

## ASSESSMENT OF *ISARIA FUMOSOROSEA* SPORES LOSS IN HYDRAULIC AGITATORS

### Summary

The study was conducted assessing the losses of spores of the fungus species *Isaria fumosorosea* during the flow of liquid through the nozzle of the hydraulic agitator. The liquid flow containing spores was  $2.5 \text{ dm}^3/\text{min}$  and the pressure 6.0 bar. The liquid circulated 100 times in the system comprising a pump, hydraulic mixer, and reservoir. It has been found that the multiplicity of fluid flow has a significant effect on reducing the number of spores of the fungus *Isaria fumosorosea* contained in a liquid. The maximum reduction of spores was 89%.

**Key words:** sprayer, biological pesticides, insecticides fungi

## OCENA STRAT ZARODNIKÓW GRZYBA *ISARIA FUMOSOROSEA* W MIESZADLE HYDRAULICZNYM

### Streszczenie

Przeprowadzono badania oceny strat zarodników grzyba gatunku *Isaria fumosorosea* podczas przepływu cieczy przez dyszę mieszadła hydraulicznego. Natężenie przepływu cieczy zawierającej zarodniki wynosiło  $2,5 \text{ dm}^3/\text{min}$ , a jej ciśnienie 6,0 bar. Ciecz krążyła w instalacji (składającej się z pompy, mieszadła hydraulicznego i zbiornika) do 100 razy. Stwierdzono, że krotkość przepływu cieczy ma istotny wpływ na redukcję ilości zarodników grzyba *Isaria fumosorosea* zawartych w cieczy. Maksymalna redukcja zarodników wyniosła 89%.

**Słowa kluczowe:** opryskiwacz, biologiczne środki ochrony roślin, grzyby owadobójcze

### 1. Introduction

The popularity of the use of biological methods of plant protection increases the demand for food derived from organic farming. The possibility of using bio-preparations is an alternative for pesticides which cause environmental degradation. Biopesticides used for pest control take advantage of natural phenomena that occur in nature between organisms such as parasites or predation. Biological methods rely heavily on the use of viruses, bacteria, protozoa, fungi, parasites, insecticidal nematodes, mites and predatory or parasitic insects to fight pathogens, plant pests and weeds [1, 4, 14, 17].

An entomopatogenic fungus is used to combat agricultural pests, inter alia, in forests, orchards, crops, greenhouse and field. For best recognized group of microorganisms that cause disease insects, parasitic fungi are commonly found in soil and ground plant parts [10, 16, 18]. One of their most important features consists in the ability to limit the number of soil fauna harmful to crops [11]. The most common species of fungi insecticides include: *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces farinosus*, *Paecilomyces fumosoroseus*, *Verticillium Lecanii* [2, 15, 19]. *Stadium* is an infectious fungus spore, which germinates on the surface of the insect's body, and then during the growth phase penetrates into it and fill the body causing death [12]. Bio-fungi do not require the use of specialized equipment to disperse them. For this purpose, the same apparatus is used as in the case of the application of chemicals. These are known to farmers as well as widely used sprayers that were constructed for the use of chemical plant protection. Studies on the biological destruction of plant protection agents in the system spraying was carried out mainly on the insecticidal nematode [5, 6]. They showed negative influence of

