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The Effectiveness of Tobacco Oil Fungicidal Preparation Towards Powdery Mildew of Wheat (*Blumeria graminis f. sp. tritici*)

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Abstract: The pesticide formulation prepared as "ready to be used plant protection product" on the basis of tobacco oil was created and tested in two years field trials against powdery mildew of wheat (*Blumeria graminis f. sp. tritici*). The results showed strong fungicidal effect without any phytotoxic activities (chemotherapeutic coefficient = 10) onto treated plants. This indicates the future development of this pesticide formulation as effective, natural friendly and cheap product for plant protection, which can be used both in commercial and organic agriculture.

Keywords: Fungicides, natural fungicide, organic agriculture, pesticides, powdery mildew of wheat, tobacco.

INTRODUCTION

The extracts prepared from plants by using different extraction agents for the purposes of pest management are fairly popular since ancient times. During the recent years with increasing popularity of the organic agriculture there is an increased interest for pesticides made by natural recourses and in particular - plant extracts [1 - 3]. However, most of the researches in this area are for application of the extracts and biologically active substances as insecticides [4 - 6]. Although there are more and more investigations which reveal the promising effectiveness of such kind of products towards the other type of agricultural pests: such as mollusks [7 - 9]; nematodes [10, 11] and plant pathogens [12 - 14].

Tobacco plant and pesticide formulations made by it are famous for a long time ago both in an organic and commercial agriculture [15, 16]. However, although that as culture this plant is extremely popular and widely grown in Bulgaria with a huge economic and social significance especially for the region of Rodopa Mountain, till now there are no commercial products for plant protection registered on this basis and used in the country. In present investigation, we found that preparations of tobacco oil can show a strong fungicidal action towards powdery mildew of wheat (*Blumeria graminis f. sp. tritici*) which is one of the major disease on that culture for the country of Bulgaria. The wheat is the most economical important culture plant for Bulgaria as well as bread is the major food for Bulgarians and Bulgarian cuisine.

Tobacco water or ethanol extracts have some significant disadvantages as non-persistence, non-stability, phytotoxicity, variation of the effectiveness towards abiotic and biotic conditions of the environment and bad physico-chemical properties as wetting and sticking ability. Such kinds of preparations express some interest for scientific research and trials, but in the real agricultural practice and pest management no one will use them as pesticides. The purpose of present investigation was to conduct a scientific-practically-applicable research, and not a pure scientific work.

For elimination of such disadvantages of the water and ethanol tobacco extracts, mentioned above, a stable and

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durable pesticide formulation was created as is described in section Material and Methods. This formulation is completely "ready to be used as plant protection product" in the agricultural area. With these formulations were conducted, several preliminarily *in vitro* tests type "Leaves Disks" and on their base - two years field test with wheat cultivar Sadovo 1 which is one of the most popular in Bulgaria.

MATERIALS AND METHODS

The active substance used is not soluble in water. For the purposes of agriculture and converting it into water soluble, one special formulation was prepared. The formulation procedures included saponification of active substance and its emulsification. Saponification procedure - mixture of coconut oil, potassium hydroxide and water was heated up to 50° C and stirred in a mixer at a rotation speed of 1 500 min^{-1}. After soap preparation active substance and glycerin were added. The mixture was heated up again to 40° C and stirred in a mixer at a rotation speed of 1500 min^{-1}.

The formulation consistence was:

- Tobacco oil (active substance) - 0.12 kg kg^{-1}
- Coconut oil - 0.05 kg kg^{-1}
- Potassium hydroxide-0.13 kg kg^{-1}
- Distilled water- 0.05 kg kg^{-1}
- Glycerin - 0.10 kg kg^{-1}.

The specifications of used materials are: glycerin G2289 Sigma-Aldrich, potassium hydroxide P5859 Sigma-Aldrich, distilled water - single distilled own production, coconut oil brand “Zoya” bought from local market. The active substances, such as essential oil, have been prepared in our laboratory steam atmospheric hydrodistillation unit with volume 30 l. The only reagent used for essential oil production was water. The production procedure includes - loading of raw material in perforated bottom basket, contact of raw material with superheated water steam and evaporation of volatile compounds, condensing of water steam and volatile compounds by means of cooling, decanting of oil (volatile compounds) from distillate by means of density differences, removing of decanted oil, dewatering of oil by potassium sulphate, and filteration of oil. The parameters of distillation process were:

- Distillation rate (steam flow rate per retort volume) -0.06 -0.08 kg dm^{-1};
- Superheated steam temperature - 135-140° C;
- Distillate temperature 30-32° C;
- Distillation time - 120 min;
- Yield (essential oil per kg of raw material) - 6.8 g kg^{-1};

The specifications of used raw material were: tobacco (Nicotinum tabacum L.) Oriental variety - dried leaves and stems, and waste from local tobacco processing factory crop 2012.

