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**AgroSym**  
2022

A large, oval-shaped photograph of a sunflower field under a blue sky with light clouds. The sunflowers are in various stages of bloom, with bright yellow petals and dark brown centers. The green leaves of the plants are visible in the foreground.

**BOOK OF  
PROCEEDINGS**

*XIII International Scientific Agriculture Symposium  
"AGROSYM 2022"  
October 6-9, 2022*

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**AGRO 2022**  
**sym**

# **BOOK OF PROCEEDINGS**

**XIII International Scientific Agriculture Symposium  
“AGROSYM 2022”**



**Jahorina, October 06 - 09, 2022**

## **Impressum**

XIII International Scientific Agriculture Symposium „AGROSYM 2022“

### **Book of Proceedings Published by**

University of East Sarajevo, Faculty of Agriculture, Republic of Srpska, Bosnia  
University of Belgrade, Faculty of Agriculture, Serbia  
Mediterranean Agronomic Institute of Bari (CIHEAM - IAMB) Italy  
International Society of Environment and Rural Development, Japan  
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CIP - Каталогизacija у публикацији  
Народна и универзитетска библиотека  
Републике Српске, Бања Лука

631(082)(0.034.2)

### **INTERNATIONAL Scientific Agriculture Symposium "AGROSYM" (13 ; Jahorina ; 2022)**

Book of Proceedings [Електронски извор] / XIII International  
Scientific Agriculture Symposium "AGROSYM 2022", Jahorina, October 06  
- 09, 2022 ; [editor in chief Dusan Kovacevic]. - Onlajn izd. - El. zbornik. -  
East Sarajevo : Faculty of Agriculture, 2022. - Ilustr.

Sistemski zahtjevi: Nisu navedeni. - Način pristupa (URL):

[http://agrosym.ues.rs.ba/article/showpdf/BOOK\\_OF\\_PROCEEDINGS\\_2022\\_FINAL.pdf](http://agrosym.ues.rs.ba/article/showpdf/BOOK_OF_PROCEEDINGS_2022_FINAL.pdf). - El. publikacija u PDF formatu opsega 1432 str. - Nasl. sa naslovnog ekrana. - Opis izvora dana 30.11.2022. - Bibliografija uz svaki rad. - Registar.

ISBN 978-99976-987-3-5

## THE INFLUENCE OF SOME BIO-PRODUCTS ON GERMINATION AND PROTECTION OF BASIL SEEDS

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### Abstract

Basil (*Ocimum basilicum* L.) is an annual herb with medicinal, edible and economical values. To reach a yield potential, among other things, seed high germination percentage and seed vigor are required. The aim of the study was to discover the most efficient biopreparations with a beneficial effect on basil seed vigor, germination and seed health. Testing was conducted with two plant protection products, permitted in organic production ('Exstrasol F', 'Polyversum'), and three herbal preparations (fermented valerian extract, fermented extract of yarrow, and fermented LAB mix). Seed germination testing followed the standard procedure suggested by the Rule book on seed quality control, while 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 three replications, and the seeds were observed on the 4<sup>th</sup> and 14<sup>th</sup> day following the treatment. The seed germination was increased in all treatments, in comparison to control. The treatments with fermented extract of yarrow had the highest effect on seed vigor. Based on the symptoms, the presence of *Alternaria* sp. was confirmed on the seed surface. Compared to control, the infection of seeds was reduced in all treatments, but the fermented extract of yarrow had the highest effect on disease reduction.

