IN VITRO COMPATIBILITY OF DIFFERENT HERBICIDES WITH ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA*

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Abstract. The *in vitro* effect of three herbicides commonly used in wheat or corn plant protection programs in Romania on mycelial growth of entomopathogenic *Beauveria bassiana* (BbTd1) were investigated. The formulations with Isoxaflutole 225 g/L + Thiencarbazone methyl 90 g/L + cyprosulfamide (safener) 150 g/L (Adengo), Nicosulfuron 40g/L (Nicogan) and dimethylamine salt 600g/L (Dicopur) were tested at three different concentrations (field recommendation- FR, half and twice the FR) both on solid and liquid media. On the solid media, the radial growth of the fungus was measured for 11 days. On the liquid media mycelial biomass was weighted after 7 days of incubation on a rotary shaker. Dicopur significantly inhibited the vegetative growth of *B. bassiana* BbTd1 in both culture media at tested concentrations. In liquid media, mycelial growth was stimulated by Adengo and Nicogan at different concentrations. No significant differences caused by Adengo and Nicogan compared to control of radial growth was detected. The results showed that Adengo and Nicogan could be used simultaneously with this entomopathogen in integrated control programs. Further in vivo studies are necessary to establish if Dicopur formulation can cause damage to field populations of entomopathogenic fungi.

Key words: *Beauveria bassiana, compatibility, herbicides, in vitro*

INTRODUCTION

Control in agriculture of diseases and pests, achieved mainly through the use of pesticides, although proven to have a high success rate for numerous crops worldwide, reducing pest outbreaks or the installation of certain diseases with the potential for high impact on crops, led at the same time due continuously use of the same chemical methods to the emergence of some phenomena (environmental pollution, insecticide resistance, negative effects on pollinators, natural predators) which inevitably led to decreased harvests, putting even more pressure on food security.

Entomopathogenic fungi are gaining popularity as insect pest biocontrol agents, and they are being explored as supplements or replacements to synthetic pesticides. The most researched entomopathogenic fungus is *Beauveria bassiana*, which is often used in commercially marketed mycoinsecticides.

The use of microbial bio-pesticides and their successful integration in the integrated management of diseases or pests together with other techniques cannot be achieved without prior testing in various compatibility studies. The need for these studies becomes all the more important as the persistence and effectiveness of entomopathogenic fungi can be compromised by the action of pesticides (Mochi et al., 2006), thus affecting the entire IPM program, while choosing pesticides optimally can minimize their harmful effect (Luz et al., 2007). The generation and collection of such data can allow farmers to adopt effective strategies based on

the selection of the appropriate compounds, thus programming the microbial fungi with chemical pesticides (Wari et al., 2020).

Pesticides are anthropogenic factors that have a synergistic or antagonistic effect on pests and their pathogens (entomopathogenic fungi), and thus also on their efficiency. Pesticides that are used properly can have a low impact on entomopathogenic fungus. Regular pesticide use in agronomical practice affects the efficacy of the entomopathogenic fungus *B. bassiana* incorporated in soil, because it frequently leads to pesticide accumulation in soil. To use mycoinsecticides with *B. bassiana* in integrated crop protection, pesticides (including herbicides) must be compatible with entomopathogenic fungus. The effects of herbicides on entomopathogenic fungi have generally been observed under laboratory conditions (Vanninen & Hokkanen, 1988; Poprawski & Majchrowicz, 1995; Tkaczuk & Safaryan, 2019; Celar & Kos, 2012, 2016), while some other were followed by field tests (Hassan et al., 1994). A helpful criterion for determining compatibility is the growth inhibition of entomopathogenic fungi. Over the years, studies concluded that *B. bassiana* is highly affected by some herbicides even at very low rates.

In this study we selected chemicals herbicides commonly used in wheat or corn crops protection programs in Romania. Our laboratory investigation was conducted to determine the effects of three herbicides on mycelial growth of a selected strain of *B. bassiana* in liquid and solid media.