The chemical compositions of active substance determined on GC-MS method was (compounds above 0.01 kg kg^{-1} only):

Tobacco oil:

Phytol acetate - 0.09 kg kg^{-1}

Acetic acid - 0.087 kg kg^{-1}

Nicotine - 0.062 kg kg^{-1}

Norambreinolide - 0.057 kg kg^{-1}

3-oxo-α-ionone - 0.021 kg kg^{-1}

Benzyl alcohol - 0.016 kg kg^{-1}

Solanone - 0.016 kg kg^{-1}

β-Caryophyllene - 0.014 kg kg^{-1}

3-methyl valeric acid - 0.013 kg kg^{-1}

Sabinene - 0.011 kg kg^{-1}

The created formulation is a completely accomplished plant protection product, which is ready to be used in the agricultural practice as well as ready to be manufactured in the future after successful passing of the obligatory toxicological and ecotoxicological tests respectively - European Union and Bulgarian Government plant protection product registration process.

Two years (growing seasons) field test was conducted at Field Research Center of the Agricultural University Plovdiv - Plovdiv district in 2013 and 2014. The complete randomized block design test of five replications was used. The wheat cultivar used in test was Sadovo 1. Test formulation was examined in 10 different concentrations with 2x factor starting from 0.01 % (m/v) each representing different test variant. One of the variants was empty (untreated) control, other - full (treated with distilled water plus surfactant) control. One variant (standard or negative control) was sprayed with ® (azoxystrobin-C3:Qol fungicide) - 0.08% (800 ml ha^{-1}). Silwet $^{L-77®}$ spray adjuvant based on a trisiloxane ethoxylate was used as a superspreading surfactant in order to low the surface tension of spray solutions at 0.025% (v/v). The ethanol or water based tobacco extracts were not used as comparative variants (negative controls) due to the above mentioned disadvantages of them. Currently, in Bulgarian agricultural practice nobody will use such kind of extracts for pest management in the farms.

Two foliar applications were used to evaluate a test formulation efficacy. Powdery mildew was allowed to develop naturally and after that foliar applications were applied. Disease severity was determined by using Percentage Disease Index before and after each treatment with five ball scale:

- 0 ball - 0 % infestation (healthy leaves - plants)
- 1 ball - 1÷25 % infestation
- 2 ball - 26÷50 % infestation
- 3 ball - 51÷75 % infestation
- 4 ball - over 75 % infestation

The percent of infestation was established by 0.5 * 0.5 mm square transparent pieces of laminating film - the general number of all squares covering the given leaf and the number of squares with disease manifestation were counted. Then, the percentage of the "infested" squares were calculated and the ball of infestation of each plant were estimated as mean value of PDI values of all leaves of the plant according to the above mentioned five ball scale. The mean values from the two years experiments were used for the final statistical analyses of the trial.

Data analyses - Areas Under Disease Progressive Curve (AUDPCs) were calculated by R language for Statistical Computing [17], agicolae package [18], on the basis of established Percentage Disease Indexes [19] and ANOVA analyses. On the basis of the values of the Relative AUDPC, the percentages of effectiveness were calculated by using the formulae of Abbot [20] with values received from full control variant. Dose - Response Modeling by drc package [21] was conducted in order to be established as the four major toxicological points:

- LD_{90}
- LD_{50}
- LOAEC (Lowest Observed Adverse Effect Concentration) - LD_{25}
- NOAEC (No Observed Adverse Effect Concentration) - LD_{05}

RESULTS

Table **1** represent the mean PDI values received from two years of the trial for each variant.

Table 1. Mean PDI values of the field test after treatment with tobacco preparation.

Number of replication	Empty Control	Full Control	0.01 %	0.05 %	0.07 %	0.1 %	0.5 %	0.7 %	1.0 %	1.5 %	1.7 %	2.0 %	Amistar 25 SK® - 800 ml ha-1
1	35	41	40	34	32	24	13	12	8	2	1.5	0	10
2	40	40	32	34	32	25	14	11	9	0.6	1.3	0	9
3	42	33	30	35	32	24	13	12	8	2	1.4	0	9
4	48	35	35	38	31	24	13	12	9	0.7	1.2	0	10

(Table 1) contd.....

