Field test of *Beauveria bassiana* and Neem extract on *Ips typographus* and *Trypodendron lineatum* – preliminary results

The spruce bark beetle *Ips typographus* is the most significant pest in Europe (Grodzki et al. 2004, Overbeck & Schmidt 2012, Wermelinger 2004). The ambrosia beetle *Trypodendron lineatum* is an invader of dead and dying trees and becomes problematic in areas with high volumes of the suitable host material (Hoover et al. 2000). The conifer-infesting species outbreaks have significant economic, ecologic, and social implications.

Over time, numerous methods have been tested to control populations of mentioned pests. Chemical insecticides have proven effective (Gajendiran & Abraham 2018, Hardy et al. 2020). After intensive use, insects have developed a resistance to their action (Hawkins et al. 2019). At the same time, a negative effect on the environment, including on people's health, was observed (Ansari et al. 2014).

In these conditions, in the last decades, scientists have been looking in nature to find solutions to control populations of harmful insects and to avoid economic losses because of insects' mass multiplication. Similar to the protection of agricultural and forest plants, new "products" considered "environment friendly" are identified and tested under laboratory and field conditions to ensure certain effectiveness. *Beauveria bassiana* and Neem tree extract are some of them.

Beauveria bassiana is a fungus that grows naturally in soils and acts as a parasite on various insect species, among which there are beetles (Rehner et al. 2011). The entomopathogenic fungi *Beauveria bassiana* is used as a biological insecticide to control a large number of pests (Zimmermann 2007). Neem, scientifically known as *Azadirachta indica*, is a tree native to the Indian subcontinent and Africa, whose oil extracted from the seeds and fruits has an antifeedant effect on harmful insects (Fora & Lauer 2008). The active compounds as Azadirachtin A and B have an insecticidal effect (Isman et al. 1990).

Beauveria bassiana and Neem tree extract are effective against pests (Barta et al. 2020, Campos et al. 2016, Carrillo et al. 2015, Wegensteiner et al. 2015) and can be an alternative control to the "classical" active ingredients as Lambda-cyhalothrin or Alfa-cypermethrin. Because the efficacy of biological compounds depends significantly on local conditions, a new test was developed to find an adequate method to control the bark and wood beetles in sensitive forest stands.

In the present study, we test *Beauveria bassiana* and Neem tree extract on two coleopteran species, *Ips typographus*, and *Trypodendron lineatum*, under field conditions, in the Apuseni Mountains (Western Romanian Carpathians). Their effect on the new generation of beetles F1 was evaluated.

The research took place during the growing season of 2022 in the Norway spruce-managed forests of the Forest District Belis, Forest Directorate Cluj, Romania. In the area, the last strong storm occurred in 2017 and had as a result, more than 150,000 cubic meters of felled trees. In some areas, the trees felled by the storm could not be extracted in the short time and represented the starting point for a potential permanent infestation in the years that followed. Scattered felling of a smaller volume of woody mass occurs almost every year and keeps pest populations active.

To evaluate the efficacy of *Beauveria bassiana* and Neem on two coleopteran species, *Ips typographus*, and *Trypodendron lineatum*, 24 portions of fresh uninfested stems of 1 meter long and 20 cm in diameter were installed inside a forest insect outbreak. These samples, formed in this way, were hung in a vertical position on a wooden support inside the forest (Figure 1A) and treated on the 2nd of June with the below ingredients: variant 1 - treated with pure water; variant 2 - treated with *B. bassiana* 1 x 10^9 CFU / g (50 g / l); variant 3 - treated with *B. bassiana* 1 x 10^9 CFU / g (50 g / l) + Neem 98% (10 ml / l); variant 4 - treated with *B. bassiana* 4 x 10^9 CFU / g (50 g / l); variant 5 - treated with *B. bassiana* 4 x 10^9 CFU / g (50 g / l) + Neem 98% (10 ml / l); variant 6 - treated with Neem 98% (10 ml / l).

The amount of solution applied was 200 ml / square meter of bark. The treated samples were left in the forest to be populated by insects for more than a month. On the 11th of July, the samples were inserted into the `eclectors` (Figure 1B), permanently monitored, and every 2 weeks, the cached insects were collected. After another 2 months, on the 5th of September, the samples were removed and portions of 30 cm from each stem were unbarked entirely in the laboratory (Figure 1C) and the existing alive beetles of new generation F1 were collected. The samples of beetles were kept in the refrigerator at -20°C until examination.

From the climatic point of view, the spring and summer of the year were warmer, and dryer compared with previous years. The temperature and air humidity values were recorded with the Hexo device throughout the experimental period (Figure 1D).

Data analysis.