MATERIAL AND METHODS

An isolate of *B. bassiana* (BbTd1) obtained from a dead adult of *Tanymecus dilaticollis* was used in this study. The fungus was multiplied in PDA (Potato Dextrose Agar) medium in the dark, at 24°C.The tests were performed both on solid and liquid media.

The herbicides The active substance, brand name, formulation, recommended rates and manufacturers of the herbicides tested in this study are listed in Table 1.

Table 1. Herbicides used in the bio-compatibility study

Active substance	Brand name	Chemical name (IUPAC)	Formulation	Recommended rate	Manufacturer
Isoxaflutole 225 g/L + Thiencarbazone-methyl 90 g/L + cyprosulfamide (safener) 150 g/L	Adengo 465	(5-cyclopropyl-1,2-oxazol-4-yl)(α,α,α-trifluoro-2-mesyl-ptolyl)methanone +Methyl 4-[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,2,4-triazol-1-yl)carbonylsulfamoyl]-5-methylthiophene-3-carboxylate+ N-[4-(cyclopropylcarbamoyl)phenylsulfonyl]-o-anisamide	CS	0,30-0,35 l/ha	Bayer AG 51373 Leverkusen, Germany
Nicosulfuron 40g/L	Nicogan 40	2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-N,N-dimethylnicotinamide	CS	0,8-1,5 l/ha	Alchimex
2,4-D dimethylamine salt 600g/L	Dicopur D	2-(2,4-dichlorophenoxy) acetic acid	SL	1l/ha	Nufarm GmbH&Co KG, Austria

The herbicides were evaluated for compatibility with *B. bassiana* at the field recommendation (FR) rate of the active ingredient, as well as 2-fold higher (2 x FR) and half 1/2-fold lower (1/2 x FR) field recommendation rates.

In vitro Tests of *B. bassiana* in liquid media For this trial each herbicide at the appropriate concentrations (1, 2/1 and ½ field recommendation rates) was applied to 100 ml of Goral medium ($NaNO_3$ 5g/l, KH_2PO_4 5g/l, Mg SO_4 2g/l, corn syrup 0,8g/l) previously autoclaved and cooled, in 500-ml Erlenmeyer flasks. Then the medium was inoculated with 1 ml of *B. bassiana* aqueous conidial suspension ($1x10^6$ UFC/ml). The control consisted in unamended Goral medium inoculated with the fungus. Each treatment had three replicates. The flasks were incubated for 7 days at 24°C, on an orbital shaker (150 rpm). After incubation, the contents of each flask were vacuum filtered using previously weighed filter membranes (Sartorius, 0.2 µm). The biomass was oven-dried (at 90°C for 24 hours) and weighed. Data were used to calculate the percentage of inhibition of germination using the formula described by Hokkanen and Kotiluoto (1992):

$$I(\%) = \frac{C - P}{C} \times 100$$

Where

I= percentage of growth inhibition,

C=growth of fungus in the control

H=growth of fungus in (pesticidal) medium with herbicide

The herbicides were further classified in evaluation categories of 1- 4 scoring index. 1 = harmless (<25% reduction in beneficial capacity), 2 = slightly harmful (25-35%), 3 = moderately harmful (36-50%), 4 = harmful (>50%) in toxicity tests in vitro according to Ambethgar classification (2009).

In vitro Tests of *B. bassiana* in solid media For this trial each herbicide at the appropriate concentrations (1, 2/1 and ½ field recommendation rates) was added to 100 ml of PDA medium after autoclaving at a temperature of approximately 45°C.From the periphery of actively growing (10-day old) Petri dish cultures, 0.5 cm diameter discs were cut with a cork-borer and a single disc was placed in the center of a new dish of herbicides amended PDA medium. The dishes were sealed with Parafilm and incubated at 24ºC in the dark for 11 days. For each treatment 5 Petri dishes (replicates) were used. The control consisted in unamended PDA medium inoculated with the fungus. The radial growth was assessed every 2 days by measuring and averaging two perpendicular diameters of the fungus colonies.

