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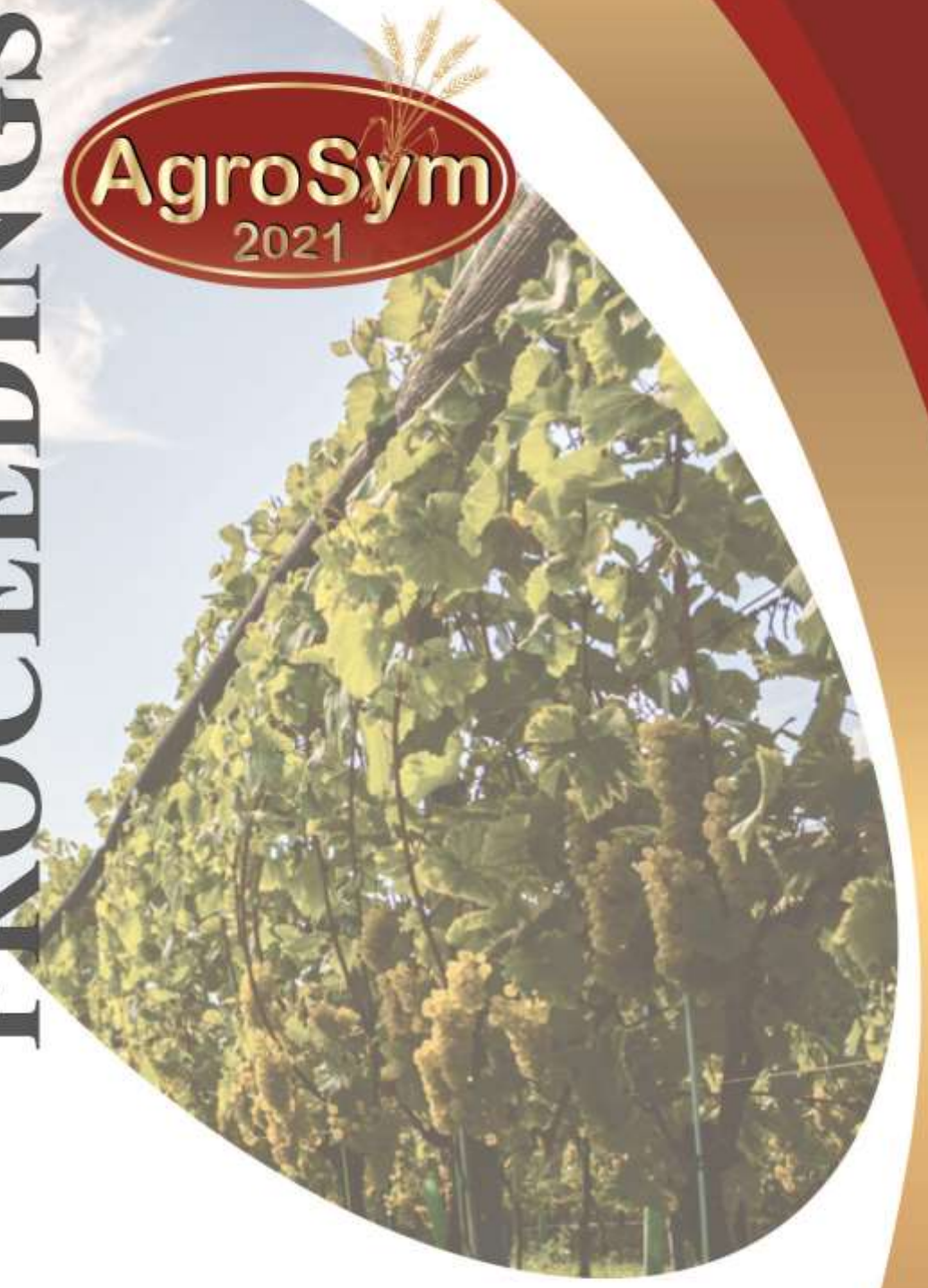

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THE INFLUENCE OF SOME BIO-PRODUCTS ON GERMINATION AND PROTECTION OF *CHAMOMILLA RECUTITA* (L.) RAUCH SEEDS

