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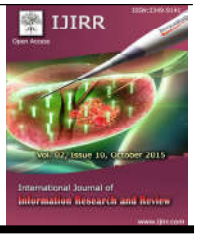

Research Article

ASSESSMENT OF EXTRACTS OF PLANTS ON THE GROWTH AND SPORULATION OF THE AGENT OF BROWN LEAF SPOTS ON TOMATOES (ALTERNARIA SOLANI NEES)

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ABSTRACT

The present research is conducted in 2015, in the Phytopathology laboratory of the University of Shumen – Shumen, Bulgaria. During the research was tested the effect of water extracts from nettle (*Urtyca dioica*), poplar (*Populus ssp*), sour dock (Rumex obsitifolius) and mustard (Sinapis alba)**,** on the growth of the agent of brown leaf spots on tomatoes – *Alternaria solani Nees.* The effect of the plant extracts is compared with the effect of the fungicide Rydomil Gold(Mancozeb 640g./kg and Metalaxyl-M 40g./kg) in the approved disease control dose, on the growth and germination of the pathogen's spores. The plant extracts from poplar (leaves), in dose 1.5ml/100 ml., nutrient medium; nettle (leaves) in dose 2.0ml/100 ml., nutrient medium; mustard (leaves) in dose 2.0ml/100 ml., nutrient medium, inhibit the growth of the agent of brown leaf spots – *Alternaria solani Nees.,* during its cultivation on potato dextrose agar.

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INTRODUCTION

Together with the potato mildew (*Phythophtora infestans*), the brown leaf spots (*Alternaria solani Nees*) are the most common disease in Bulgaria, that spread on tomatoes, cultivated in open field. (Bacharov, 1988). In contrast with the rest of the tomato leaf spots, this disease forms symptoms on the stems, petioles, flower buds, and fruits, which makes it highly injurious. This disease damages not only the tomatoes, but also the different culture and wild plants from the family *Solanaceae.* The disease damages potatoes, peppers, and eggplants, which is a risk of accumulating infections in the plant areas, and risk of spreading the infection during the critical phases of the culture development (Polyxenova, 2001). Another precondition for the development of brown leaf spots in the tomato plantations is the agent's (*Alternaria solani Nees*) high resistance to the unfavorable environmental conditions. The disease agent retains its infectious nature in wide range of environmental acidity; it develops equally well in acid, neutral, and slightly alkaline environments, and does not have any specific requirement for the nutrient medium.

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The successful disease control includes preventive agro – technical measures, and protective sprays. According to Pattel *et al*., (2005), the fungicides based on copper and mancozeb are good preventive resourse for inhibiting the mycelium development and sporulation of *Alternaria solani Nees*. They control the development of alternariosis on tomatoes, but without taking into account the possible risk for the environment. The conclusion, drawn from the intense agricultural practices, is that they are not capable to ensure ecologically clean and healthy food for the consumer, due to their instability., especially regarding the production, meant for direct consumption, as for example the tomatoes (Karov, 2006).

The usage of plant products is an essential part of the contemporary plant protection systems, which are adapted with the environment and are safe for humans (for example the biological agriculture). These are extracts, essential oil, flavonoids, etc, which improve the physiological status and ensure the satisfactorily protection from diseases, and preserve the natural connection in the agrobiocenosises. (Ujvary, 2002; Jakimov *et al*., 2008). The substances, synthesized from higher plants and toxic against the disease agents, are often applied in the form of plant extracts and infusions for preparation of garden sprays, or are spread as a dust; whereupon all plant

waste are used (Stancheva, 2009). A water extract from nettle (*Urtyca dioica*), in dose 2.5ml/100 ml., nutrient medium, and essential tea tree oil (*Melaleuca alternifolia*), in dose 0.5ml/100 ml., nutrient medium, satisfactorily inhibit the agent of alternariosis in the medical plant stevia (*Stevia rebaudiana Bertoni*)- *Alternaria stevae*(Georgieva-Andreeva *et al*., 2010).
In Bulgaria, there are natural preconditions for the development of biological agriculture as an alternative of the highly intense production of fresh fruit and vegetables, including tomatoes. According to data of the Ministry of Environment and Water, over 80% from the agriculture fields are suitable for biological production, and, with a few exceptions, there can be cultivated tomatoes as well (Panaiotov, 2004). The ambition of the manufacturers of biological tomato fruits is clean environment and production, as a result. The aim of the research is to test the inhibiting effect of the water extracts from four plants for the agent of brown leaf spots on the tomatoes – *Alternaria solani Nees.,* and to evaluate the potential control possibilities.