Data analyses The data were analyzed with unpaired t-test and Welch's correction with a significant value of $p < 0.05$. The statistical analysis was performed by using Prism software (GraphPad, version 7.0).

RESULTS AND DISCUSSIONS

In vitro Tests of *B. bassiana* in liquid media The herbicides tested in this study did not negatively affect the growth of *B. bassiana* BbTd1 with the exception of Dicopur, on the contrary, a stimulation of mycelium growth was registered. Nicogan stimulated the biomass accumulation in liquid medium at all tested concentrations with no statistical difference between FR and 2x FR concentrations (Table 2). The amendment of liquid medium with Adengo at ½ field recommendation rate exhibited a stimulatory effect on the mycelium growth more than Nicogan did. With all this, a growth inhibition of the fungus less than 6% was registered at FR and 2 x FR concentrations, respectively. Dicopur inhibited the mycelium growth of the isolate BbTd1 in the liquid medium more than 77 % at all tested concentrations.

Table 2. Percent of mycelium growth inhibition (positive values) or increased growth (negative values) of entomopathogenic fungus *Beauveria bassiana*, cultured on liquid Goral media treated with selected herbicides, after 7 days

Product name	Concentration		
	½ FR	FR	2 x FR
Nicogan	-15.20±12.33 a	-52.77±15.92 b	-40.24±4.96 ab
Adengo	-97.73±4.17 a	1.18±12.07 b	5.75±4.93 b
Dicopur	77.47±0.48 a	82.35±1.89 b	85.13±0.47 b

Percentages are given as mean ± standard deviation. On the same raw, means fallowed by the same letter are not statistically different (t-test).

In vitro Tests of *B. bassiana* in solid media *Beauveria bassiana* growth in solid media was almost completely inhibited by Dicopur at ½ FR (75%; t=3.903; p=0.0154) and completed inhibited at 1FR and 2 x FR concentrations compare to control, after 11 days (t=6.179; p=0.0035 and t=6.179; p=0.0035, respectively) (Figure 1).