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Abstract

The effects of six biological plant protection products, permitted in organic production ('Extrasol F', 'Polyversum', 'Timorex gold',' Vegard', 'Ozoneem trichul', 'EcoBooster Calcium'), in addition to two herbal preparations ('LAB 3' and 'LAB 4'), were examined on germination and seed quality of *C. recutita* cv. "Banatska". The aim of study was to discover the most effective bio-product with beneficial effects on quality and health of German chamomile seeds. Seed germination testing was conducted according to the standard procedure suggested by a Rule book on seed quality control, while the seed health was examined by the filter paper method. The seeds were treated with 15 ml of bio-product solution, while the same amount of distilled water was used as a control. The experiment was conducted in triplicates, and the seeds were observed on 14th day following the treatment. In comparison to control, seed germination rate was increased in following treatments: 'LAB 4' by 11%, 'Extrasol F' by 9%, 'EcoBooster Calcium' and 'LAB 3' by 2%. Based on the symptoms observed on the seed surface, presence of *Alternaria* sp. was confirmed. Compared to control, the infection of seeds was reduced by 1% in treatments with 'Vegard', 'LAB 3' and 'LAB 4'. However, doubling the concentration didn't reduce infection but reduced germination in comparison to control. Since 'LAB 4' and 'Extrasol F' affected the most germination and to a certain extent prevented seed infection, both bio-products could be recommended for safe application on chamomile seeds.

Keywords: *German chamomile, germination and seed health, biological products, Alternaria* sp., *organic.*

Introduction

Chamomilla recutita L. is one of the most famous medicinal plants in Serbia, belonging to the Asteraceae family. It is cultivated for its inflorescences. Once properly dried, they represent well known herbal drug *Chamomilae flos*, which is widely used in pharmaceutical and cosmetic industries. It contains 0.2-1% essential oil (*Aetheroleum chamomilae*) whose color, depending on temperture, varies from dark blue to green (Glamočlija et al., 2015). As the plant is well-known for it antiseptic, antibacterial and antiviral properties the demands for its herbal drug is continuosly increasing. Apart that German chamomille is used in various herbal preparations for plant nutrition and protection it is also used in the composting procedure (Oljača et al., 2020). Therefore, it is quite important to enable conditions for production of healthy and safe herbal raw material, free from pesticide residues, heavy metals and economically harmful pests and pathogens. In order to produce a sufficient amount of such raw material, healthy and good quality seeds of German chamomile should be used.

Apart to commonly applied conventional measures, in attempt to increase seed germination various bio-agrotechnical measures use to be examined, particularly the "bio-products". The term bio-products stands primarily for toxicologically harmless and ecologically completely acceptable products. They might not be as effective as synthetic chemicals but they use to keep the level of harmful microorganisms below the threshold of economic harmfulness and within acceptable limits (Milenković, 2015). Among them stand out, 'Extrasol F' is a bio-product composed of rhizosphere nitrogen-fixing bacteria (*Bacillus subtilis* strain Č13) intended to improve seed germination. The bio-fungicide 'Polyversum' is based on the fungus *Pythium oligandrum* and is recommended for protection of seeds from economically important phytopathogenic fungi. The active ingredient of 'Timorex gold' bio-product is a tea tree essential oil (*Melaleuca alternifolia*) dissolved in paraffin oil (15 x 10^{10} CFU/ml), and apart to conventional it could be also used in organic production for protection against fungal pathogens causing symptoms of gray mold and potato blight (Kołodziejczik, 2018).

The application of bio-products based on beneficial fungi, bacteria and plant extracts can prevent the appearance of phytopathogenic fungi causing decay of cultivated plants but also damping of seedlings if the plantation use to be established via nursery plants. Soil pathogens such as *Pythium debarianum*, *Fusarium* spp., *Sclerotinia* spp., *Phytophtora* spp., *Aspergillus* spp., *Alternaria* spp., *Rhizoctonia* spp. and others are mainly causing of such symptoms.

The aim of study was to examine the influence of various bio-products - biofungicides, biostimulants and bioinsecticides, and some herbal preparations on quality and health of German chamomile seeds.