MATERIALS AND METHODS

Isolation and preservation of *Alternaria solani Nes*

This pathogen is isolated from diseased plants from a field in Pamukchi village – district Shumen. The isolation samples came from plants, re – harvested in 2015, from leaves and fruits with clearly pronounced symptoms. The isolation is performed in the Phytopathology laboratory of the University of Shumen – Shumen, by the know phtyopathology methods, on environment of potato dextrose agar (PDA). The preservation of the isolates is performed in thermostat, at a temperature of 22^0- 24^0 (Popkova, 1987).

In vitro testing the effect of extracts of plants on the growth and sporulation of the agent of brown leaf spots on tomatoes (*Alternaria solani Nes.*)

In 2015., in the Phytopathology laboratory of the University of Shumen – Shumen, was tested the biological effect of extracts from four kinds of higher plants in different concentration, added in the nutrient medium for cultivating the agent of brown leaf spots – *Alternaria solani Nes*. The extracts are added in ratio drugs/ water – 1/1. The tests include the following variants, and the tested concentrations are in accordance with the recommendations (Stancheva, 2009; Georgieva-Andreeva and Tanova, 2010):

Variant 1: Nettle (*Urtyca dioica*) – leaves, in dose 2ml for 100 ml., nutrient medium.

Variant 2: poplar (*Populus ssp*.) – leaves, in dose 1.5 ml for 100 ml., nutrient medium.

Variant 3: Sour dock (Rumex obsitifolius) – leaves, in concentration 2ml for 100 ml., nutrient medium.

Variant 4: Mustard **(**Sinapis alba**)** – leaves and stems, in dose 2ml for 100 ml., nutrient medium.

Variant 5: (Standard) – Rydomil Gold in dose 0,002ml/100 ml., nutrient medium (0.025% working solution).

Variant 6: (Control) – pure nutrient medium.

The laboratory experiment includes two test and each one of them includes the abovementioned identical variants of the tested resources, introduced on hard nutrient medium (PDA), in the correspondent concentrations.

Test 1

On nutrient medium of PDA, from each variants, is made a culture from seven – days daily culture on potato dextrose agar on the methods of the agar block (Popkova, 1987). The cultures are cultivated in thermostat, at temperatures of 24-26 °C, throughout 7 days. During this period, reports for determine the mycelium growth were made twice, through measuring the mycelium stocks, and calculating the speed of the mycelium growth.

Test 2

Mycelium- spore suspension was prepared by the Drygalski method (Popkova, 1987) – from culture of monospore cultivation on potato dextrose agar. A diluted suspension was used, with titre 25 p. spores/1 visual microscopic field (25.10^3 p. Spores for 1 ml suspension). It was transferred in solutions from the tested extracts in the correspondent doses. Thus prepared cultures were put in thermostat at temperatures of 26-28^0 C (± 2^0 C), for 48 h. The germination of the spores is reported after microscoping the cultures (10 microscopic preparations per variant), after 24 h, 36 h and 48 h after the incubation. The data processing of the received results from the researches is performed by the correspondent statistic method (Zaprianov, 1987). After the statistic processings, the results are presented in tables.

RESULTS AND DISCUSSION

The results from the laboratory tests are presented in Table 1. According to these results, most effective for the growth of the cultivations are the extracts from nettle – 2 ml/100 ml., nutrient medium; poplar – 1.5 ml/100 ml., nutrient medium, and mustard – 2ml/100 ml., nutrient medium. Regarding the diameter of the mycelium colony, the highest inhibition is caused by the addition of poplar extract – calculated colony diameter – 3.76 cm, with4.80 cm for the cultivated variant without additions. The inhibition of the control variant is 21.6%**.**

This effect is followed by the variant with nettle extract – colony diameter – 4.56 cm, and inhibition effect for the control variant – 5.21%); mustard extract – colony diameter – 4.79 cm and inhibition effect for the control variant – 1.05**%**). The sour dock extract in the tested dose did not show any effect regarding the colony diameter. The data for Table 1 undoubtedly show that the inhibition of the mycelium growth with addition of plant extracts in the cultivation medium, in the variant with nettle and poplar extracts, is equal to the inhibition of the fungicide Rydomil Gold, in dose 0.002ml/ 100ml., nutrient medium – 0.025% work solution.

