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Effectiveness of Combined Biological Control in Protecting Maize from the European Corn Borer and Fungal Infections

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Summary: Maize (*Zea mays* L.) is one of the most important agricultural crops worldwide, but its production is often threatened by insect pests and pathogenic fungi. The European corn borer (ECB) *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) is one of the most damaging pests of maize. Its larvae damage maize ears, causing direct yield losses and creating entry points for fungal pathogens, primarily species from the genera *Fusarium* and *Aspergillus*. These fungi are major causes of ear rot and can seriously affect grain quality and the safety of its consumption. Biological control represents an environmentally friendly alternative to chemical pesticides. The aim of this study was to evaluate the effectiveness of biological control of the ECB in reducing *Fusarium* and *Aspergillus* infections. A field experiment was conducted using a randomized block design with four replications. In addition to the control, the treatments included the application of parasitic wasps of the genus *Trichogramma* Westwood, 1833 (Hymenoptera: Trichogrammatidae), as well as larvae of the predatory green lacewing *Chrysoperla carnea* Stephens, 1836 (Neuroptera: Chrysopidae), and a combination of both biological agents. The results showed a statistically significant effect of treatments on *Aspergillus* infections. Plants treated with the combination of parasitic wasps and predators had significantly lower infection rates compared to the control and other treatments, suggesting a synergistic effect in suppressing ECB populations and reducing fungal entry points. Conversely, the treatments had no statistically significant effect on *Fusarium* infections, likely due to the overall low incidence of *Fusarium* during the experiment. These results highlight the importance of biological control in reducing damage and preserving maize grain quality.

Keywords: *Aspergillus*, biological control, *Chrysoperla carnea*, European corn borer, *Fusarium*, maize, *Trichogramma brassicae*

Introduction

Maize (*Zea mays L.*) is one of the most important agricultural crops, cultivated on extensive areas worldwide, with the sown area increasing annually, making it the leading crop in global production (FAO., 2025). In Serbia, maize is also one of the most significant field crops. However, despite its importance and widespread cultivation, maize production faces serious constraints. One of the key factors affecting both yield and grain quality is the presence of harmful organisms, particularly the European corn borer (ECB) *Ostrinia nubilalis* Hübner, 1796 (Lepidoptera: Crambidae), as well as pathogenic and mycotoxigenic fungi from the genera *Fusarium* and *Aspergillus*. *Ostrinia nubilalis* plays a significant role in the epidemiology of these fungi.

The larvae of the second generation of the ECB play a crucial role in the development of ear rot in maize caused by *Fusarium* species, as the feeding damage they cause substantially increases the risk of mycotoxin contamination in the kernels (Saladini et al., 2008). Some studies also emphasize the importance of the ECB in the epidemiology of *Fusarium* and *Aspergillus* fungi (Saladini et al., 2008; Niculina et al., 2019). The larvae of these insects can carry spores of mycotoxigenic fungi beneath the husks, in the kernel zone, which favorably affects the development of infections (Negrut et al., 2019). Also, damage to the ear caused by ECB larvae creates entry points for spores.

The life cycle of *Aspergillus* spp. involves both saprophytic and pathogenic stages. As a saprophyte, these fungi can survive and colonize the soil and decomposing organic matter, which serves as an overwintering reservoir and a source of primary inoculum for infestation (Abbas et al., 2009). Under favorable conditions, usually in spring, sclerotia exposed at the soil surface germinate and release conidia inoculum. The conidia can be transmitted by air or insects, acting as a new source of inoculum on host plants, such as corn silks, especially under environmental conditions favorable to their development, including temperatures and moisture deficit (Zakaria, 2024). Infected plant tissues during the growing season produce secondary conidial inoculum, which can colonize newly developed plant tissue. Besides causing mechanical damage that increases plant stress, insects play a significant role in this process by acting as vectors for the dispersal of conidia and secondary inoculum (Abbas et al., 2009). The wounds they create also serve as entry points for pathogenic fungi (Horn, 2007). *Aspergillus* ear rot is characterized by development of distinct conidial mass on corn kernels, typically at the ear tip or at sites of mechanical injury. The color of these conidial masses can serve as a primary visual indicator for species identification: *Aspergillus flavus* produces olive-green conidial masses, whereas *A. niger* produces black conidial structures (Rodrigo and Jackson-Ziems, 2017). The presence of infection in corn does not necessarily imply aflatoxin contamination, as infection can occur without toxin accumulation, while aflatoxin production may also occur in outwardly healthy kernels (Pruter et al., 2019). Under suitable conditions, aflatoxin may be produced within 24 h after infestation (Gwinner et al., 1996).