Number of replication	Empty Control	Full Control	0.01 %	0.05 %	0.07 %	0.1 %	0.5 %	0.7 %	1.0 %	1.5 %	1.7 %	2.0 %	Amistar 25 SK® - 800 ml ha-1
5	37	38	37	40	32	24	13	12	7	1.8	1.4	0	8
Mean	40.4	37.4	34.8	36.2	31.8	24.2	13.2	11.8	8.2	1.93	1.36	0	9.2

Fig. (**1**) shows conducted ANOVA analysis with R language IDE:

```
> summary(aov(lm(values~ind, data=test_ts)))
             Df Sum Sq Mean Sq F value Pr(>F)
ind          12  14058  1171.5   244.8 <2e-16 ***
Residuals    52    249     4.8
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
>
```

Fig. (1). ANOVA analyses.

The *p*-value is far below 0.05 = 2.0 e-16 which proves the validity of the conducted trial.

Fig. (**2**) shows the ANOVA analyses with Tukey HSD algorithm and graphic:

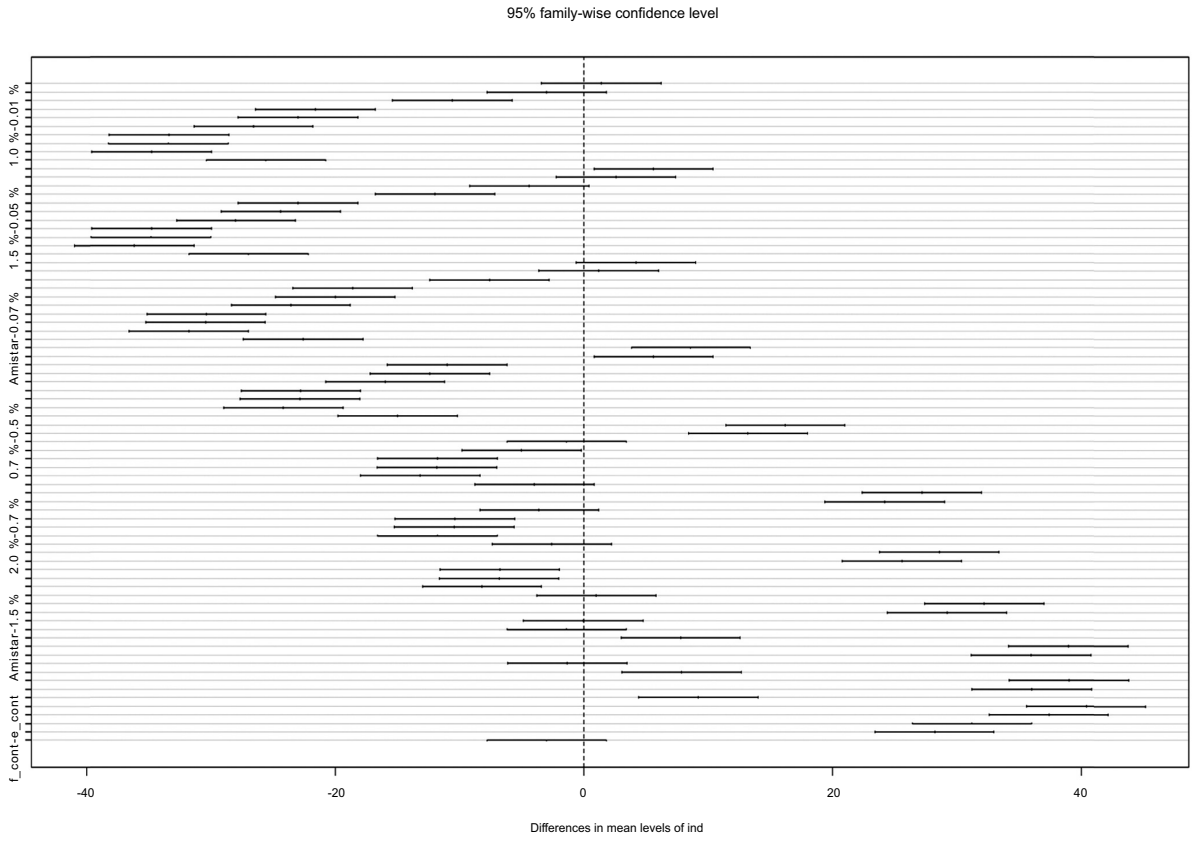

Fig. (2). ANOVA TukeyHSD analyses.