**Keywords:** *Ocimum basilicum* L., bio-products, fermented extracts, germination, seed health, *Alternaria* sp.

### Introduction

Basil (*Ocimum basilicum* L.) is an annual herbaceous, a thermophilic plant species belonging to the Lamiaceae family. It is an ornamental, spicy, and medicinal plant (Stepanović and Radanović, 2011), used as fresh or dried for flavoring food, making tea, and liqueurs. The above-ground part of the plant (*Basilici herba*) is used in traditional and homeopathic medicine for the treatment of a number of diseases, and it is also used as a carminative, sedative, lactagogue and to improve appetite as well (Filipović et al., 2016). Basil essential oil (*Basilici aetheroleum*) is well-known for its bactericidal, fungicidal, antiviral, repellent, antioxidant, antidiarrheal, chemopreventive, and radioprotective properties (Kišgeci et al., 2009). It is mostly grown in Italy at about 80 ha, then in France on 30 ha and in Israel on 20 ha. In Serbia, basil has not been grown on larger areas in the past, but in recent years it has been grown more, especially in the Vojvodina region (Kišgeci et al., 2009; Stepanović and Radanović, 2011). Basil is often grown by direct sowing due to their good germination energy and total germination. The effect of lower

temperature on basil growth decreasing is well known (Walters and Currey, 2019), but the effect of bio-products in mentioned conditions has not been well characterized.

In an attempt to increase seed germination various “bio-products” has been commercially used (Filipović et al., 2021). The bio-products based on plant extracts can prevent the appearance of phytopathogenic fungi, as well. 'Exstrasol F' is a bio-product composed of rhizosphere nitrogen-fixing bacteria (*Bacillus subtilis*) intended to improve seed germination. The 'Polyversum' is based on the fungus *Pythium oligandrum* and is recommended for protection of seeds from economically important phytopathogenic fungi such as *Fusarium* spp., *Pythium* spp., *Peronospora belbahrii* and *Alternaria alternata* are mainly causing decay of cultivated plants and damping of seedlings (McLeod et al., 2006; Taba et al., 2009; Garibaldi et al., 2011).

The aim of this study was to examine the influence of some herbal preparations on germination and health of basil seeds on lower temperature.

### 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 (Serbia). The seeds of basil (*O. basilicum* L.) cv. Genovese, produced at the experimental field of the Institute, have been subjected to tests on seed vigor, germination, and seed health, during 2022. Two bio-products (from the Lists of plant protection and plant nutrition products and the list of soil improvers, permitted in organic production) and three herbal preparations (created in the Institute), are used in the treatments of basil seed.

#### Testing of biopreparations on the seed germination

The energy (EG) and total seed (TG) germination were examined as suggested by the Rule book on seed quality control („Official Gazette of the Republic of Yugoslavia“, no. 23/2009, 64/2010, 72/2010 and 34/2013). The seed germination testing was conducted with 100 seeds on filter paper in Petri dishes, in triplicates. The seeds were treated with 15 ml of previously prepared solutions: 'Exstrasol F' (recommended concentration 0.20 ml/kg), 'Polyversum' (recommended concentration 0.50 g/kg), fermented valerian extract, fermented extract of yarrow, and fermented LAB mix (5 ml herbal preparations being dissolved in 45 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 a binocular loupe on 4<sup>th</sup> and 14<sup>th</sup> day from the day of setting up the experiment (ISTA, 2010). Preliminary research on germination at a temperature of 22°C was conducted in triplicates, as well. Germination was 94%. The experiment was conducted in a humidity chamber at T 19/16°C day/night regime.

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

Bio-product	Active substances		Short business name	
	name	content	manufacturer	representative
Exstrasol F	<i>Bacillus subtilis</i> strain Č13	1 x 10 <sup>8</sup> CFU/cm <sup>3</sup>	BioGenesis, Bačka Topola and Jugo Hem, Leskovac	-
Polyversum	<i>Pythium</i>	3% (1 X 10 <sup>6</sup> - 10 <sup>7</sup> oospore/g)	Biopreparaty,	Vins 2000,