Fusarium species were ranked among the ten most important plant-pathogenic fungi worldwide, particularly affecting cereals (Dean et al., 2012). This genus includes many species that vary both morphologically and phylogenetically (Timmusk et al., 2020). *Fusarium* spp. are widespread, primarily soil-borne pathogens that negatively affect plant development throughout the entire growing season. *Fusarium* spp. can affect germination, emergence, and early growth. Typical symptoms include brown discoloration, chlorosis, wilting and stunted seedlings (Oldenburg et al., 2017). In addition to seedling damage, *Fusarium* spp. cause significant infections during the growing season. There are three main pathways for ear infection: spore germination on the silk, followed by downward growth to kernels and rachis; wounds caused by insects or hail and systemic infection from infected seed (Foroud et al., 2014). Since *Fusarium* ear infections can be initiated through multiple pathways, including systemic spread from seedlings, preventing only mechanical injuries and insect damage is not sufficient to fully control the disease. The pathogenic activity of *Fusarium* spp. involves infection and colonization of husks, leaf sheaths, and other ground tissues. These infections lead to characteristic symptoms such as white mycelial growth or pink spore masses on the ears (Oldenburg et al., 2017).

Maize crops face dual threats from these toxigenic fungi, which not only reduce yields through infection but also compromise grain quality through mycotoxin contamination such as aflatoxins, fumonisins, and deoxynivalenol, significantly threatening both, agricultural productivity and grain quality (Mesterhazy et al., 2022). Mycotoxins can occur in agricultural commodities before or after harvest and pose significant health risks to humans and animals due to their mutagenic, carcinogenic, teratogenic, estrogenic, hemorrhagic, immunosuppressive, nephrotoxic, hepatotoxic, and cytotoxic properties (CAST, 2003). Studies show that mycotoxins contaminate about 25% of the world's grain supply, making them one of the most serious food safety threats according to the EU's food safety warning system. This contamination has resulted in the destruction of one billion metric tons of food and other products (Yu et al., 2021).

Larvae of the ECB damage the maize ear, and such injuries often serve as entry points for *Fusarium* and *Aspergillus* spores, thereby increasing the likelihood of infection (Franeta et al., 2025a). Therefore, it is crucial to pay special attention to the control of this species to reduce the risk of fungal diseases and preserve the safety and health of the crops.

Until recently, chemical insecticides have been the most used method to control the European corn borer. However, chemical protection negatively impacts the environment, disrupts ecological balance, and poses health risks to humans (Gošić-Dondo et al., 2016). Consequently, scientific research has increasingly focused on identifying sustainable and environmentally friendly crop protection methods. One such method is biological control, which involves the use of natural enemies or bioinsecticides to suppress pest populations. Parasitic wasps from the genus *Trichogramma* Westwood, 1833 (Hymenoptera: Trichogrammatidae) are commonly used to control the ECB. Female wasps locate the pest's egg masses and oviposit their own eggs into them using their ovipositor, effectively parasitizing the pest eggs (Ruberson and Kring, 1993). In addition to parasitoids, generalist predators from the family Chrysopidae (Neuroptera), such as common green lacewings *Chrysoperla carnea* Stephens, 1836 (Neuroptera: Chrysopidae), also play a significant role in biological control. These insects are known to effectively reduce *O. nubilalis* populations and can attack both eggs and early larval stages of the pest species (Franeta et al., 2025b).

The aim of this study was to evaluate the effectiveness of two biological control agents; the parasitic wasp *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae) and the predatory green lacewing *Chrysoperla carnea* in reducing maize ear infections caused by *Fusarium* and *Aspergillus* species.

