



# Examination of the Effect of Four Pesticides Used in Practice on *Beauveria* Strains Under Laboratory Conditions



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## ABSTRACT

Biological pest control is increasingly crucial and emphasized in research, leading to the frequent use of entomopathogenic fungi such as *Beauveria bassiana* and *B. brongniartii*. Integrated pest management often requires multiple control agents to address various species simultaneously, raising the question of the interaction between the utilized fungi and the other active agents applied simultaneously. The present study examined the interactions between active ingredients and entomopathogenic fungi in laboratory conditions. The results indicate that insecticides and herbicides containing diazinon or glyphosate have neutral or positive effects on the examined *Beauveria* species. However, fungicides with the active ingredients penconazole or sulfur demonstrated adverse effects when used alongside the tested entomopathogenic fungi. The combined use of fungicides and fungi deserves examination because, in many cases, fungal diseases appear simultaneously with pests, e.g., powdery mildew.

## TANULMÁNY INFÓ

### Kulcsszavak:

Erdővédelem  
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## KIVONAT

**Négy, a gyakorlatban használt növényvédő szer hatásának vizsgálata különböző *Beauveria* törzsekre laboratóriumi körülmények között.** Az erdővédelemben egyre inkább előtérbe kerül a biológiai védekezés. Ennek keretein belül gyakran alkalmaznak entomopatogén gombákat, mint például a *Beauveria bassiana* és a *B. brongniartii*. Sokszor szükséges kombinált módon a károsítók és kórokozók ellen egyaránt védekezni, melynek során különböző hatóanyagok egyidejű kijuttatása elkerülhetetlen. Felvetődik a kérdés, hogy ezen gombafajok és a leggyakrabban velük együttesen használt egyéb növényvédő szerek hatóanyagai milyen hatással vannak egymásra. Különböző *Beauveria* törzsek növényvédőszer-hatóanyagokkal való kölcsönhatását vizsgáltuk laboratóriumi körülmények között. A gyakorlatban használt diazinon, illetve glifozát hatóanyagú rovar- és gyomirtó szerek egyidejű alkalmazása többnyire semleges, vagy egymást erősítő hatást mutatott. A penkonazol vagy kén hatóanyagú gombaölő szerek esetében viszont az azonos területre történő kijuttatás nem célravezető, mert a gomba hatását gátolta mindkét hatóanyag. A gombaölő szerek és a gomba együttes használatát azért kell vizsgálni, mert sok esetben megjelenik a kártevőkkel párhuzamosan gombabetegség is a kezelni kívánt állományban pl. lisztharmatok.

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## 1 INTRODUCTION

Soil-dwelling pests have significant adverse effects on forest management and agriculture in Europe. Various Melolonthinae species larvae are particularly major limiting factors of any afforestation, reforestation, or tree planting activities because they feed on the roots of freshly planted seedlings (Fröschle, 1996; Luisa and Mauro, 1996; Hirka and Csóka, 2011). Pest control methods are numerous, including mechanical, chemical, and biological control agents, but most are complicated to apply due to the complex biology and life cycle of the target insect pests. Although biological control is favored over chemical and mechanical methods, efficient and user-friendly methods are often lacking, emphasizing the need to develop new biocontrol agents and methods. Unfortunately, climate change only exacerbates the problem.

The European Union aims for a climate-neutral continent by 2050 and has recently implemented its Farm to Fork Strategy, which supports the transition towards sustainable food systems by reducing chemical pesticide use by 50% and encouraging organic farming. (European Green Deal, 2019-2020-2022; European Commission - Farm to Fork strategy, 2020).

Various herbicides, insecticides, fungicides, and soil disinfectants are utilized in chemical control to enhance crop and tree growth. Agriculture on large, connected monocultural fields increases disease frequency and infestation severity. Applying soil disinfectants and fungicides prepares the soil for the seeds and seedlings by protecting vulnerable roots and sprouts. Such disinfectants and fungicides are usually only harmful to the target organisms. However, herbicides and insecticides often contain substances that can accumulate and harm beneficial creatures like bees (it raises concerns lately) and, over the long term, mammals, including humans (Nikolopoulou-Stamati et al., 2016; Pathak et al., 2022; Kaur et al., 2024).