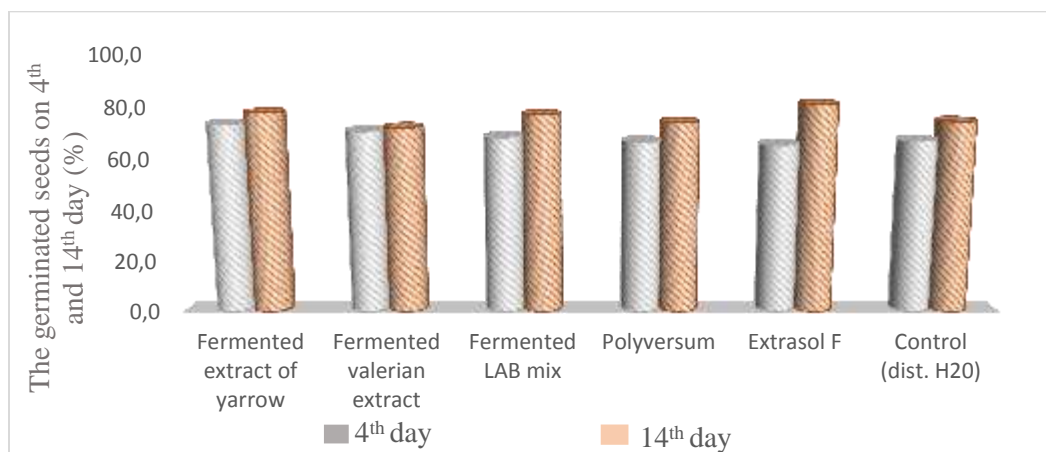
<i>oligandrum</i>		Czech Republic	Belgrade
LAB mix	herbal preparation for protection and plant nutrition	fresh aerial parts of chamomile 30%, horsetail 20%, valerian 15%, nettle 15% and comfrey 20%	Institute for Medicinal Plants Research “dr J. Pančić”, Belgrade

*The seed health status of basil*

The seed health status was examined by the filter paper method. The experiment was conducted on previously sterilised (110°C/1h) and moistened filter paper, using 100 unsterilised seeds, in triplicates. Macroscopic and microscopic seed examinations were performed 4<sup>th</sup> and 14<sup>th</sup> day following incubation. 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 Olympus EP50 (Olympus, Hamburg, Germany).

**Results and discussion**

The efficacy of five bio-products, with different active substances (Tab. 1), was tested in recommended concentration, on the energy germination and the total germination of basil seeds. Observed was conducted on 4<sup>th</sup> and 14<sup>th</sup> experimental days (Graph 1).



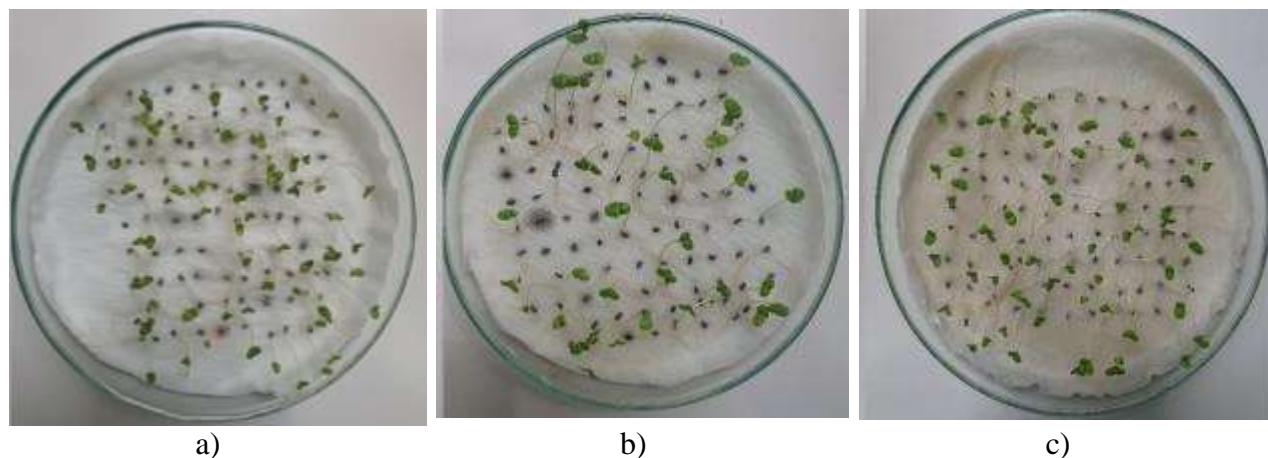
Graph 1. The energy germination and the total germination of basil seeds observed on 4<sup>th</sup> and 14<sup>th</sup> days of the experiment (%).