Material and methods

The study was conducted in the laboratory of Agricultural Research and Development of the Institute for Medicinal Plants Research "Dr Josif Pančić" in Belgrade. The two-years old seeds of German chamomile (*C. recutita* L. Rauch.) cv. "Banatska", produced at the experimental field of the Institute, have been subjected to testings on seed germination and seed health, during 2021. The details on six bio-products (from the list of plant protection and plant nutrition products and the list of soil improvers, permitted in organic production) and two herbal preparations (created in the Institute), are provided in Table 1.

Table 1. Bio-products used in the treatments of German chamomile seed

Bio-product	Active substances		Short business name	
	name	content	manufacturer	representative
Extrasol F	*Bacillus subtilis* strain Č13	1 x 10^8 CFU/cm^3	BioGenesis, Bačka Topola and Jugo Hem, Leskovac	-
Polyversum	*Pythium oligandrum*	3% (1 X 10^6 - 10^7 oospore/g)	Biopreparaty, Czech Republic	Vins 2000, Belgrade
Timorex gold	tea tree oil *(Melaleuca alternifolia)*	222.5 + 194.5 g/l	Stockton Israel, Israel	Stockton, Belgrade

	+ paraffin oil			
Ozoneem trichul 1% EC	Azadirachtin	10 g/l	Ozone Biotech, India	BioGenesis, Bačka Topola
EcoBooster Calcium®	organic nitrogen fertilizer of animal and plant origin	3-3.5% total, 1.5-2% organic N, 5% organic C, CaO min. 2.5%, max. 12 C/N	Ekopatent, Vrbas	Ekopatent, Vrbas
Vegard	fertilizer and special products	amino acids 1-2%, fulvic acids 5%, humic acids 5%, other organic materials 20%	Beijing Kingbo Biotech, China	Timings, Belgrade
LAB3	herbal preparation for protection and plant nutrition	fresh aerial parts of chamomile 20%, horsetail 20%, valerian 20%, dandelion 20%, yarrow 10%, nettle 10%, comfrey 5% and liquorice 5%	Institute for Medicinal Plants Research "dr J. Pančić", Belgrade	
LAB4	herbal preparation for protection and plant nutrition	fresh aerial parts of chamomile 50% and valerian 50%	Institute for Medicinal Plants Research "dr J. Pančić", Belgrade	

Testing of bio-products on the quality of German chamomile seeds

The energy and total seed germination were examined as suggested by the Rule book on seed quality control („Official Gazette of the Socialist Federal Republic of Yugoslavia", no. 47/87, 60/87, 55/88 and 81/89, „Official Gazette of the Socialist Republic of Yugoslavia", no. 16/92, 8/93, 21/93, 30/94, 43/96, 10/98, 15/2001 and 58/2002 and „Official Gazette of the Republic of Yugoslavia", no. 34/2013). In short, the seed germination testing was conducted with 100 seeds on filter paper in Petri dishes, in triplicates. The seeds were previously treated with 15 ml of previously prepared solutions of the following single bio-products prepared in two concentrations: 'Extrasol F' (0.20 and 0.40 ml/kg), 'Polyversum' (0.50 and 1 g/kg), 'Timorex gold' (1 and 2 ml/kg), 'Ozoneem trichul' (1 and 2 ml/kg), 'EcoBooster Calcium' (0,50 and 1 ml/kg), 'Vegard' (0,50 and 1 ml/kg), 'LAB 3' (1 and 2 ml/kg) and 'LAB 4' (1 and 2 ml/kg), all being dissolved in 100 ml of distilled water. The same amount of distilled water (15 ml) was used in the control treatment. The number of germinated seeds was counted by the use of binocular loupe on 4^{th} and 14^{th} day from the day of setting up the experiment (ISTA, 2010).