Material and Methods

The field trial was conducted at the experimental fields of the Institute of Field and Vegetable Crops in Rimski Šančevi, Novi Sad, Serbia (N 45°19'17.81"; E 19°50'1.857"). The maize hybrid used in the study was NS 6000, belonging to the FAO maturity group 550. Sowing was carried out on May 7, 2024. The experimental design was completely randomized, comprising four blocks that included three different biological treatments and an untreated control. Two-meter-wide paths were established between blocks, and each experimental plot measured 30 m × 6 m, with treatments applied to the central rows. The flight activity of the ECB was monitored using a commercial light trap model "RO Agrobečej." The biological treatments applied were as follows:

C: Control (no biological agents applied),
Treatment 2: Application of parasitic wasps, *T. brassicae*
Treatment 3: Application of predatory lacewing larvae, *C. carnea*,
Treatment 4: Combined treatment involving simultaneous application of parasitic wasps and lacewing larvae.

Parasitic wasps were released in capsule form, with 4 capsules placed in the two central rows of each plot, each containing approximately 2,000 individuals. Lacewing larvae at the second instar stage (L2), totaling 500 larvae per plot, were evenly distributed across the treated areas, also in the two central rows. Treatments were repeated after seven days to enhance efficacy. The biological agents were acquired from Bioline AgroSciences Ltd. (Little Clacton, UK). In mid-September, 20 maize ears were harvested from each plot, corresponding to each treatment and replication, resulting in a total of 320 ears. The incidence of fungi from the *Fusarium* and *Aspergillus* genera was assessed using the scale developed by Reid et al. (1996). The scoring scale was as follows: 0 – 0% infected kernels per ear; 1 – 1-3% infected kernels per ear; 2 – 4-10% infected kernels per ear; 3 – 11-25% infected kernels per ear; 4 – 26-50% infected kernels per ear; 5 – 51-75% infected kernels per ear; 6 – 76-100% infected kernels per ear. For the evaluation, 20 ears per replication and treatment were used, with 10 ears taken from each of the two central rows. The assessment was conducted twice: first in mid-September, and the second seven days later, to achieve greater accuracy and reliability of the results.

Results

Statistical Analysis of Data

All statistical analyses were performed using R software (4.4.2 GUI 1.81 Big Sur ARM build). Prior to inferential analysis, assumptions of normality and homogeneity of variance were assessed. The Shapiro–

Wilk test was used to examine the normality of residuals for each dependent variable, while Levene's test was employed to assess the homogeneity of variances among treatments. Where data met parametric assumptions, one-way analysis of variance (ANOVA) was performed to determine the effect of treatment on pathogen incidence. Given the ordinal nature and potential deviations from normality in the data, non-parametric tests were additionally applied. The Kruskal–Wallis test was used to assess differences among treatments for both pathogens. Where significant differences were detected, post-hoc pairwise comparisons were conducted using Dunn's test, with Bonferroni adjustment for multiple comparisons. Effect sizes for the Kruskal–Wallis tests were estimated using eta-squared (η^2), as implemented in the *rstatix* R package, to quantify the magnitude of treatment effects. Boxplots were constructed to visualize the distribution of *Aspergillus* and *Fusarium* colony counts across treatments. Significant pairwise differences, as identified by Dunn's test, were annotated on the plots. All graphical visualizations were generated using the *ggplot2* and *ggpubr* R packages.

Weather conditions

Temperature and precipitation data were collected from the nearest FieldClimate station (number 00000E89) throughout the growing season (May–September) of the experiment and compared to optimal conditions for maize growth, defined as optimal daily temperatures between 16 °C and 22 °C and minimum monthly precipitation of 40–100 mm, depending on the month. The observed temperatures were consistently above the optimal range, indicating suboptimal thermal conditions for maize growth. The analysis revealed temperature extremes and periods of insufficient precipitation during critical growth phases, resulting in plant stress. The maximum temperatures consistently exceeded 30 °C, peaking around 40 °C in July, August, and September. Such high temperatures likely caused heat stress, reducing pollen viability and disrupting pollination, particularly during the flowering period in July. These conditions were also favorable for *Aspergillus* infections (Figure 1).

