozukov (2005) Trilogy 70 EK is bioproduct with high efficiency and prolonged effect on supression the tobacco pathogens - *Peronospora tabacina* and *Erysiphe cichoracearum*. But in our research these treatments had failed.

Neem oil affects mitochondrial oxidative phosphorylation, which inhibit the respiratory chain (Biswas et al., 2002). The mechanism of action is thus causes strong drying / suffocating of the pathogen. Therefore, the greatest effect has applied immediately after inoculation (average intensity of attack - 31,83% (Graph 1).

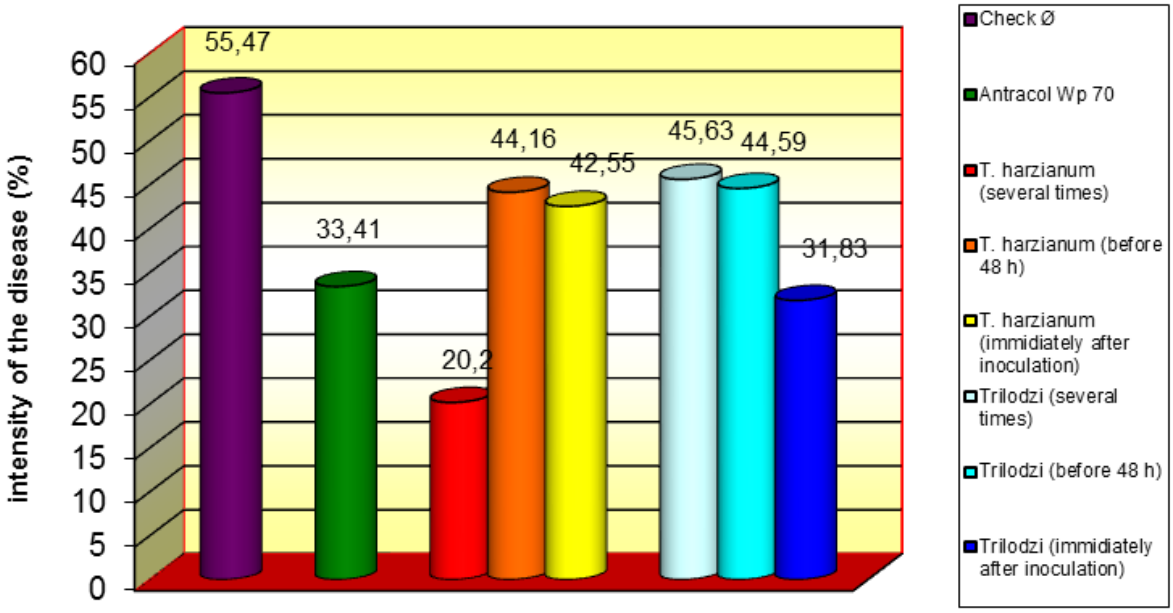
Table 2. The influence of biological and chemical way for control of *Alternaria alternata* (IInd year)

Variant	Total number of leaves	% of infecte leaves	Intensity of attack (%)
Контрола Ø	570	87,72	52,46
Antracol WP-70 + *A. alternata*	195	64,10	35,38
T. harzianum (several times)+ *A. alternata*	331	58,01	21,09
T. harzianum (before 48 hours) + *A. alternata*	350	86,00	45,71
T. harzianum.+ *A. alternata*	487	85,22	44,59
Trilogy 70 EK (several times) + *A. alternata*	252	85,56	44,31

Trilogy 70 EK (before 48 hours) + *A. alternata*	357	82,54	46,63
Trilogy 70 EK + *A. alternata*	335	73,13	32,89

The other two variants with Trilogy 70 EK gave poor results (averages - 45.63 and 44.59%). Its effect has the bigest expression on the surface of leaves. According to the declaration for Neem oil (Annonimus, 2015), thorough coverage of the leaves provides good protection because prevents contact and adhesion of the fungus on the leaf. This means that aplication several times or 48 hours before inoculation can not prevent the penetration of this endotrophic fungus, which results in higher values of intensity of attack by disease.