Oak forests experience the most soil-living insect damage in forestry, regardless of the reforestation method. Young plants are significantly impacted by powdery mildew infection and cockchafer grub feeding on their roots in nurseries and during natural reforestation. Simultaneously controlling powdery mildew and cockchafer damage is the main focus of oak reforestation efforts. When using entomopathogenic fungi for insect damage control, it is also crucial to focus on the agents involved in powdery mildew control and examine the interactions of these agents.

Herbicides and insecticides are less likely to interact with the fungi but still require inspection. Soil disinfectants and fungicides applied in the same medium – soil – are in direct contact with such agents, entailing a considerable likelihood of interaction between the chemicals and the fungi. Diazinon is a widespread and commonly used active soil disinfection ingredient. Some studies have labeled diazinon as harmful to fungi (Khun et al., 2020), but this claim requires investigation because it is likely that the sterile circumstances secured by the disinfectant hold more benefits than harms for fungal growth. Since fungicides destroy or hinder fungi, they also harm *Beauveria* species. The open question is, to what degree?

As 'good fungi,' *Beauveria* species are neutral to plants and a good biological alternative to insecticides. Simultaneous application with the above-mentioned chemical control agents needs to be studied to decide which can be used safely together without affecting the other negatively.

*Beauveria* species are asexually reproducing, cosmopolitan, and entomopathogenic; such characteristics are all pathogenic to insects. The majority of *Beauveria* species breed in soils where their target organisms live. The most common of these fungi is *Beauveria bassiana*, widely used as a biocontrol agent in agriculture. Another species of the genus, *Beauveria brongniartii*, appears to be a specific pathogen of scarab beetles (Zimmermann, 2007).

Unlike bacteria and viruses, fungi act via contact. and infect directly through insect exoskeletons. Hyphae grow from the spores and produce enzymes that dissolve the cuticle, thus providing entry into insect bodies. Once inside, *B. bassiana* produces a variety of toxins such

as beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein and oxalic acid, calcium oxalate, and many beauvericin analogs (Wang et al., 2021; Charnley, 2003; Kučera – Samšišňáková, 1968; Quesada-Moraga – Vey, 2004). Beauvericin is the most significant toxin because it weakens the host's immune system via nutrient deprivation, eventually killing the infected individual (Fan et al., 2017).

*Beauveria* spp. has been used in experiments for decades. The first recorded research dates to 1956, when the target species was *Cylas formicarius* (Li, 2007). Other species, such as *Dendrolimus punctatus*, *Scolytus multistriatus*, long-horn beetles, *Tetranychus evansi*, *Myzus persicae*, *Corythucha arcuata*, *Hylobius abietis*, and many others, were also experimented on (Li, 2007; Barson, 1977; Higuchi et al., 1997; Bugeme et al., 2008; Kim et al. 2013; Sönmez et al., 2016; Lalík et al., 2021), revealing that the fungus has a wide range of application potential.

Many experiments with *Beauveria bassiana* and *B. brongniartii* against *Melolontha* and other species have also been conducted in Italy, Switzerland, Austria, Poland, Turkey, and China (Keller, 2000; Enkerli et al., 2004; Kessler et al., 2004; Chałańska et al., 2017; Malusá et al., 2020; Yaman, 2019; Zhou et al., 2020).

In addition to proving that *Beauveria* species can withstand different environmental conditions, these studies confirmed that pest problems are present all over Europe and Asia.

The present study investigated the following hypotheses to discover how *Beauveria bassiana* and *B. brongniartii* are integrable as biological control agents in pest management:

- H1.) The reactions of *Beauveria bassiana* and *B. brongniartii* strains differed (inhibitory or supportive) during the various treatments.
- H2.) Fungicide treatments (penkonazol and sulfur) inhibit the growth of both *Beauveria bassiana* and *B. brongniartii* strains.
- H3.) The soil disinfectant diazinon creates a sterile environment for *Beauveria bassiana* and *B. brongniartii* strains, thus enhancing their growth.

## 2 MATERIALS AND METHODS

The experiment was conducted in the University of Sopron laboratory. The samples were grown in parafilm-sealed Petri dishes that ensured constant humidity. The dishes were kept at constant room temperature (22±1 °C) with natural light conditions without shading and received approximately 10–11 hours of light daily.

