THE APPEARANCE OF *SCLEROTINIA SCLEROTIORUM* ON GREEN BEANS AND THE EXAMINATION OF ANTIFUNGAL EFFECT OF EXTRASOL®

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Abstract. *The causer of white mold (Sclerotinia sclerotiorum (Lib.) de Bary) represents a very significant pathogen on numerous cultivated plant species, especially vegetables. The possibility of pathogen control is very limited and the crop rotation is extremely narrow due to distinct poly – pathogenicity. Recent studies of control of white mold are aimed towards the use of biological products. During 2014 the appearance of white mycelular cover with black sclerotia was noteiced on ground part of the stem and first pods in the crop rotation of green beans on Zmajevo locality (45° 27' 08" NGW, 19° 41' 05" EGL). Examining the stubble beans during September, on a certain parts of the lot with the symptoms of the necrosis on the ground part of the stem and the first pods, oases were noticed. Within necrotic areas, appearance of the thick white mycelium coating with large, black sclerotia became visible. After Sclerotia collecting and isolation on potato dextrose agar (PDA), which was followed by acquiring of pure cultures, six isolates. Fungus mycelium was distinctively white, and during six days of cultivation at 25°C it covered Petri dish of 90 mm in diameter, and the appearance of sclerotia on colonies' edges was noticed on the eleventh day. With the aim of examining the biological control of S. sclerotiorum, Extrasol® (Biogenesis), based on Bacillus subtilis Č 13 was used. The efficiency evaluation of the product was conducted in vitro, by transmitting of round shaped fragments, of 5 mm in size, of 7 days old S. sclerotiorum colonies, to the surface of the PDA base in which the product in 10, 15 and 20% concentration has been added, prior to the gelation. After 7 days incubation period, the measurement of mycelia growth diameter was performed at 25°C in cases with different concentrations and control cases. The product with 20% concentration showed efficiency of 100%, with concentration of 15% the efficiency was 35.8%, while the product with concentration of 10% showed poor efficiency.*

Key words: *Sclerotinia sclerotiorum, green beans, Bacillus subtilis*

INTRODUCTION

The white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary), belongs to the group of polyphagous pathogens widely distributed around the world, and it attacks over 400 plant species, among which numerous crops, and especially vegetable ones [8; 11; 13]. The disease occurs around the world, most often in the areas with moderate climate and the plants can be attacked in all stages of development [14]. Wet and chilly weather conditions that are prevailing in the moderate climate regions are favorable to the development of the pathogens [19]. In Ontario, the average representation of *S. sclerotiorum* on beans (*Phaseolus vulgaris* L.) was 25 % [3]. According to HUNTER et al (1984), the white mold is present in beans in Alberta,

due to cultivation of plants in irrigation conditions which is a favorable factor for the development of the disease.

On the territory of Vojvodina there are two possible harvests annually, depending on the choice of the crops [15] and irrigation conditions [6]. In last few years, the sowing structure of stubble beans (*Phaseolus vulgaris* L.) occupies a significant area, and due to regular irrigation, single pathogens appear in more significant intensity. Diseases in a combination with conditions of production, the tolerance of the used assortment, climate and weather conditions, are often the cause of hesitant and low yields [12].

MATERIALS AND METHODS

During 2014. in stubble beans crop, type Nirvana, in the Zmajevo locality (45° 27′ 08" NGW, 19° 41′ 05" EGL) massive appearance of white mold on the above part of the stem was recorded. In some places black sclerotia with in mycelium were noticed. The sclerotia were collected and stored in a refrigerator for 10 days at 4°C.

After that period, surface disinfection with 4% NaOCl was performed and sclerotia were washed with sterile distillated water. In a Petri dish of 90 mm in diameter, potato dextrose agar was spilled (PDA) and vertically cut sclerotinia were placed on the surface. The incubation lasted for 6 days at 25°C, and after that a sifting was made in order to get clear cultures.

During this operation, antifungal efficiency of the product Extrasol® (Biogenesis) based on *Bacillus subtilis* Č 13 *in vitro* was observed. The product was applied in the concentration range of 10, 15 i 20% on 7 day's old *S. sclerotiorum* cultures. After sterilization, flasks of 250 ml volume with PDA base were placed in a water bath at 50°C, and 30 minutes later Extrasol® was added in the above mentioned concentrations. The flasks were placed in a magnetic stirrer for 5 min and the contents was diffused in Petri dishes of 55 mm in diameter. After the gelation of the base, round fragments of *S. sclerotiorum* mycelium of 5 mm in size were placed in the middle. A base without additional product along the fragment of mycelium was used as control. The incubation in a thermostat at 25°C lasted 7 days. After this period, measurement of the diameter of the mycelium growth was performed and the degree of inhibition was calculated. The experiment was in three replications.