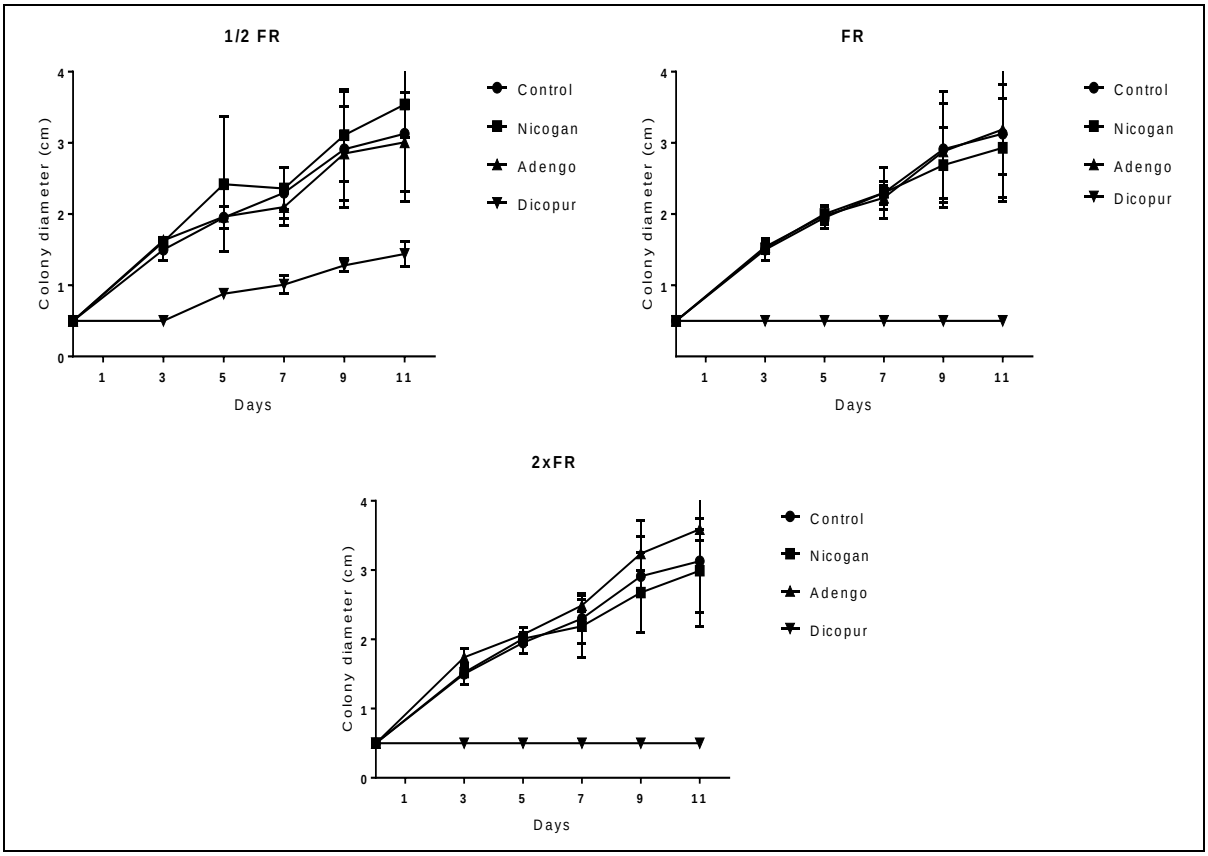

Figure 1. Colony diameter of entomopathogenic fungus *Beauveria bassiana*, cultured on PDA media treated with selected herbicides at different concentrations

Nicogan slightly stimulated the radial growth of *B.bassiana* BbTd1 in solid medium at ½ FR but insignificantly compared to control (-14.13%; t=0.8673; p=0.4204). At 1FR and 2 x FR concentrations this herbicide did no suppressed significantly the fungus radial growth (2.28%; t=0.3801; p=0.7147 and 3.1%; t=0.2787; p=0.789, respectively). The amendment of solid medium with Adengo at tested concentrations did not significantly affected the radial growth of *B.bassiana* BbTd1 compared to control, after 11 days (0.86%; t=0.2273; p=0.8264; -0.41%; t=0.1176 and - 14.8%; t=1.067; p=0.3434).

To our knowledge the effect of these herbicides on the entomopathogenic Hyphomicetes has not been documented. The compatibility of *B. bassiana* with isoxaflutole was evaluated only by Celar and Cos (2016). According to their findings, it may be compatible with *B. bassiana* in the field because it had the fewest side effects. However, 82% of entomopathogen germination was inhibited, according to the results.

There are studies that generally demonstrate the inhibitory effect of herbicides on the entomopathogenic fungus as we found for Dicopur (dimethylamine salt), even more that fungicides due. Herbicides may reduce or increase the effectiveness of *B. bassiana* and disturb natural epizootics of this pathogen. Celar and Kos (2012) concluded that the entomopathogenic fungus *B. bassiana* is extremely susceptible to some herbicides. Herbicides have a high fungistatic or even fungicidal action, especially at approved, but even at lower field dosages. In vitro experiments conducted by Gardner and Storey (1985) revealed that 20 of the 21 herbicides affected *B. bassiana* conidial germination, mycelial growth, or both.

CONCLUSIONS

Our test *in vitro* found that the herbicide Dicopur (dimethylamine salt) is not compatible with *Beauveria bassiana* BbTd1 strain.

The Nicogan (nicosulfuron) and Adengo (isoxaflutole + thiencarbazone-methyl + cyprosulfamide) herbicides could be used simultaneously with this entomopathogen in integrated control programs. In order to finally validate or reject the results of laboratory tests, an in vivo field test must be performed.