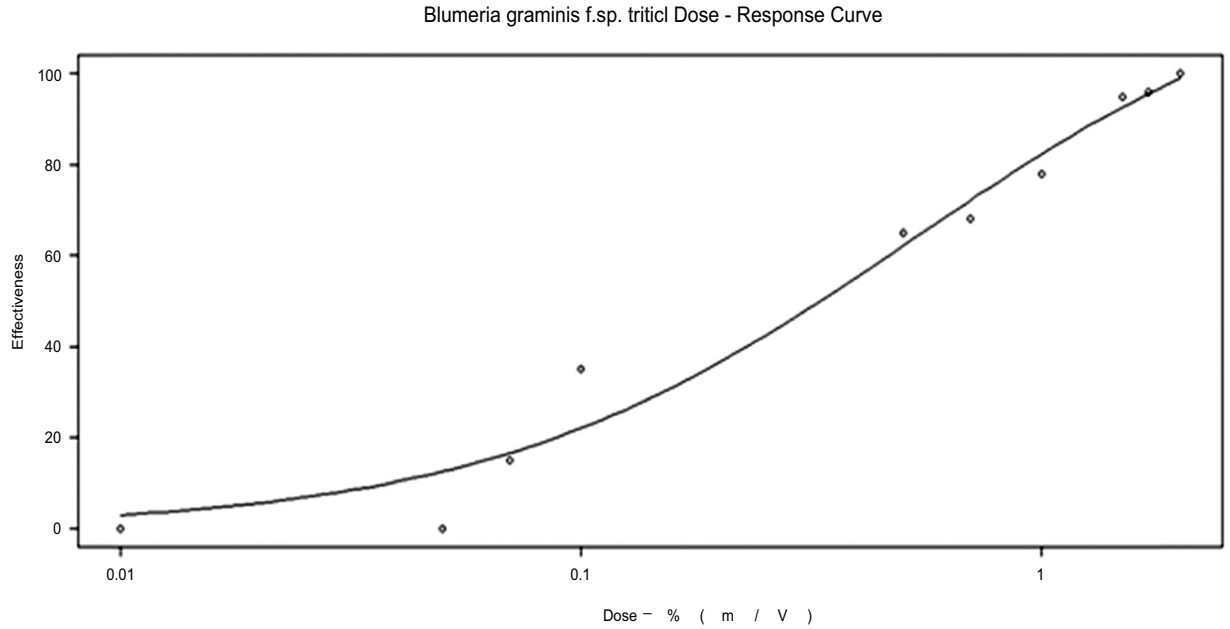

Fig. (3). Dose - Response Curve of tested tobacco oil formulation towards powdery mildew of wheat.

The Dose - Response Modeling Fig. (**3**) indicated that powdery mildew of wheat (*Blumeria graminis f. sp. tritici*) was significantly affected by created formulation on basis of tobacco oil. The concentration of 2% (20 l ha^{-1}) - LD_{90}(LD_{50} = 0.6 %, LD_{25} = 0.075 %, LD_{05} = 0.015 %) was able to completely inhibit (eradicate) disease and protect the plants. During the test, no phytotoxic manifestation onto tested plants were observed (chemotherapeutic coefficient = 10). ANOVA analyses prove that there is a significant difference between variants in the test (p<0.05). There is no significant difference found between PDI values of given variant conducted during the two years of the trial (p>0.05). No significant differences were found between empty and full control variant (p<0.05) during the field test – p=0.61.

CONCLUSION

The created natural pesticide formulation on the basis of tobacco oil is promising for future investigations and development as a natural fungicide after successfully passing the obligatory toxicological and ecotoxicological examinations. This will have also significant economic and social effect for a large part of people in Bulgaria who are involved in tobacco plant breeding, which during the recent years is constantly decreasing due to international and nation smoking restriction and prevention programs. Therefore, it is important to mention that in most cases in the regions in Bulgaria where there is tobacco breeding, the environmental conditions do not allow growing of other crops. This mean that in most cases almost all income of families involved in the tobacco farming depends on tobacco plant yields in this remote poor mountain region. Even more - the development of synthetically pesticides, requires a lot of chemical engineering capacities and investigations, which is not possible in the relatively poor countries like Bulgaria. In the section "Materials and Methods" everyone can see, that creation and manufacturing of this natural pesticide formulation is not a complex and expensive process, which means that such kinds of pesticides are extremely suitable for the current economic conditions of Bulgaria and other developing countries. Development of pesticides on the basis of tobacco plant extracts (oil) must be the priority for the national government of Bulgaria and other countries where people income depends heavily on this plant culture.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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