Results of testing immunomodulators against phomopsis stem canker in sunflower

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ABSTRACT

- The induction of protective plant mechanisms by using natural and synthetic agents attracts ever growing attention of researchers due to an urgent need in environmentally safe technologies for crop production. Previous efforts of scientists in this area of research led to developing a number of products based on chitosan, such as Phytochit, AgroChit, Chitozar, Narcissus, and others which are applied in Russia nowadays.
- The aim of the study was to determine biological and economic effectiveness of two formulations based on Phytochit, WSP, against one of the most damaging sunflower diseases – Phomopsis stem canker, *Phomopsis helianthi* Munt.-Gret. et al.
- Research was conducted in 2009 and 2010 by using two sunflower cultivars: Rodnik that is susceptible to Phomopsis stem canker and Master that is tolerant to this disease. Mean daily temperatures, relative air humidity and precipitation were recorded each day. The flight of ascospores was evaluated by using two spore traps SP-1. Observation frames in these spore traps were changed twice a week and each time after rainfall. The numbers of ascospores on the observation frames surface smeared with mixture of gelatin and glycerin were determined while examining them under microscope.

Phytochit, a biogenic disease resistance inducer, and Albite and Kendal (a stimulator of endogenous defence mechanisms in plants), plant growth regulators, were used for this experiment. Phytochit was also applied in mixture with one of these plant growth regulators: Phytochit + Albite and Phytochit + Kendal. These mixtures and their individual components were applied for seed treatment as well as for combination of seed treatment and spraying vegetating sunflower plants. Maxim was used as standard for preplant seed treatment, and Vermiculen - as standard for the combination of preplant seed treatment and spraying vegetating plants. Untreated seeds and plants served as control.

- In the period of formation and ripening of sunflower heads, first the leaf form and then the stem form of *P. helianthi* appeared. In control, the stem form of Phomopsis canker reached 12 - 18 % severity in Rodnik and 7-10 % in Master.

Analysis of the two-year results shows that applications of the mixed formulations based on Phytochit and Albite or Kendal allowed to increase their biological activity against *P. helianthi*. Seed treatments in combination with spraying vegetating plants with these formulations appeared to be most efficient. Biological effectiveness against *P. helianthi* reached 100% for these treatments. Sunflower seed yields were significantly higher compared both to control and to treatments of only seeds with these formulations. In cv. Rodnik the yield amounted to 3.0-3,1 t/ha and in cv. Master to 3.5-3.6 t/ha.

- The use of biocontrol formulations based on a disease resistance inducer Phytochit and plant growth regulators Albite or Kendal improves sunflower resistance to *P. helianthi* and increases yields. Biological effectiveness of the formulations reaches 100% at 7-18 % of Phomopsis canker severity and sunflower seed yields for these treatments are 0.44-0.87 t/ha higher than in control.
- High biological effectiveness of the formulations based on Phytochit that was achieved during their field tests makes it possible to include them into technologies aimed at controlling pests, increasing sunflower yields and obtaining ecologically pure products.

Key words: canker - disease - formulations - induction - resistance - sunflower.

INTRODUCTION

The induction of protective plant mechanisms by using natural and synthetic agents attracts ever growing attention of researchers due to an urgent need in environmentally safe technologies for crop production. One of these agents – chitosan is a cationic polysaccharide that is known as a disease resistance inducer. Previous efforts of scientists in this area of research led to developing a number of products based on chitosan, such as Bion, AgroChit, Chitozar, Narcissus, Phytochit and others which are applied in Russia nowadays (Novozhilov, Tyuterev et al., 1999; Tyuterev et al., 2001; Khatskevich, Gamgadze et al., 2001). The protective activity of chitosan is based on the activation of natural (immune) resources in plants as a response to attacks of plant pathogens (Kurosaki and Tashiro, 1987; Maucd and Hadwiger, 1988). The defense reaction mechanism in plants is launched as soon as receptors in plant cells have recognized biologically active units in the primary structure of chitosan (Ozeretskovskaya, 1994).

The stimulation of these resistance-inducing mechanisms in plants provides a number of advantages compared with applications of fungicides and other chemicals which have the mode of action that consists mainly in inhibiting or eliminating both pathogens and beneficial flora and fauna and which are mostly toxic. The chitosan-based products have the following advantages:

1. They provide environmental safety;
2. They are applied at low concentrations;
3. They have systemic and prolonged activity.

However, these biotic elicitors are less efficient at high severity of plant diseases. To minimize these disadvantages it is necessary not only to select and improve chitosan-based products, but also to create their formulations with compounds having similar structures with "protective substances" within plants (Begunov & Kolomiets, 1994; Tyuterev, Yakubchik et al., 1997). The efficiency of these formulations may be improved by including correctly selected additives of protective activity (Tyuterev & Dorofeeva, 1989Begunov & Krasnikova, 1994).