RESULTS AND DISCUSSIONS

Examining the stubble beans during September, on a certain parts of the lot with the symptoms of the necrosis on the ground part of the stem and the first pods, oases were noticed. Within necrotic areas, appearance of the thick white mycelium coating with large, black sclerotia became visible. By isolating the fragments of infected green beans tissue on nutritive base, mycelium covered the surface of Petri dish of 90 mm diameter in six days. The beginning of sclerotia forming was recorded on the ninth day, in a form of silvery-transparent droplets. Appearance of hard black sclerotia on the edge of Petri dish was noticed on the eleventh day.

Antifungal activity of the product Extrasol®, when applied at concentrations of 15 and 20% was noticed. The inhibition percentage of 100% was noticed at 20% of product concentrations. In this version, an increase of mycelium on nutritive base out of the conveyed disc was not noticed. The product concentration of 15% gave the inhibition of 31,1%, while use the 10% of the product concentration as well as in a control cases, the percentage of inhibition was not recorded (Table 1).

Due to inadequate levels of host resistance, fungicides are the main method of white mold control (BARDIN AND HUANG, 2001). However, fungicide control is sometimes inadequate (STEADMA, 1979), the reason is insufficient coverage of leaves with the fungicide, and in correctly chosen time of application related to ascospores release (MUELLER et al., 2002). Use of biological agents in a herbal pathogens control might be ecologically acceptable and economically worthwhile. The possibility of *S. sclerotiorum* suppression by implementation of biological products is stated by several authors (BOLAND, 1997; HUANG ET AL., 2000; HU et al., 2011). Strains of the genus *Bacillus*, especially *Bacillus subtilis* are exceptional candidates for biological struggle and production of formulations in a commercial purposes (JACOBSEN et al., 2004). Earlier studies in the field has shown that foliar application of *B. subtili* decreases the frequency on *S. sclerotiorum* on the beans (TU, 1997). According to the THEODULOZ et al. (2003) researches, after applying on the plant surface, the cells of *B. subtilis* begin to produce antibiotics while antifungal substances keep the efficiency for 15 days.

The product Extrasol® exhibited antifungal effect *in vitro* conditions in increased concentration. Due to knowledge of the mechanism of parts that are classified as a competition and antibiosis (FRAVEL, 1988), the biological agent has to be applied to the plants earlier before the realization of infection with the askospores *S. sclerotiorum*. The majority of the authors agree that the primary infections are developing in the phenophase of beans flowering, due to possible colonization of the flower petals, which are the source of nutritious components, and then the infection spreads to the whole plant (TU, 1989; BARDIN AND HUANG, 2001). With confirmation of the biological product efficiency *in vitro* conditions, need of examination in open fields is imposed, which will be the subject of examination in the following period.

Table 1

Diameter of mycelium and percentage of inhibition at different product concentration

Isolates	Extrasol®							
	10%		15%		20%		Control	
	DM (mm)	PI (%)	DM (mm)	PI (%)	DM (mm)	PI (%)	DM (mm)	PI (%)
Sc1	55	0	3,42	37.7	0	100	55	0
Sc2	55	0	3,72	32.2	0	100	55	0
Sc3	55	0	3,30	40.0	0	100	55	0
Sc4	55	0	3,42	37.7	0	100	55	0
Sc5	55	0	3,48	36.6	0	100	55	0
Sc6	55	0	3,78	31.1	0	100	55	0
∑	55	0	3,51	35.8	0	100	55	0

DM - Diameter mycelium; PI - Percent inhibition

CONCLUSIONS

Sclerotinia sclerotiorum at stubble beans might be significant pathogen, especially if the production is in irrigation conditions. By isolation of the pathogen on a nutritious base, a fast increase was recorded, in six days the fungus covered Petri dish of 90 mm diameter, and on the eleventh day the appearance of the sclerotia was confirmed. The product Extrasol with 20% concentration showed efficiency of 100%, with concentration of 15% the efficiency was 35.8%, while the product with concentration of 10% showed poor efficiency

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