The data were normally distributed; therefore, ANOVA followed by Tukey testing was used for each species to compare variants (treatments) using the average data of the four replicates. Alpha diversity profiles were computed for both species and each treatment variant. Diversity profiles were compared statistically using t-test. All data analyses were made in PAST, version 4.02.

In total, 10.674 beetles of *Ips typographus* and 382 beetles of *Trypodendron lineatum* were captured during the trial development. These figures show that although treatments have been applied, the number of new adults from the F1 generation remains high; for both species, the trend of some decline in abundance can be detected. However, no statistically significant differences in abundance can be observed (Figure 2A, B).

Diversity profiles were computed to assess differences in abundance during the season when treatments are applied. Significant differences in *I. typographus* abundance were detected between variants 2, *B. bassiana* 1 x 10^9 CFU / g (50 g / l); ($t = 9.1$, $p<0.001$), and variant 5 *B. bassiana* 4 x 10^9 CFU / g (50 g / l) + Neem 98% (10 ml / l) ($t = 6.6$, $p<0.001$) and the other treatments (Figure 3A). No such differences were detected for *T. lineatum* (Figure 3B).

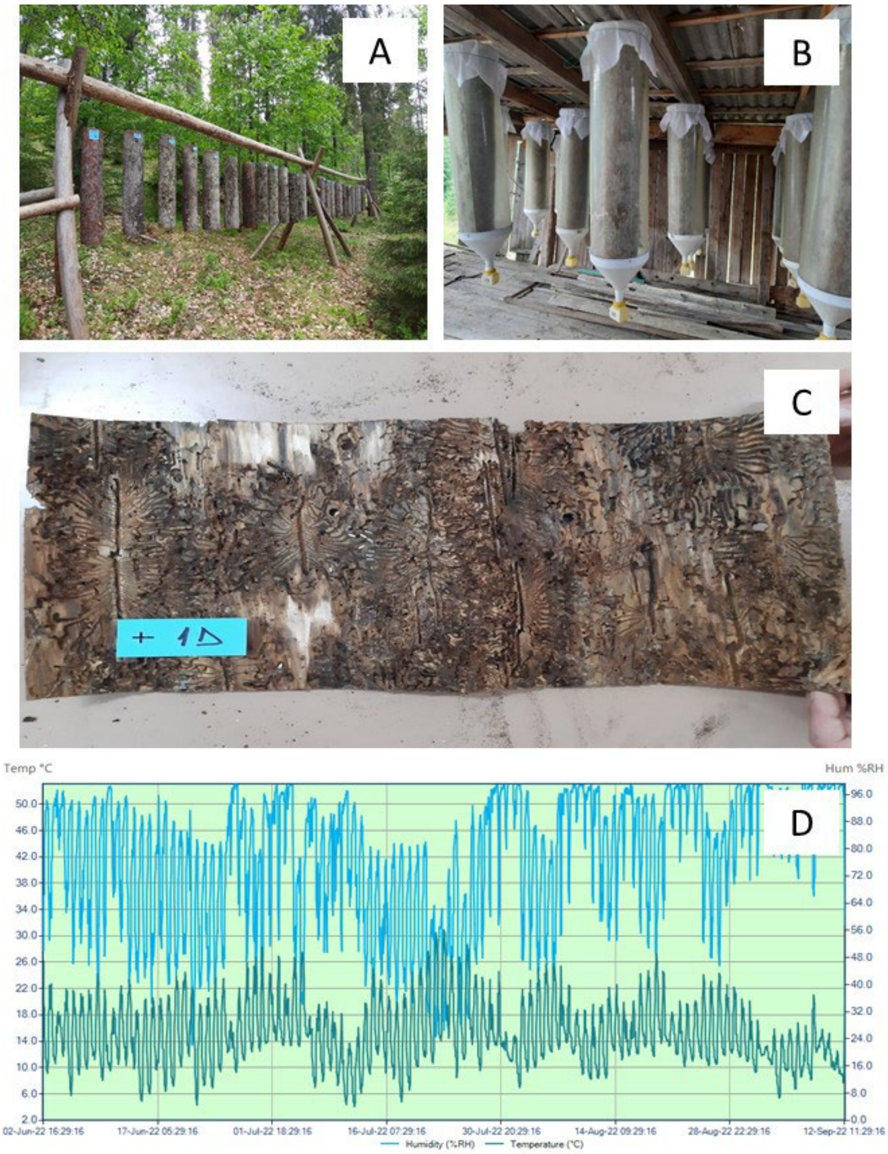
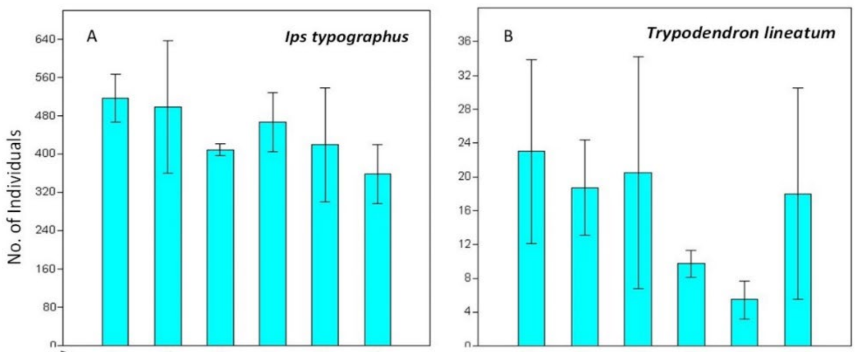