The aim of this study was to determine the efficiency of treating seeds alone or in combination with spraying vegetating plants with formulations based on Phytohyte as a disease resistance inducer and plant growth regulators (Albite and Kendal).

MATERIAL AND METHODS

The study was conducted by using Rodnik as a cultivar susceptible to Phomopsis canker (growing season: 80 days, potential yield: 3.2 t/ha, oil content: 55%) and Master as a tolerant cultivar (growing season: 94 days, potential yield: 4.0 t/ha, oil content: 54%).

The area of experimental plots was 4.9 m^2; the experiment had a randomized design and included four replicates.

The soil in the experimental plots was leached chernozem. The humus amount in the plough-layer came to 3.8 %. The soil was of heavy loam texture and cloddy and grainy structure. The soil pH value was 6.8. Winter wheat was a preceding crop.

The sunflower seeds were planted on May 4. For the preplant seed treatment and spraying vegetating plants (at the early budding stage) the following products were used: Phytochit, WSP (chitosanium succinate mannite, water soluble power): 0.2 kg/t, ha; two separately applied plant growth regulators – Albite, TRP (poly beta hydroxybutyric acid, thin runny paste): 0.1 kg/t, ha and Kendal, L (a liquid plant defense biostimulator): 0.2 kg/t, ha; two mixtures - 0.2 kg/t, ha of Phytochit + 0.1 kg/t, ha of Albite and 0.2 kg/t, ha of Phytochit + 0.2 kg/t, ha of Kendal. Maxim, SC (fludioxonyl, suspension concentrate) at a rate of 5 L/t of seeds was used as standard for preplant seed treatment, and Vermiculen, CL a biological product in the form of cultural liquid (CL) based on conidia, mycelium and metabolic products of *Penicillium vermiculatum* Dangeard (strain PK-1-3) at a rate of 3 L/t, ha, was used as standard for a treatment including preplant seed treatment and spraying vegetating plants. A plot with untreated sunflower plants was used as control.

Mean daily temperatures, relative air humidity and precipitation were recorded each day.

The tests of Phytochit as a disease resistance inducer, Albite and Kendal as plant growth regulatotrs and their formulations were conducted under the conditions of the natural infection by Phomopsis canker.

Sunflower residues infected by *Ph. helianthi* in previous years which were located close to the experimental field at the side of prevailing wind served as the infection source. The flight of ascospores was recorded by using two spore traps SP-1. Observation frames in these spore traps were changed twice a week and each time after rainfall. The numbers of ascospores on the observation frames surface smeared with mixture of gelatin and glycerin were determined while examining them under microscope. The hydrothermic coefficient (HTC) was determined by the formula:

$$HTC=\sum R*10/\sum t;$$

where ∑R – rainfall amount (milliliters) for a certain period of time with air temperatures above 10°C;

∑t – accumulated daily temperatures for the same time [Petrushina et al., 2002].

The evaluation of Phomopsis canker infection was made by using the scale developed in the All-Russian Research Institute of Oil Crops (VNIIMK) [Lukomets et al., 2008]. Biological effectiveness was calculated by the formula:

$$Б = (P_к - P_в / P_к) \cdot 100\%$$

,

where Б – biological effectiveness;

$P_к$ – severity of *Ph. helianthi* in control; степень развития фомопсиса в контроле;

$P_в$ – severity of *Ph. helianthi* in the experimental treatments.

Sunflower heads in each treatment were harvested by hand. The heads were threshed with a mini-combine Hege.

The data obtained were processed by using the analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The flight of ascospores was observed repeatedly every 2-4 days after rain, even an insignificant one (less than 0.05 mm). Every ascospore emission corresponded to the maximum or minimum or close values of relative air humidity. The maximum number of ascospores above the experimental sunflower field did not exceed 180,000,000 per square meter and was observed in May in 2009 and in June in 2010 at HTC > 1. The maximum number of ascospores was no more than 80,000,000 per square meter and was calculated in August (HTC < 1).

In 2009, a cool weather that started on April 10 kept until late May. The mean monthly air temperature in May was 16.2°C. In early May, daily air temperatures ranged between 17 and 22°C, in the second half of the month only on some days the temperature reached 25-27°C. At night the minimum air temperatures sank to 8-13°C. The first and second decades of May were rainy, the precipitation was 31 mm (174% of standard) and 47 mm (237% of standard), respectively. Frequent rains created favorable conditions for flight of ascospores and infection of plants. HTC was > 1, and relative air humidity no less than 60 %. In June, the mean monthly temperature was 23.7°C. In the third decade of June, the mean decade air temperature reached 26.7°C. Namely, on June 22, the first Phomopsis canker symptoms were observed on individual plants. However, the maximum air temperatures rose to 34-37°C later and that did not favor the disease progress.