One *Beauveria bassiana* strain (labeled BORA) and three *Beauveria brongniartii* strains (labeled ART8, ART64, and ART315) were chosen based on earlier field trial results (Merő, 2016) for this experiment.

The strains originated from mycelia grown in Petri dishes on PDA (potato dextrose agar), with 15 replications per strain. Mycelia were placed in the middle of the petri dish to allow for the radial growth of the fungi. After two weeks, the samples were sprayed with four different chemicals, 3-3 repetitions each, and three control samples were left untreated per strain. *Table 1* lists the main attributes of the chosen chemicals.

Solutions were made of each of the four chemicals dissolved in distilled water, based on the recommended usage amount calculated for the Petri dish area (63.6 cm<sup>2</sup>), as given in *Table 2*.

Table 1. Main attributes of the used chemicals

Name	Active ingredient	Target	Mode of action
Taifun 360	360 g/l glyphosate	herbicide	contact
Basudin	5 % diazinon	insecticide, soil disinfectant	contact
Vegesol eReS	23 % m/m sulfur	fungicide	contact
Topaz	10 % penkonazol	fungicide	absorbable

Table 2. Applied concentrations of the chemicals

Name	Recommended usage amount	Amount/Petri dish	In solution	
			Chemical	Water
Taifun 360	5 l/ha	3.2 µl	64 µl	6.4 ml
Basudin	35 kg/ha	22.4 µg	448 µg	6.4 ml
Vegesol eReS	5 l/ha	3.2 µl	64 µl	6.4 ml
Topaz	0.5 l/ha	0.32 µl	0.64 µl	6.4 ml

Each sample was sprayed with 320 µl solution. Three samples per chemical per strain and three control samples of each strain were left untreated. Measurements The surface area of the fungal culture was measured after seven days. Every sample was photographed and placed on millimeter paper. Manual calculations were completed based on this scale.

The following statistical methods were used to evaluate results:

- The Shapiro-Wilk normality test to determine if the samples represent normal distribution
- Cochran C homogeneity test of variance

The above-mentioned tests are preconditions for any parametric statistical test. According to the normality test and homogeneity of variances test results, ANOVA tests can be used for the samples.

- ANOVA (=Analysis of Variance) to assess the effect of the chemicals
- Dunnet post hoc test to compare treated samples with control samples because this test has been developed especially for comparing samples or sample groups to one given sample or sample group (usually control group) (Lee and Lee, 2018) and
- Tukey HSD (= Honest Significant Difference) post hoc test to compare the treatments and the reaction of the strains to each other because this post hoc test is used to compare different samples or sample groups with each other. This widely used and accepted post hoc test has a high trust index to determine where and how the significant effect occurred (Lee and Lee, 2018).

Statistica software (version 13.5.0.17) was used with default settings for the above-mentioned evaluations.