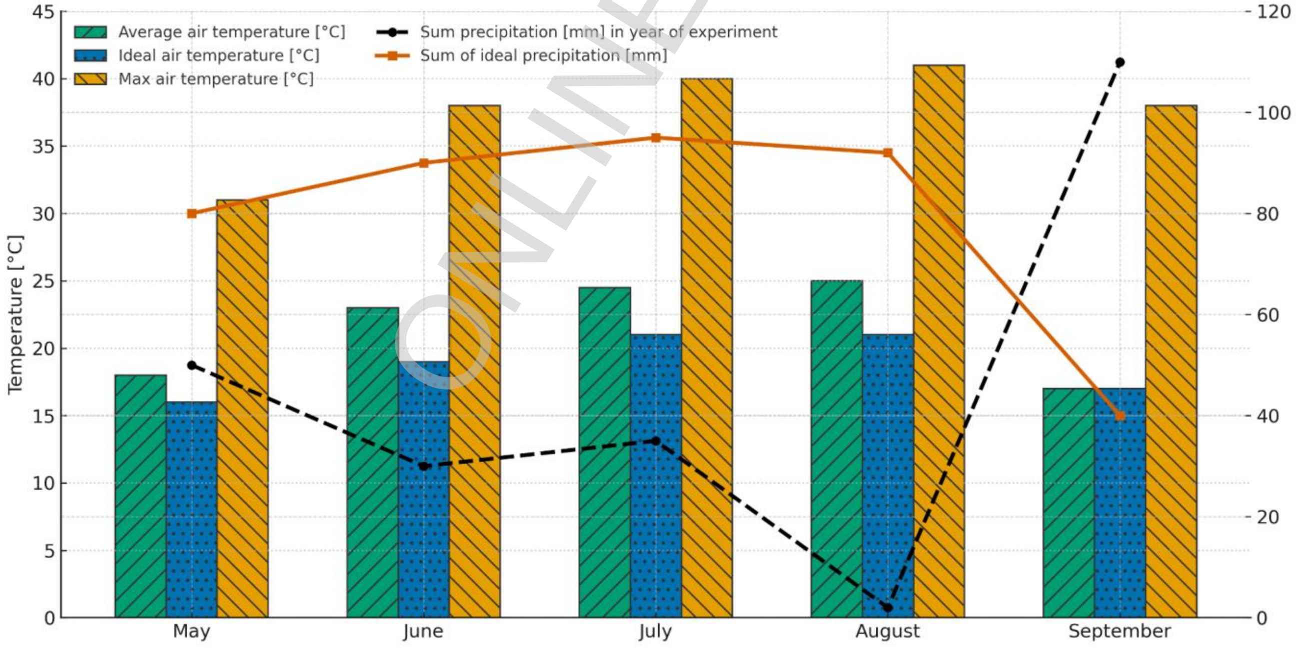

Figure 1. Weather conditions in 2024 when the experiment was conducted

Treatment Effects on Aspergillus and Fusarium Incidence

The Shapiro–Wilk test indicated that residuals from the one-way ANOVA for *Aspergillus* were not normally distributed (W = 0.93, p-value < 0.001) (Table 1). Levene's test showed a significant difference in variance among treatments (F = 13.0, p-value < 0.001). For *Fusarium*, normality was also violated (W = 0.60,

p-value < 0.001), while homogeneity of variance was borderline non-significant ($F = 2.62$, p-value $= 0.051$). Due to violation of normality and homoscedasticity assumptions for both *Aspergillus* and *Fusarium*, the non-parametric Kruskal–Wallis test was applied.

Table 1. Shapiro–Wilk and Levene's Tests for *Aspergillus* and *Fusarium*.

	Shapiro-Wilk test		Levene's Test		
	W	p-value	Df	F value	Pr(>F)
Aspergillus	0.93	2.433e-11	3	13.025	4.789e-08
Fusarium	0.60	2.2e-16	3	2.6159	0.05113

* W - Shapiro–Wilk test statistic, p-value - probability under the null of perfect normality, Df - degrees of freedom for the F test, F value - Levene's F-statistic, Pr(>F) - p-value for the F test.