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REFERENCES

AMBETHGAR, V., SWAMIAPPAN, M., RABINDRA, R.J., RABINDRAN, R. (2009). Influence of some herbicides on in vitro vegetative growth of *Beauveria bassiana* (Balsamo) Vuillemin. *Resistant Pest Management Newsletter*, 19, 1, 11-14.

CELAR, F.A., KOS, K. (2016). Effects of selected herbicides and fungicides on growth, sporulation and conidial germination of entomopathogenic fungus *Beauveria bassiana*. *Pest Management Science*, 72, 11, 2110-2117. DOI: 10.1002/ps.4240

CELAR, F. A., KOS, K. (2012). Compatibility of selected herbicides with entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. A*cta Agriculturae Slovenica*, 99, 1, 57. DOI:10.2478/v10014-012-0007-2

COREMANS-PELSENEER, J. (1994). Laboratory tests on the entomopathogenic fungus *Beauveria*. *IOBC/WPRS Bulletin*, 17, 10, 147-155.

GARDNER, W.A., STOREY, G.K. (1985). Sensitivity of *Beauveria bassiana* to selected herbicides. *Journal of Economic Entomology*, 78, 1275-1279. DOI: 10.1093/jee/78.6.1275

HASSAN, S.A., BIGLER, F., BOGENSCHUTZ, H., BOLLER, E., BRUN, J., CALIS, J.N.M., COREMANS-PELSENEER, J., DUSO, C., GROVE, A., HEIMBACH, U., HELYER, N., HOKKANEN, H., LEWIS, G.B., MANSOUR, F., MORETH, L., POLGAR, L., SAMSOE-PETERSEN, L., SAUPHANOR, B., STAUBLI, A., STERK, G., VAINIO, A., VAN DE VEIRE, M., VIGGIANI, G., VOGT, H. (1994). Results of the sixth joint pesticide testing programme of the IOBC/WPRS working group: pesticides and beneficial organisms. *Entomophaga*, 39, 107-119. DOI:10.1007/BF02373500

HOKKANEN, H.M.T., KOTILUOTO, R. (1992). Bioassay of the side effects of pesticides on *Beauveria bassiana* and *Metarhizium anisopliae*: standardized sequential testing procedure. In: Hassan et al. 1992. Guidelines for testing the eff ects of pesticides on beneficial organisms. Working group "Pesticides and Beneficial Organisms". *IOBC/WPRS Bulletin* 15, 3, 148-151.

LUZ, C., BASTOS, N., NUNES, R. (2007). In vitro susceptibility to fungicides by invertebrate-pathogenic and saprobic fungi. *Mycopathologia*, 164, 39-47. DOI: 10.1007/s11046-007-9020-0

MOCHI, D.A., MONTEIRO, A.C., DE BORTOLI, S.A., DORIA, H., BARBOSA, J.C. (2006). Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in soil with different pesticides. *Neotropical Entomology*, 35, 382-389. DOI: 10.1590/s1519-566x2006000300014

POPRAWSKI, T.J., MAJCHROWICZ, I. (1995). Effects of herbicides on in vitro vegetative growth and sporulation of entomopathogenic fungi. *Crop Protection*, 14, 81-87. DOI: 10.1016/0261-2194(95)91116-W

TKACZUK, C., MAJCHROWSKA-SAFARYAN, A. (2019). The effect of herbicide use on the occurrence of entomopathogenic fungi in the soil of blackcurrant plantations. *Applied Ecology and Environmental Research*, 17, 3003-3011. DOI:10.15666/aeer/1702_30033011

VANNINEN, I., HOKKANEN, H. (1988). Effect of pesticides on four species of entomopathogenic fungi in vitro. *Annales Agriculturae Fenniae*, 27, 345-353.

WARI, D., OKADA, R., TAKAGI, M., YAGUCHI, M., KASHIMA, T., OGAWARA, T. (2020). Augmentation and compatibility of *Beauveria bassiana* with pesticides against different growth stages of *Bemisia tabaci* (Gennadius); an in vitro and field approach. *Pest Management Science*, 76, 3236-3252. DOI: 10.1002/ps.5881