### 3 RESULTS

#### 3.1 Effects of the used pesticides

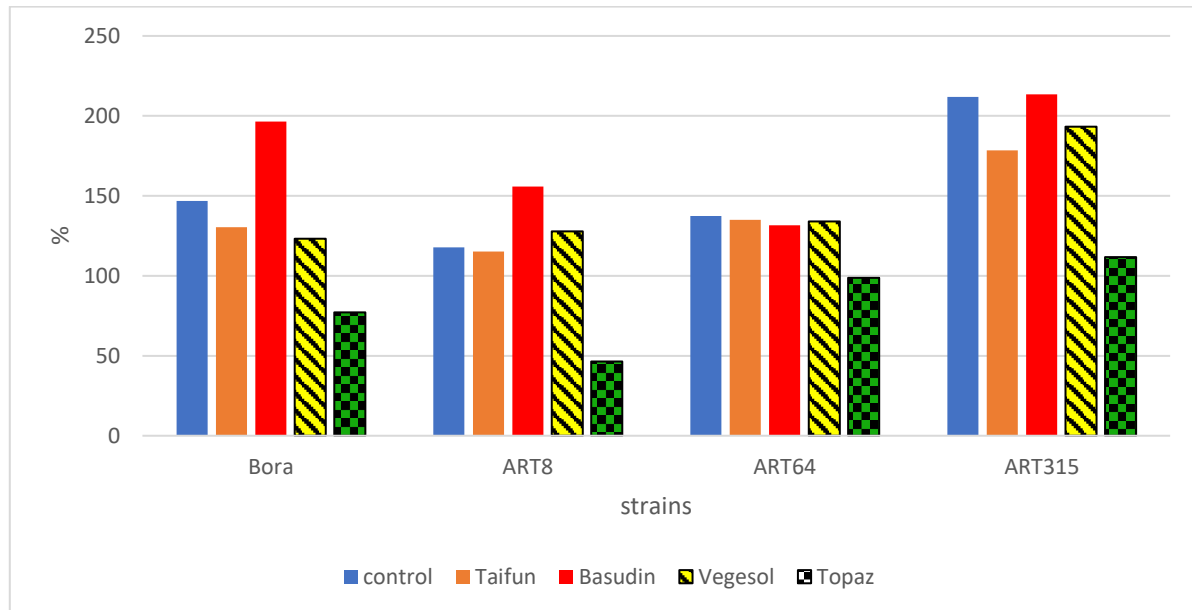


Figure 1. The effect of treatments on the growth of fungal strains seven days after spraying compared to the control samples

#### *Vegesol eReS*

As seen in Figure 1, the Vegesol eReS treatment promoted slight growth inhibition except in ART8. Since growth inhibition was insignificant, no detailed evaluation and results are provided here. ANOVA and Dunnett post hoc tests showed  $p = 0.72$  for *B. bassiana* samples and  $p = 0.83$  for *B. brongniartii* at  $p < 0.05$ .

The above-mentioned three treatments had no significant effect on the treated fungi; therefore, their data were not analyzed further.

#### *Topaz*

Samples treated with Topaz displayed growth inhibition in all cases (Figure 1), ranging from 38.58% to 100.08%, depending on the strains compared to the control samples. These Statistical analyses (Tables 3 and 4) confirm these significant results.

Table 3. Effect of Topaz treatment on strains - ANOVA,  $p = 0.05$

Effect	Univariate Tests of Significance for % Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	DF	MS	F	p
Intercept	1145475	1	1145475	2604.240	0.000
Strain	19400	3	6467	14.702	0.000
Treatment	29348	1	29348	66.723	0.000
Strain*treatment	2841	3	947	2.153	0.134
Error	7038	16	440		

SS-sum of squares; DF – degree of freedom; MS-mean square; F-value of F distribution; p-probability

Table 3 highlights the significance within the ‘strain’ row. When comparing control samples of the various strains, considerable differences can be detected among growth rates, especially when comparing any sample to the ART315 control samples (Table 4. - column {7}). The Topaz treatment shows notable growth inhibition; however, when examined with the different growth rates in the control samples, the result is insignificant. This can be explained by the outstanding value of the ART315 control samples’ growth rates.

The Dunnett post hoc test showed  $p = 0.04$  for *B. bassiana* samples and  $p = 0.002$  for *B. brongniartii* at  $p < 0.05$ . For detailed evaluation, the Tukey post hoc test was used to compare the treatments (Table 4).

### Control samples

From an average colony size of 153.435 mm<sup>2</sup> at the beginning of the experiment, the examined *Beauveria* spp. grew to a maximum of 311.78 mm<sup>2</sup> colony size within seven days. Even the slowest growing strain did more than double its size on average (ART8 – 117.88 % growth), and the fastest growing one – ART315 – showed 211.78 % growth on average, more than three times the starting size in seven days.

Several samples exhibited coloring ability, resulting in a pinkish decolorization of the agar. That was independent of the treatment, so it also appeared in the treated and the control samples.