The Kruskal–Wallis test revealed a highly significant effect of treatment on *Aspergillus* colony counts ($\chi^2 = 61.87$, df = 3, $p < 0.001$), with a large effect size ($\eta^2 = 0.185$) (Table 2). Following *post-hoc* Dunn's test with Bonferroni correction showed that C differed significantly from T2 ($p < 0.001$), T3 ($p < 0.001$), and T4 ($p < 0.001$). Additionally, T2 and T3 were not significantly different ($p = 1.00$), while T4 was significantly lower than both T2 ($p < 0.001$) and T3 ($p < 0.001$) (Figure 2). The lowest *Aspergillus* incidence was consistently observed in T4, while C had the highest counts.

The Kruskal–Wallis test showed no statistically significant differences among treatments on Fusarium colony counts ($\chi^2 = 6.13$, df = 3, $p = 0.106$), with a small effect size ($\eta^2 = 0.0098$). *Post-hoc* comparisons indicated no significant pairwise differences after Bonferroni correction, except a marginal difference between T3 and T4 ($p = 0.046$), which did not reach significance under the adjusted threshold.

Table 2. Non-parametric comparisons of group differences for *Aspergillus* and *Fusarium*.

	Kruskal-Wallis rank sum test			η^2		
	Kruskal-Wallis chi-squared	Df	p-value	n	effect size	magnitude
Aspergillus	61.873	3	2.339e-13	323	0.185	large
Fusarium	6.1299	3	0.1055	323	0.00981	small

* Df - degrees of freedom, p-value - probability value for statistical significance, η^2 - eta-squared (measure of effect size), n - sample size, magnitude - qualitative label of effect size.

Discussion

Modern agriculture faces significant challenges related to the excessive use of pesticides. Excessive use of insecticides continues to cause growing concern. One of the main reasons for this is the increasing occurrence of resistance in insects, which directly leads to reduced insecticide effectiveness. In addition, the environmental aspect must not be overlooked (Ganai., 2018). Environmental pollution is becoming an increasingly prominent problem (Ćupić et al., 2024). Due to the harmful effects of insecticide use, there is a growing need to adopt integrated production systems. The goal of integrated production is to minimize the use of chemical treatments while prioritizing biological control methods that are considerably more environmentally friendly (Baker et al., 2020).

Biological control involves the use of natural enemies or bio-insecticides to combat harmful insects. Classical biological control is particularly important for managing invasive species in agriculture, as it enables selective and effective action without causing harm to the environment (Van Driesche, 2010). Parasitic wasps from the genus *Trichogramma*, which are widely used in many countries, are among the most important biological methods for controlling the ECB (Ageev et al., 2025). Although these methods have yielded excellent results in different agroecological conditions, their application in Serbia is still not widespread enough. In Serbia, the use of *Trichogramma* wasps is partially limited due to the lack of domestic production, and their import significantly increases protection costs and the overall production costs of maize, especially sweet corn (Ivezić, 2019).