Germination was higher than 20% at T 22°C than at T 19/16°C, which is in agreement with previous studies where it was concluded that higher temperatures (>20 °C) accelerate the growth and development of basil (Walters and Currey, 2019). Although germination at lower temperatures was significantly lower it was still on a satisfying level according to the Rule book on seed quality control („Official Gazette of the Republic of Yugoslavia“, no. 23/2009, 64/2010, 72/2010 and 34/2013).

In the treatments at T 19/16°C with herbal preparations and biofungicides, the highest energy of germination was achieved with 'Fermented extract of yarrow' (averagely 73.7%) followed by

'Fermented valerian extract' and 'Fermented LAB mix' for 2.7% less. However, the biofungicides 'Exstrasol F' and 'Polyversum' exhibited weaker effects for 8% and 6.7% than the control.

The seeds treated with 'Exstrasol F' and 'Polyversum', had higher total germination values (on average by 15.6% and 7.3%) compared to those achieved after 4<sup>th</sup> day. After 14<sup>th</sup> days, the herbal preparations 'Fermented extract of yarrow' and 'Fermented LAB mix' had a similar effects on total germination seeds (78% and 77.3%). The product 'Fermented valerian extract' had a weaker effect (2.4%) compared to the control (Picture 1).

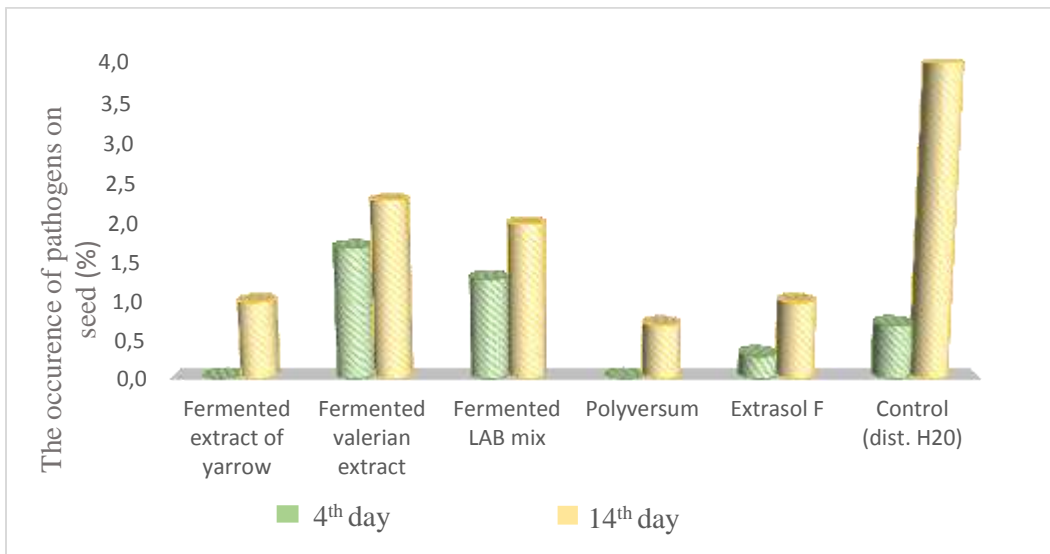


Picture 1. Total germination: a) Control treatment; b) 'Polyversum'; c) LABmix

In earlier research, 'Exstrasol F' and 'Polyversum' also, had a positive effect on the energy germination and total germination of German chamomile seeds. Also, 'LAB 4' (fresh aerial parts of chamomile 50% and valerian 50%) had an effect on the EG and TG of chamomile seeds (32.3% and 60.8%) (Filipović et al., 2021). Filipović et al. (2014) tested EG and TG on seeds of white oregano (*Origanum heracleoticum* L.) and marjoram (*Origanum vulgare* L.) with the use of herbal preparation 'LAB 1' (fresh herbs of chamomile (20%), horsetail (20%), valerian (20%), yarrow herb (10%), nettle (10%), comfrey (10%) and licorice (10%)) which has a beneficial effect on tested characters, as well.