Table 4. Effect of Topaz treatment on strains – TUKEY HSD,  $p = 0.05$

Cell No.	Strain	Treatment	Tukey HSD test; variable % Approximate Probabilities for Post Hoc Tests Error: Between MS = 439.85, df = 16.000							
			{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
			246.78	177.14	217.88	146.43	237.30	198.72	311.78	211.70
1	1	1		0.016	0.695	0.001	0.999	0.161	0.027	0.483
2	1	2	0.016		0.313	0.633	0.045	0.901	0.000	0.500
3	2	1	0.695	0.313		0.013	0.939	0.943	0.001	1.000
4	2	2	0.001	0.633	0.013		0.002	0.105	0.000	0.026
5	3	1	0.999	0.045	0.939	0.002		0.373	0.009	0.799
6	3	2	0.161	0.901	0.943	0.105	0.373		0.000	0.993
7	4	1	0.027	0.000	0.001	0.000	0.009	0.000		0.001
8	4	2	0.483	0.500	1.000	0.026	0.799	0.993	0.001	

Strain: 1 – BORA; 2 – ART8; 3 – ART64; 4 – ART315; Treatments: 1 – control; 2 – Topaz; horizontal column header numbers {1}-{8} correspond to the ‘Cell’ column respectively and the values show the growth rates in % - for example {1} 246.78 means that the average growth rate of the BORA control samples was 246.78 % in the experiment.

## 4 DISCUSSION

The study found that Topaz – the fungicide with the active ingredient penconazole – was the only one of the tested chemicals to unequivocally inhibit the growth of the used *Beauveria bassiana* and *B. brongniartii* strains (Figure 1), entailing why this treatment’s results were evaluated in more detail.

## Relation to objectives

The effect of the four chemicals used was different on the growth of the tested *Beauveria* strains. *Beauveria bassiana* and *B. brongniartii* strain reactions differed in the same treatments. Displayed inhibition in one did not entail the same in the other, contradicting the first hypothesis. The strains reacted differently in Basudin (diazinon) and Vegesol eReS (sulfur). The former increased the growth rates of *B. bassiana*, while the latter decreased them. Basudin had a variable effect on *B. brongniartii* – in one case, it caused a notable increase, while in another case, it caused only a slight increase. In the third case, a slight inhibition in mycelium growth occurred. Growth inhibition was detected with Taifun (glyphosate) and Topaz (penkonazol) in all cases.

Both fungicides inhibited fungal growth in the samples, except for ART8, which was treated with Vegesol eReS. The lack of growth was likely caused by masking or a mixing defect. Inhibition rate ranged from 3.36 % to 100.08 %. The percentage value over 100 % means that colony size was even smaller by the end of the experiment (seven days after spraying) than at the time of the treatments. The treatment caused it to shrink. This difference is induced by the various modes of action (contact and absorbable) and the efficacy of the varying active ingredients. Penkonazol was a stronger inhibitor due to its absorbability. Simultaneous usage of *Beauveria* species and penkonazol or sulfur is not recommended. This result verifies the second hypothesis that fungicidal treatments (penkonazol and sulfur) inhibit the growth of *Beauveria bassiana* and *B. brongniartii* strains used in the experiment.

Previous experiments where *Beauveria* and fungicides were used together also demonstrated this growth inhibition (Clark et al., 1982; Todorova et al., 1998; Khun et al., 2020). Daconil and maneb showed remarkable inhibition in *B. bassiana* growth (Olmert and Kenneth, 1974), but mancozeb and metiram also reduced the survival chances of the fungus (Loria et al., 1983). Zineb with copper-oxychloride caused the same effect (Majchrowicz et al., 1993). Some experiments revealed a neutral connection between certain fungicides – such as chlorothalonil and methalaxil – and mycelial development (Loria et al., 1983); however, in other experiments, they induced a positive effect in fungal growth thanks to fungicides (Anderson et al., 1989), even in case of copper-oxychloride, which earlier appeared to be inhibitory (Challa – Sanivada, 2014).

Basudin treatment exhibited a significant increase in BORA growth rate. Growth in *B. brongniartii* samples differed. One strain developed unequivocally, another was slightly supported, and the third strain showed a slight growth inhibition due to the diazinon treatment. These results modify the third hypothesis. The soil disinfection happens, but growth enhancement can firmly be stated only in *B. bassiana*.