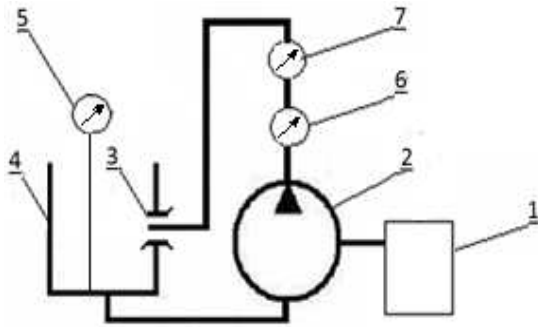
liquid pressure in the sprinkler system and the multiplicity of flow of liquid through the plant nematode survival. There are few published studies on the destruction of bio-fungi in plant sprayer. Nilsson and Gripwell [13] found that, during the flow of liquid through the spray system bio-fungal *Verticillium lecanii* contained therein may be destroyed during the circulation of the liquid sprayer which causes a reduction in the effectiveness of the treatment being performed. There is no, however, detailed investigation providing knowledge, what elements of sprayer's installation and what physical conditions of liquid flow in the system can cause the greatest loss of biological agent protection product containing insecticidal fungus. It is necessary to adapt the working elements of the apparatus, method of application of these measures and selection of optimum operating parameters to ensure the smallest losses with high efficiency at the same time [5, 8, 9]. By analogy to the destruction of insecticidal nematode, it appears that hydraulic stirrer is one of the most important elements of the installation sprayers which can affect critical for the survival of spores of the fungus insecticide [6].

### 2. Aim

The aim of the study was to evaluate the losses of spores of the fungus *Isaria fumosorosea* depending on multiples of liquid flow in use by the system containing hydraulic agitator.

### 3. Materials and methods

A measuring position was built of a tank, a jet agitator and a diaphragm pump Comet BP 40/15. The research used manual jet agitator Geoline with a diameter of the exit aperture of 1.2 mm. A block diagram of the measuring position is shown in Fig. 1.



Source: own work / Źródło: opracowanie własne

Fig. 1. The measuring stand: 1 – starter drive, 2 – diaphragm pump, 3 – jet mixer, 4 – tank, 5 – temperature gauge, 6 – pressure sensor, 7 – electromagnetic flowmeter

Rys. 1. Stanowisko badawcze: 1 – przystawka napędowa, 2 – pompa membranowa, 3 – mieszadło strumieniowe, 4 – zbiornik, 5 – miernik temperatury, 6 – czujnik ciśnienia, 7 – przepływomierz elektromagnetyczny

Parameters measuring liquid flow in the supply line mixer used connected to a computer via a card measuring DAQCARD NI-6024 pressure sensor KELLER type PA-21SR / 100 and electromagnetic flow meter ARAG, type 4621BA03333. With the flow meter a flow rate of liquid through the plant can be read, and the total volume of liquid that has passed through the system from the start of the experiment. The temperature of the liquid in the tank is measured by an electronic thermometer type DT 1. In order to not interfere with the test of multiple flows the bypass valve was eliminated, and to control the fluid pressure in the system a starter drive control of the pump rotation was used. The starter drive was connected to the pump with a jointed shaft. The fluid pressure in the system was set at 6.0 bar. At this pressure, fluid flow rate was 2.5 dm<sup>3</sup>/min. Multiplicity of the liquid flows is determined by the formula 1:

$$K = Q_c / Q_z, \quad (1)$$

where:

- K – multiplicity of the liquid flow,
- Q<sub>c</sub> – overall liquid velocity that flowed through the system from the beginning of the experiment [dm<sup>3</sup>],
- Q<sub>z</sub> – liquid velocity [dm<sup>3</sup>].

In the research a biological crop protection formulation Preferal was used, containing 2 x 10<sup>9</sup> spores of a parasitic fungus *Isaria fumosorosea* in 1 gram of agent. Preferal is designed primarily to control insects in greenhouses. According to manufacturers, formulation was mixed with water preparing a spray solution having a concentration of 0.3%; the total volume of liquid in the test system is 5 dm<sup>3</sup>.