From mid June to July 18, there was a long (34 days) rainless period with hot winds. In this period of time, Phomopsis canker remained at the leaf infection stage.

By the end of the 3rd decade of July, 75 mm of rain had fallen which made 394 % of the decade norm. This increased the incidence of Phomopsis in the experimental site and its transition to sunflower stems.

The August weather conditions did not favor the progress of *Ph. helianthi* for lack of precipitation. In September, the weather conditions were favorable for the disease development. Only in the first half of September there were good rainfalls - 120-140% of the decade norm and, as a result, the stem form of *Ph. helianthi* affected 18% in the susceptible cultivar tested and 10% in the tolerant one.

So, the conditions of Phomopsis canker development described above determined its low intensity in 2009.

Due to favorable weather conditions from the second half of May to July inclusive, the Phomopsis canker disease progressed very fast: on July 19, its incidence in control was 20%, on July 21 – 27%, on July 23 – 54% and on July 28 – 61%. The stem form of *Ph. helianthi* had already manifested itself on the susceptible cultivar plants in control on July 21. However, on July 27 the longest dry period (atmospheric drought) started and remained until August 18. During this period the maximum air temperature exceeded 30°C and relative air humidity sank to 40 %. The Phomopsis-infected sunflower leaves dried quickly and fell off. As a result, the progress of the Phomopsis canker stem form in control was 12 % on the Rodnik cultivar and 7% on Master.

No sunflower head infection had been observed for those two years.

Analysis of the two-year results shows that biological effectiveness of Phytochit as a disease resistance inducer and Albite and Kendal, plant growth regulators applied separately both for seed treatment and for combination of seed treatment and spraying vegetating plants came to 46-86.5 % in Rodnik and 67.6-73 % in Master (Fig. 1). The yield amounted to 2.7-2.8 t/ha and 2.7-2.9 t/ha, respectively (Fig. 2).

Applications of mixed formulations based on Phytochit with Albite or Kendal improved their biological activity against Phomopsis canker. Seed treatments in combination with spraying vegetating plants with these formulations appeared to be most efficient. Biological effectiveness against *Ph. helianthi* reached 100% for these treatments. Sunflower seed yields were significantly higher compared both to control and to treatments of only seeds with these formulations. In cv. Rodnik the yield was 3,0-3,1 t/ha nd in cv. Master - 3.5-3.6 t/ha.