Graph 1. Effect of use the alternative methods in control of the brown spot disease (average values of two year investigations)



CONCLUSIONS

- There is a possibility to apply ecologically frienndly methods in the control of pathogenic fungus *Alternaria alternata*.
- The best results in our investigation were obtained with the variant treated with *Trichoderma. harzianum* several times during the growing season.
- These results were better compared to chemical control with the contact fungicide Antracol WP-70.
- Bioproduct Trilogy 70 EK (1%) gave somewhat poorer results, but its best activity was achieved when applied immediately after inoculation (as well as a chemical fungicide).
- Since the results obtained with this bioproduct were identical to those of Antracol WP-70, it can be recommended as a substitute for chemical-based products.
- Comparing the biological way of protection by chemical, biological is a good alternative to the use of chemical fungicides in the control of *Alternaria alternata*, the causing agent of Brown spot disease on tobacco.
- The application of these methods, certainly

supported by the facts on the effectiveness of this way of protection, on the one hand, and eligibility to human health, on the other, would increase the ecologically sense of producers at a higher level.

REFFERENCES

1. Annonimus, 2015. Triple action of Neem Oil.
   http://www.southernag.com/docs/labels_msds/Neem-pt.pdf
2. Bozukov H., 2002. Investigation on action of biopesticide Trilogy 70 Ek against agent of economy important mycosis on tobacco. Тутун / Tobacco, Vol. 52, No 7-8, pp. 231- 233.
3. BPIA Biopesticide Industry Alliance, 2014. Advancing Knowledge About Biopesticides: Biopesticides Offer Multiple Benefits for Agricultural Dealers and Consultants. Source: Guide to Understanding and Evaluating Biorational Products. 2006. Libertyville, IL: Valent BioSciences Corporation. Valent BioSciences Corporation.
4. Govindachari T.R., Suresh G., Gopalakrishan G., Banumathy B., Masilamani S., 1998. Identification of antifungal compounds from the seed oil of *Azadirachta indica.* Phytoparasitica Vol. 26, No 2, pp.109-116.
5. Gveroska B. 2013 a. In vitro biocontrol activity of *Trichoderma harzianum* against some pathogenic fungi on tobacco. Food and Environmental safety, Volume XII, Issue 1, pp. 95-104, Stefan Cell Mare University of Suceava, Romania.
6. Gveroska B. 2013 b. Relationships of *Trichoderma* spp. quantity in soil to reducing the damping off in tobacco seedlings. *Bulg. J. Agric. Sci.,* 19: 666-674.
7. Harman, G. E, Howell, C.R.,. Viterbo, A, Chet, I., Lorito, M., 2004. *Trichoderma* species – opportunistic, avirulent plant symbionts, Nature Review Microbiology, Vol 2: 43-56.
8. Harman, G. E., 2006. Overview of Mechanisms and Uses of *Trichoderma* spp., Phytopathology 96: 190-194.
9. Isman M.B., 2000. Plant essential oils for pest and disease management. Crop protection, No. 5-10, pp. 603-608.
10. Monte E., 2001. Understanding Trichoderma: between biotechnology and microbial ecology, Int. Microbiol, No. 4, pp. 1-4.
11. Quarles W., 2000. Least – Toxic Controls of Plant Diseases. Brooklyn Botanic Garden, September.
    http:/www.bbg.org/gardening/article/least-toxic_controls_of_plant_diseases
12. Raisada RB., Srivastava MK., Kaushai RA., Singh RP., 2001. Azadirachtin, a neem biopesticide: subchronic toxicity assessment in rats. Food Chem Toxicol, Vol. 39, No. 5, pp. 477-483.
13. Roco A., Pérez Érez L.M., 2001. In vitro biocontrol activity of *Trichoderma harzianum* on *A.alternata* in the presence of growth regulators, Plant Biotechnology, 4 (2).
14. Sempere F., Santamarina M.P., 2007. In vitro biocontrol analysis of *Alternaria alternata* (Fr.) Keissler under different environmental conditions, Mycopathologia, No. 163, pp. 183-190.
15. Teaser A., 2015. Leaf spot disease control using Neem oil. Plasma Neem, EPA - Registered Cold Pressed.
    http:/www.plasmaneem.com