The seed health status of German chamomile

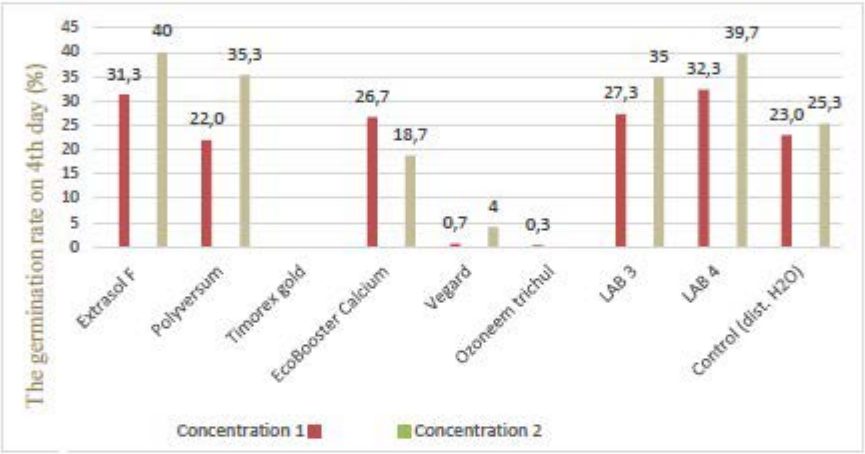
The seed health status was examined by the filter paper method. The experiment was conducted on previously sterilised (110 °C i 1h) and moistened filter paper, using 100 unsterilised seeds, in triplicates. Macroscopic and microscopic seed examinations were performed 14^{th} day following their incubation in humidity chamber at T 20±2 °C. An Olympus CX43 microscope (Olympus,

Hamburg, Germany) was used to observe the microscopic characteristics of the phytopathogenic fungi developed on the seeds, and the photographs were taken with an Axiocam ErC.5s (Zeiss, Göttingen, Germany).

Results and discussion

Comparative presentation of the efficacy of eight bio-products tested in two concentrations, on germination rate of German chamomile seeds, observed on 4th experimental day, are given in Graph 1.

Graph 1. The germination rate of German chamomile seeds observed on 4th day of the experiment (%).



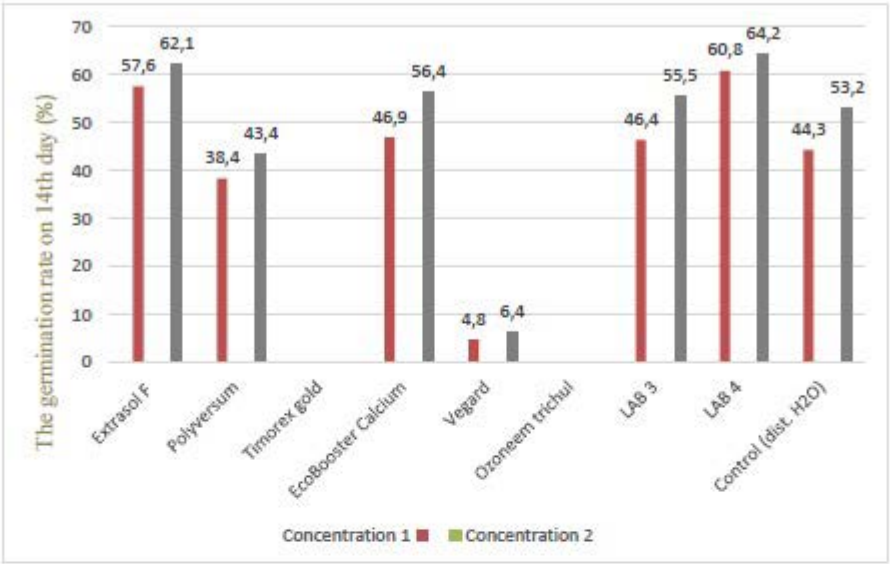
In seeds treated with the first (lower) concentrations of tested bio-products, the germination rate proved to be the highest in 'LAB 4' (averagely 32.3%) although similar results were also obtained with 'Extrasol F' (31.3%) and somewhat lesser with 'LAB 3' (27.3%).

The seeds treated with the second (higher) concentration of bio-product, generally had higher EG values (on average by 3.8%) compared to those achieved by the first concentration. In the treatment with second (higher) concentration of bio-product, the highest germination rate was achieved with 'Extrasol F' (40.0%) followed by 'LAB 4' (39.7%), although 'Polyversum' did not lag behind them a lot (35.3%).

The product 'Extrasol 55' stopped the development of economically significant plant diseases in grain crops (Jevtić et al., 2005), while the product of 'Polyversum' significantly affects on the germination rate of bean and soybean seeds (Horoszkiewicz-Janka et al., 2013).

Comparative presentation of the efficacy of eight bio-products tested in two concentrations, on the total germination of German chamomile seeds, observed on 14th experimental day, are given in Graph 2.