This is one of the most widely used fungicides for pathogen control in most of the cultures, which are his hosts. For the variant with Rydomil Gold, the calculated colony diameter is 3.70 cm, and represents 77.08% from the diameter on the control medium (clean medium).

Table .1 The effect of plant extracts on Alternaria *solani Nes.*

Variants	Mycelium development				Dynamics of spores' germination					
	Colony diameter	Relativity	Growth speed	Relativity	At the 24th hour		At the 36th hour		At the 48th hour	
	cm	%	cm/24h	%	%	Relativity %	%	Relativity %	%	Relativity %
Nettle -*Urtyca dioica* 2 ml/100ml medium	4.56***	94.79	1,06	84.80	38.0	98	54.2	98.6	65.0	92.9
Poplar -Populus ssp. 1.5 ml/100ml medium	3.76***	78.40	1,03	82.4	37,5	97	53.0	96.4	63.5	90.7
Sour dock -*Rumex obsitifolius* 2 ml/100ml medium	4.90	102.9	1,28	102.4	39.0	101	55.0	100	68.9	98.4
Mustard -Sinapis alba 2 ml/100 ml medium	4. 79	98.95	0,90	72,00	38.7	100	55.2	101	70.1	100
Control – clean medium	4.80	100.00	1,25	100,00	38.7	100	55.0	100	70.0	100
Rydomil Gold 0,002 ml/100 ml medium	3.70***	77.08	0.85	68.00	23.1	60	25.5	46.4	26.5	37.8
GD 0.1%	0.16	4.8	0.10	5,3	0,19		0.16		0,19	
P%	0,67		0,71		0,94		0,82		0,84	

The inhibition effect is one of the highest - 22.92%, and is closest to the effect, caused by the poplar extract. The reported results that show better inhibition effect of the tested extracts. Regarding the speed of the mycelium growth, with few exceptions, the effects of the tested extracts recurs. The highest inhibition effect is reported for the fungicide Rydomil Gold – 68% for the control variant, followed by the mustard – 72% for the control variant, poplar – 82.4% for the control variant, and nettle – 84.8%. The spores' germination for all variant occurs maximum after the 36th hour. The inhibition of the spores' germination is reported even before the 24th hour. The highest inhibition effect is caused by the fungicide Rydomil Gold – 60% for the control variant, followed by the poplar – 97% for the control variant, and nettle – 98%. After the 36th hour, thus described effect is slightly intensified regarding the same variants.

After the 48th hour, to the variants that inhibit the spores' germination is added the variant with sour dock extract, in the cultivation medium. The addition of sour dock extract does not affect the mycelium growth, but inhibits the spores' germination, which can be observed after the 48th hour. The inhibition amounts to 98.4% for the control variant, which, after the 48th hour, ranks after the fungicide Rydomil Gold – 37.8 %, poplar – 90.7%, and nettle - 92.9%. The lowest effect regarding the mycelium growth and spores' germination is reported for the mustard extract. The usage of plant extracts or infusions (water/ leaves 1/1) from poplar and nettle in the tested concentrations is advisable as a control resource for the tomato brown leaf spots in small areas, due to the ecological effect of these resources and the production aims. It is necessary to take into account the fact that these resources cannot substitute, at this stage, the recommended contact resources, mainly due to their instability in the nature environment (Stantcheva, 2009). Their application must be regulated and directed mostly towards the protection of the fruits in the cases of late infections.

Conclusions

The plant extracts from poplar (leaves), in dose 1.5ml/100 ml. nutrient medium; nettle (leaves), in dose 2.0ml/100 ml. nutrient medium ; mustard (leaves), in dose 2.0ml/100 ml., nutrient medium, inhibit the growth of the agent of brown leaf spots – *Alternaria solani Nees.*

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