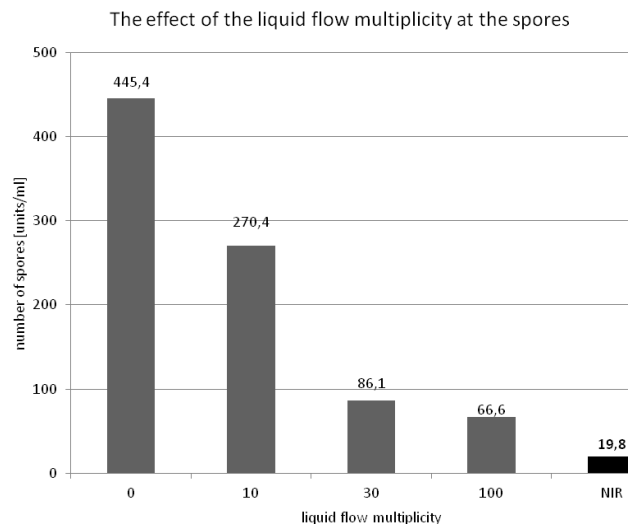
During the experiment the liquid samples were collected of a volume of 0.02 dm<sup>3</sup> before pouring it to the tank, then after 10, 30 and 100-fold flow of liquid through the system. From each sample 0.625 ml of liquid was collected for microscopic analysis of the number of spores in a liquid; and counted 50 fields of view in the eyepiece of the microscope a magnification of 100 times (10 x ocular lens 10). Number of test suspension for 50 fields of view was 0.625 ml, hence; the number of spores for 1ml of suspension stemmed from the formula 2:

$$x = \frac{\sum(50 \text{ pól})}{0,625} \quad (2)$$

For the assessment of the purity of biologicals before the experiment, tests were performed on the same water used for mixing compounds, and liquids from the spores of the fungus *Isaria fumosorosea* used in the research. Inoculation was performed through Koch method on Petri dishes with the use of Sabouraud agar with gentamycin and chloramphenicol. Inoculation of liquid from the spores was made before it was poured into the tank, then performed for each sample taken during the experiment. All cultures were performed in triplicate. The dishes were placed in an incubator at 22°C, and after 5 days of incubation the grown fungus colonies were overviewed.

#### 4. Results and analysis

In order to determine the significance of the effect of multiple fluid flow in the system on the survival of *Isaria fumosorosea* spores analysis of variance was performed using the results obtained with counting an amount of spores. The analysis of variance has shown significance effect of a test agent on the amount of spores at significance level α = 0.05. The results are presented in Fig. 2. Calculated value of the least significant difference, which amounted to NIR = 19.79.



Source: own work / Źródło: opracowanie własne

Fig. 2. Quantitative assessment of spores reduction

Rys. 2. Ocena ilościowa redukcji zarodników



Source: own work / Źródło: opracowanie własne

Fig. 3. Inoculation of spores of the fungus *Isaria Fumoso-rosea* in a Petri dish

Rys. 3. Posiew zarodników grzyba *Isaria Fumoso-rosea* na szalce Petriego

The experiment confirmed the hypothesis that the hydraulic stirrer and fluid flow multiplicity through hydraulic sprayer agitator system can destroy spores of the fungus *Isaria fumosorosea* insecticide. It was found that the higher fluid flow multiplicity the lower number of spores. The largest decrease was observed after 30-hundredfold flow of liquid, the amount of spores was less by 359.3 units/ml as regards the references to attempts taken before pouring the liquid into the tank (0). Then the decline has stabilized, and 100-fold fluid flow, decrease in the number of spores was lower by 378.8 units/ml.

An example of a Petri dish inoculated colonies of fungus *Isaria fumosorosea* is shown in Figure 3. The review of examined fungus colonies grown on Petri dishes did not find a group of fungi than the one used in the study, so the liquid is mixed with Preferal used for test including only the fungus *Isaria fumosorosea*.

## 5. Conclusions

Analysis results of measurements indicate that the multiplicity of fluid flow through the nozzle of the hydraulic mixer has a significant impact on reducing the number of spores of the fungus *Isaria fumosorosea* contained in the circulating liquid.

The study clearly showed that the use of the sprayer to perform agricultural treatments using biologicals - the fungus *Isaria fumosorosea*, can cause loss of the active agent. It is necessary for the selection of preparations application for the treatment such parameters to multiple circulation of the liquid in the sprayer as low as possible.

## 6. References

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