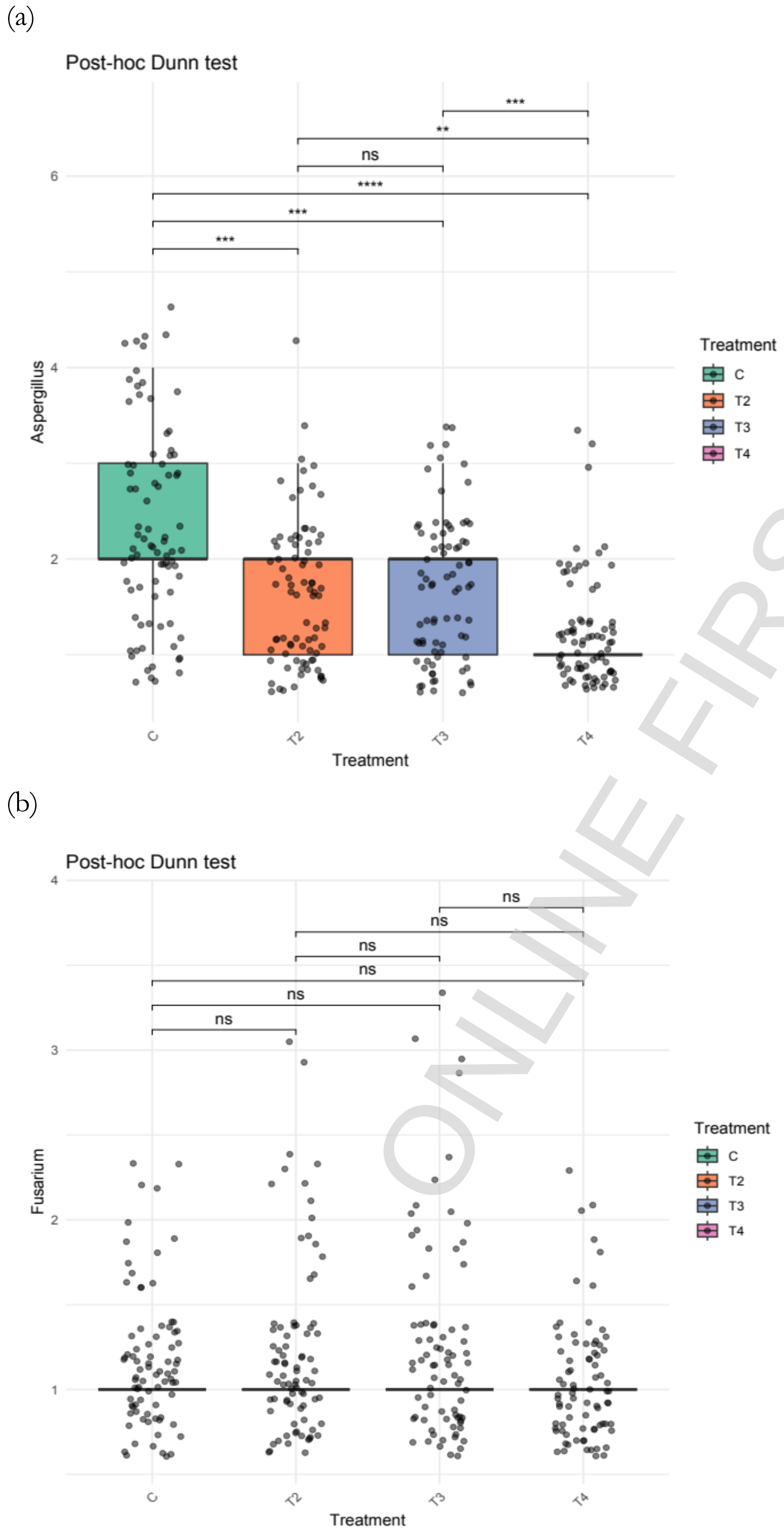

Figure 2. Distribution of (a) Aspergillus and (b) Fusarium infection across treatments. Boxplots with overlaid data points showing Aspergillus and Fusarium abundance under four treatments (T1–T4). Each box shows median and interquartile range; whiskers span the minimum to maximum. Individual dots represent single samples. Asterisks denote significance levels: ns, $p \geq 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

The efficacy of field releases of the egg parasitoid *Trichogramma* in controlling the ECB has been confirmed by numerous studies, which have demonstrated high activity of *Trichogramma* wasps mostly *Trichogramma brassicae*, with parasitism levels ranging from 12.5% to 80.0% (Ivezić et al., 2020). Our results support these findings, showing that the use of *Trichogramma* significantly reduced the population of *Ostrinia nubilalis*, as well as *Aspergillus* infection in maize ears when combined with other biological agents.

Among these, the larvae of green lacewings (*Chrysoperla* spp. and *Chrysopa* spp.) are particularly notable for their high efficiency in targeting multiple pest developmental stages (Berteloot et al., 2024). *Trichogramma* wasps, as egg parasitoids, have been successfully employed for over 120 years in the biological control of numerous harmful Lepidoptera species (Smith, 1996; Van Lenteren, 2000). In contrast, larvae of *Chrysoperla carnea* feed on both unparasitized eggs and early larval stages, thereby complementing the pest control effects of *Trichogramma*. This synergistic interaction between the two biological agents enhances overall pest management efficiency while simultaneously reducing potential entry points for fungal pathogens (Franeta, 2025b)

In the 2024 study, the combination of *Trichogramma brassicae* and *Chrysoperla carnea* was the most effective in controlling the ECB.This treatment achieved the highest yield, which was significantly higher compared to other treatments. Additionally, there was a notable reduction in ear damage and the lowest number of tunnels in stalks and ears. These results indicate a synergistic effect between the two biological agents, allowing for effective pest control and reduced risk of secondary infections (Franeta, 2025b)