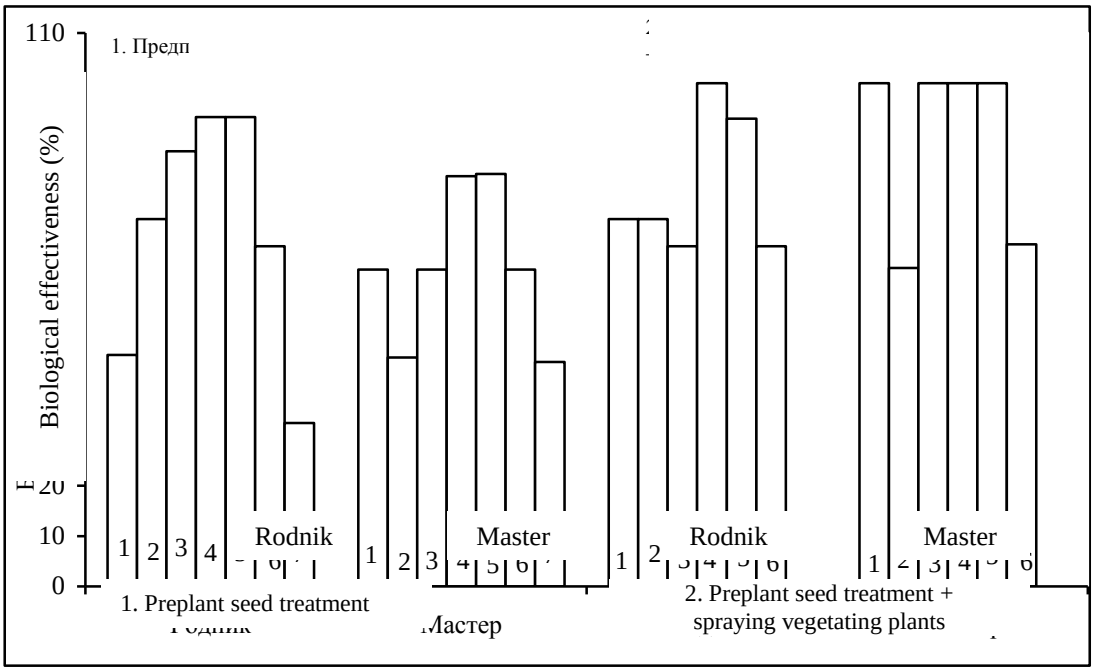
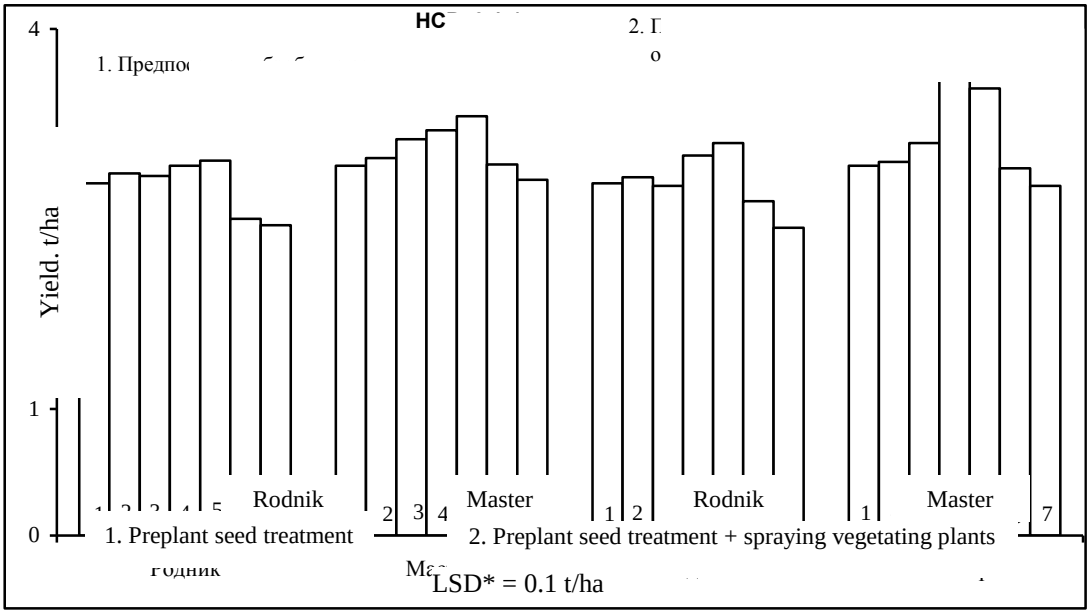


Fig. 1. Biological effectiveness of biologically active compounds and formulations based on Phytochit against Phomopsis canker of sunflower in experimental plots (Phomopsis canker severity: 12-18 % in Rodnik and 7-10 % in Master)



*LSD – least significant difference

Fig. 2. Economic effectiveness of biologically active compounds and formulations based on Phytochit under the field experiment conditions in sunflower

1. Phytochit, WSP – 0.2 kg/t
2. Albite, thin runny paste – 0.1 L/t
3. Kendal, L – 0.2 L/t
4. Phytochit, WSP + Abite, TRP – 0.2 kg/t + 0.1 L/t
5. Phytochit, WSP + Kendal, L – 0.2 kg/t + 0.2 L/t
6. Vermiculen, CL – 3 L/t
7. Maxim, SC – 5 L/t

1. Phytochit, WSP – 0.2 kg/t
2. Albite, thin runny paste – 0.1 L/t
3. Kendal, L – 0.2 L/t
4. Phytochit, WSP + Abite, TRP – 0.2 kg/t + 0.1 L/t
5. Phytochit, WSP + Kendal, L – 0.2 kg/t + 0.2 L/t
6. Vermiculen, CL – 3 L/t
7. Control

Biological effectiveness of the products taken as standards was lower and came to 44.6% for preplant seed treatment with Maxim and 67% for preplant seed treatment and spraying vegetating plants with Vermiculen. Sunflower yields in these treatments were 2.4-2.8 t/ha and 2.6-2.9 t/ha, respectively.

CONCLUSION

The flight of ascospores and their great number over sunflower fields are observed both under the conditions favorable for *Ph. helianthi* and during a drought.

The use of biocontrol formulations based on a disease resistance inducer Phytochit (rate of application: 0.2 kg/t, ha) and plant growth regulators Albite (0.1 kg/t, ha) or Kendal (0.2 kg/t, ha) improves sunflower resistance to *Ph. helianthi* and increases yields. Biological effectiveness of the formulations reaches 100% at 7-18 % of Phomopsis canker severity. Sunflower seed yields for these treatments are 0.44-0.87 t/ha higher than in control.

The higher biological effectiveness of preplant seed treatments and spraying vegetating plants with the formulations based on the composition of Phytochit as an immunomodelator and Albite or Kendal that was achieved during their field tests makes it possible to include them into technologies aimed at controlling Phomopsis canker, increasing sunflower yields and obtaining ecologically pure products.

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