Contrary to our experiment, Khun et al. (2020) found diazinon highly toxic to the fungi. In insecticides, Anderson and Roberts (1983) established that the studied pyrethroids all inhibited *B. bassiana* growth. Clark et al. (1982) stated that permethrin was the least harmful concerning this trait. Furlong and Groden (2001) described imidacloprid as synergistic with *B. bassiana*. Alizadeh et al. (2007) demonstrated less than 27% growth inhibition on the fungus and recommended parallel application. Other experiments suggested that chlorpyrifos can be used simultaneously with *Beauveria* species without inhibitory effects (Amutha et al., 2010). Wari et al. (2020) also determined the simultaneous applicability of *Beauveria* strains and various insecticides.

Experiments with herbicides showed the inhibitory effect on *Beauveria* growth, flurochloridone and prosulfocarb implementing the strongest inhibition (Celar – Kos, 2016). Todorova et al. (1998) described diquat as harmless to *B. bassiana*.

In all four chemicals, the appearance of the mycelia changed the most, even when the effect on growth was not indicated. This is likely connected to the hydrophobic feature of the mycelia, as the chemicals were dissolved in distilled water for application, and the contact chemicals

(Taifun 360, Basudin, Vegesol eReS) could only take effect on the small surface areas where the droplets touched the mycelia. Topaz could go deeper and cause a more severe effect due to its absorbability.

After we applied the recommended amount, none of the chemicals exceedingly inhibited fungi growth; however, the fungicides, and mainly Topaz with the active ingredient penkonazol, are not recommended for simultaneous application with *Beauveria* species because even the slightest growth inhibition can cause severe efficacy loss.

### **Novelties and recommendations for further experiments**

Only *Beauveria bassiana* strains have been applied in forestry practice in Hungary, thus far without any remarkable effect, so studying *B. brongniartii* strains can be considered a first attempt to introduce this species in practice. The results can be used effectively in plant protection practice and integrated pest management, but additional experiments are welcome. Concerning the control samples, the growth of ART315 – a *B. brongniartii* strain – was the most powerful; therefore, further experiments with this strain are highly recommended. Studying the interaction between other agents and the fungi, the effect of the used strains on other target species or applying another application amount to boost effectiveness can provide utilizable information. Experiments with different application timing procedures are proposed. Even if simultaneous application is feasible, a few weeks' difference in usage may be more economical in the long run than the double (or triple) cost of returning to the site. This contributes to fungicides or other chemicals that could potentially hinder the development of *Beauveria* species. The present study recommends experiments in applying *Beauveria bassiana* and *B. brongniartii* simultaneously to determine if they have any effect on each other, something similar to the experiment of Canfora et al. (2017), which found that the two fungus species have some interaction related to their different ecological niches.

### **Recommendations for practical use**

Previous studies showed that *Beauveria* has a maximum growth rate and infectability at higher temperatures. The optimum range for *B. brongniartii* is 20–25 °C, according to Kessler et al. (2003). Fargues et al. (1997) posit a 25–28 °C range for *B. bassiana*. This range varies from 25–30°C in Ekesi et al. (1999) and Bugeme et al. (2008), rising to 25–32°C in James et al. (1998). Based on these studies, it is recommended to apply the fungi when the average soil temperature reaches 20–25 °C. Traditionally, these temperatures correspond with the end of May, but climate change can push the temperatures earlier. The application mode depends on the properties of the stand to be treated and the land features. Planting together with watering pipes, using an interrow cultivator, or direct injection are all effective. Upon application, simultaneous usage with substances supporting the fungus (e.g., *Trichoderma* sp.) or treatment with higher concentrations are recommended. Adding moisture-fixing substances or continuous water supplementation is essential if applied in late spring. Simultaneous application with Taifun 360 and Basudin is encouraged, but Vegesol eReS and Topaz fungicides should be avoided if not crucial.

## **5 CONCLUSIONS**

The present study examined *Beauveria bassiana* and *B. brongniartii* species and their simultaneous application with several chemicals used in plant protection practice.

The main result is the affirmation that these fungi experimentally used in biological control as part of integrated pest management are not recommended to be applied together with



fungicides, especially if the active ingredient is penkonazol. This part of the experiment is notable, and the data also produce spectacular and significant statistical results.

The growth-enhancing effect of Basudin soil disinfectant is beneficial; however, the main goal is not fungal growth inhibition. The helping feature is a ‘bonus’ in this case.

The two fungus species are not interchangeable because their reactions differed under the same treatments.

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