Graphs 2. The total germination of German chamomile seeds observed on 14th day of the experiment (%).



In the treatments with two concentrations of bio-products, the highest total of germination was achieved with 'LAB 4' (60.8% and 64.2%) followed by 'Extrasol F' (57.6% and 62.1%), while 'EcoBooster Calcium' had an effect (46.9% and 56.4%). The bio-products 'Timorex gold' and 'Ozoneem trichul' did not effect on seed germination. Filipović et al. (2014) showed that herbal preparation 'LAB 1' is shown beneficial effect on the EG and TG of two medicinal plant species, white origano (*Origanum heracleoticum* L.) and marjoram (*Origanum vulgare* L.). The biofungicides 'Extrasol F' and 'Polyversum' applied in the recommended concentrations (0.20 ml/kg and 0.50 g/kg, respectively), had an effect on fennel seed germination 73.3% and 64.7% (Filipović et al., 2021).

Comparative presentation of the efficacy of eight bio-products tested in two concentrations, on the occurence of German chamomile seeds pathogens, observed on 14th experimental day, are given in Graph 3.

Graphs 3. The occurence of pathogens on German chamomile seed observed on 14th day of the experiment (%).

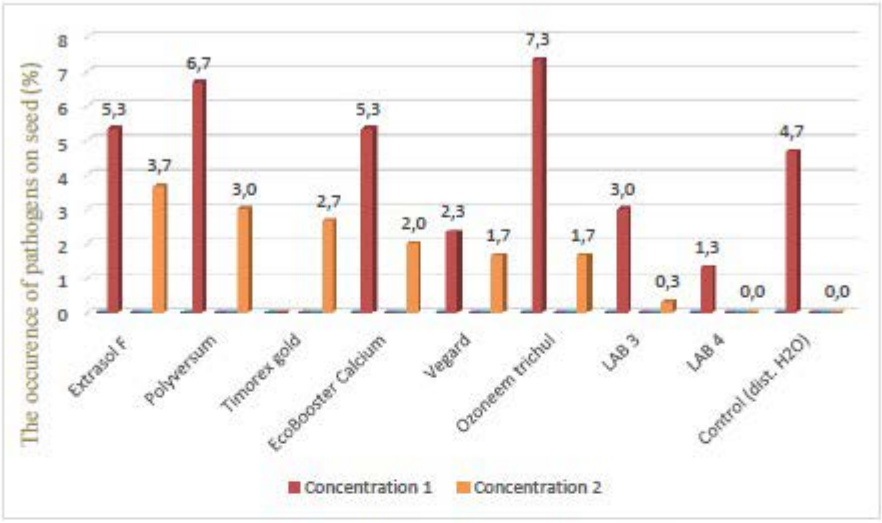


Photo 1. The symptoms of dark mycelia on the seed surface

Photo 2. *Alternaria* sp.

Macroscopic examinations revealed the symptoms of dark mycelia on the seed surface, while the microscopic confirmed the presence of *Alternaria* sp. 'Timorex gold' in a lower concentration was prevented the occurence of seed infection, while the most infected seeds were recorded in the treatment with bioinsecticide 'Ozoneem trichul' (7.3%). In the treatment with higher concentration of 'LAB 4', the pathogens of genus *Alternaria* did not appear on the seed. Similar effect was achieved with 'LAB 3' (0.3%). The occurence of *Alternaria* sp. on the seed was confirmed after the treatment with 'Extrasol F' (3.7%), 'Polyversum' (3.0%) and in the control treatment (3.0%). In the research Filipović et al. (2021), the phytopathogenic fungi on fennel seed identified as *Alternaria* sp. The bio-products 'Extrasol F' and 'Polyversum' in the recommended concentrations (0.2 ml/kg i 0.5 g/kg) affected on reduce infected seeds. The higher concentrations did not reduce occurence of infected seeds.

Conclusions

The several bio-products showed positive effect on germination and reduction of pathogens on tested German chamomile seed cv. „Banatska". Bio-products 'LAB 4', 'Extrasol F' and 'EcoBooster Calcium' could be recommended for improvement of the germination rate and total seed germination of German chamomile seeds, while the 'Timorex gold' and 'LAB 4' could be recommended for the safe use in prevention of the seedborne diseases of *Ch. recutita* cv. „Banatska".

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