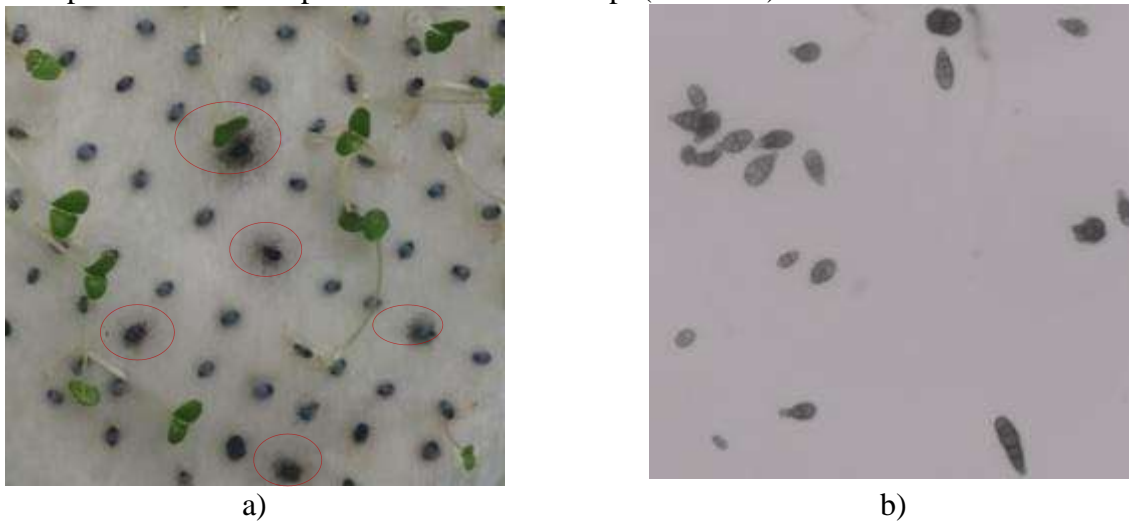
The efficacy of five bio-products tested in recommended concentration, on the occurrence of basil pathogens, observed on 4<sup>th</sup> and 14<sup>th</sup> experimental days, are given in Graph 2.





Graphs 2. The occurrence of pathogens on basil seed observed on 4<sup>th</sup> and 14<sup>th</sup> days of the experiment (%).

Macroscopic examinations revealed the symptoms of dark mycelia on the seed surface, while the microscopic confirmed the presence of *Alternaria* sp. (Picture 2).



Picture 2. The symptoms of diseases: a) dark mycelia on the seed surface; b) *Alternaria* sp.

'Polyversum' and 'Fermented extract of yarrow' prevented the occurrence of seed infection after 4<sup>th</sup> day. A similar effect was achieved with 'Extrasol F' (0.3%). The most infected seeds were recorded after 14<sup>th</sup> days, in the treatments with 'Fermented valerian extract' (2.3%), 'Fermented LAB mix' (2.0%) and in the control treatment (4.0%). The pathogens of the genus *Alternaria* appeared on the seeds of fennel and chamomile (Filipović et al., 2021).

## Conclusions

The several biopreparations showed a positive effect on germination and reduction of pathogens on *Ocimum basilicum* L. Herbal preparations 'Fermented extract of yarrow', 'Fermented valerian extract' and 'Fermented LAB mix' could be recommended for improvement of the germination energy and total germination of basil seeds, while the 'Polyversum' and 'Exstrasol F' could be recommended as one of the measures to prevent the occurrence of basil seed disease.

## Acknowledgement

The research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/ 200003, 200011 and 200032).

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