Figure 1. Experimental design with vertical position of stems on a wooden support (A), samples inserted into the \`eclectors\` (B), stem unbarked and assessed in the laboratory (C), temperature and air humidity in field during the research (D).



	V1	V2	V3	V4	V5	V6
V1		1	0.9442	0.9983	0.963	0.7757
V2	0.2053		0.9734	0.9998	0.9843	0.8471
V3	1.258	1.053		0.9963	1	0.9981
V4	0.5778	0.3725	0.6805		0.9985	0.941
V5	1.138	0.9327	0.1203	0.5602		0.9953
V6	1.854	1.648	0.5954	1.276	0.7157	

	V1	V2	V3	V4	V5	V6
V1		0.9994	1	0.9037	0.7529	0.9987
V2	0.4647		1	0.9801	0.9037	1
V3	0.2733	0.1913		0.9577	0.8498	1
V4	1.449	0.984	1.175		0.9994	0.9865
V5	1.913	1.449	1.64	0.4647		0.9228
V6	0.5467	0.082	0.2733	0.902	1.367	

Figure 2. Survived individuals of *I. typographus* (A) and *T. lineatum* (B) after treatments, the table represents statistical analyses, ANOVA, and Tukey test; p values are presented above the line are F values are presented below.

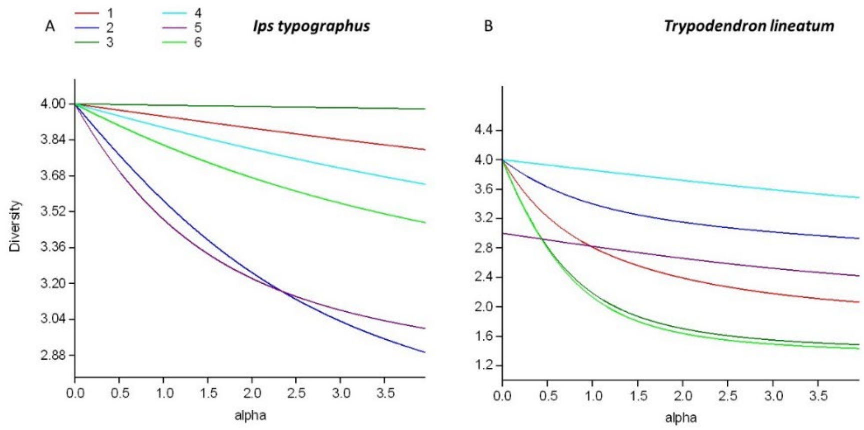


Figure 3. Diversity profiles (alpha diversity) of the survived individuals of *I. typographus* (A) and *T. lineatum* (B) after treatments. Each line represents one treatment variant.

The laboratory test results with entomopathogenic fungi to control bark beetles were promising, but just a few were successfully applied to manage bark beetles under field conditions (Mann & Davis 2021).

In our tests, the result differences were not significant. The relative low humidity and high temperatures during the whole experimental period probably reduced the effect on fungal and neem treatments, facts similarly demonstrated by earlier authors (Bugeme et al. 2008, Jenkins et al. 2003, Mann & Davis 2020, Togbé et al. 2014, Yeo et al. 2003). Even if statistically, there were no significant differences between the variants, some trends could be observed. *Ips typographus* was influenced by the application of Neem extract (10 ml/l), the beetle number being, in this case with 30% lower than in the untreated variant. In contrast to this, in the case of the species *Trypodendron lineatum*, some influence was observed in the case of the use of *Beauveria bassiana* in the concentration of 4 x 10^9 CFU / g (50 g / l), respectively of the use of *Beauveria bassiana* 4 x 10^9 CFU / g (50 g / l) in combination with Neem extract 98% (10 ml / l), suggesting a presence of synergy between them. Therefore, *Beauveria bassiana* decreases the beetle number by 60%, and in addition with Neem extract by 75%, compared with the untreated variant.

New tests with higher product concentrations and repeated treatments at intervals of 7-10 days during the flight period are necessary.

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