Interestingly, while biological control agents had a significant impact on reducing *Aspergillus* infections in maize ears, the incidence of *Fusarium* infections was too low to permit evaluation of treatment efficacy. This differential response can be attributed to the different infection pathways utilized by these two fungal species. While *Aspergillus* primarily requires insect-induced entry points (Horn, 2007), *Fusarium* species utilize multiple infection routes, including silk infection, insect wounds, and systemic spread from infected seeds (Foroud et al., 2014). Therefore, biological treatments focused on insect-induced wounds may have a limited impact on *Fusarium*. Moreover, extreme weather conditions during 2024, with temperatures exceeding 40°C and insufficient rainfall, were favorable for the development of *Aspergillus*. High temperatures combined with plant heat stress created optimal conditions for colonization (Abbas et al., 2009). These conditions likely increased the importance of preventing insect damage through biological control, making wound prevention even more crucial for infection management.

Conclusion

The results of the research have shown that the combined application of biocontrol agents, using the parasitic wasps *T. brassicae* and the larvae of the green lacewing *C. carnea*, significantly reduced infections caused by fungi from the genus *Aspergillus* on maize. The synergistic effect of these two biological agents successfully suppresses the population of the ECB, thereby reducing entry points for *Aspergillus* and *Fusarium* species and increasing the safety and quality of the grain. On the other hand, the effect of the treatment on infections caused by fungi from the genus *Fusarium* was not statistically significant, which is likely due to the low incidence of these infections during the experiment period, as well as the different infection pathways these fungi use. These results confirm that biological control represents an effective and environmentally friendly alternative to chemical pesticides. Further development of integrated approaches combining biological control with other disease management methods. Future research should focus on the long-term efficacy of biological control and its impact on mycotoxin occurrence in maize, as well as on the development and application of combinations of multiple biological control agents to achieve comprehensive protection of maize against harmful organisms and to maintain high grain yield quality and safety.

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Efikasnost kombinovane biološke kontrole u zaštiti kukuruza od kukuruznog plamenca i gljivičnih infekcija

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Sažetak: Kukuruz (*Zea mays* L.) je jedna od najvažnijih poljoprivrednih kultura u svetu, ali je njegova proizvodnja često ugrožena napadima insekata i patogenih gljiva. Jedan od najznačajnijih štetočina kukuruza je kukuruzni plamenac - *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae). Larve ovog insekta oštećuju klipove kukuruza, što dovodi do direktnih gubitaka u prinosu i stvara ulazne tačke za infekciju gljivama, prvenstveno iz rodova *Fusarium* i *Aspergillus*. Ove gljive uzrokuju trulež klipa i mogu ozbiljno narušiti kvalitet i bezbednost zrna za korišćenje. Biološka kontrola predstavlja ekološki prihvatljivu alternativu primeni hemijskih pesticida. Cilj ovog istraživanja bio je da se proceni efikasnost biološke kontrole kukuruznog plamenca u smanjenju infekcija gljivama *Fusarium* i *Aspergillus*. Poljski ogled je postavljen po principu slučajnog blok sistema sa četiri ponavljanja. Pored kontrole, tretmani su obuhvatali primenu parazitskih osica iz roda *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae), larvi predatorske vrste zlatooka - *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), kao i kombinaciju ova dva biološka agensa. Rezultati su pokazali značajan uticaj tretmana na infekcije gljivama *Aspergillus*. Biljke tretirane kombinacijom parazitskih osica i zlatooke imale su značajno nižu stopu infekcije u poređenju sa kontrolom i ostalim tretmanima, što se može pripisati sinergističkom efektu u suzbijanju populacije kukuruznog plamenca i smanjenju broja ulaznih tačaka za gljive. S druge strane, tretmani nisu imali statistički značajan uticaj na infekcije gljivama *Fusarium*, delom zbog niske ukupne pojave ovih gljiva tokom ogleda. Dobijeni rezultati ukazuju na značaj primene biološke kontrole u smanjenju šteta i očuvanju kvaliteta zrna kukuruza.

Ključne reči: *Aspergillus*, biološka zaštita, *Chrysoperla carnea*, *Fusarium,* kukuruz, kukuruzni plamenac, *Trichogramma brassicae*