

EFFECT OF DIFFERENT PESTICIDES AND EUROPEAN CORN BORER ATTACK ON PHYTIC PHOSPHORUS CONTENT IN MAIZE GRAIN

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Abstract

This work aimed to evaluate changes in phytic phosphorus content as the response of three maize hybrids (ZP 457, ZP 5601, and ZP 606) to different pesticide treatments for European Corn Borer (ECB) attacks and, as well as the correlation between damage caused by ECB attack and Pphy content. In the experimental field, maize ears were sampled and dried at a moisture content of 14% for the determination of changes in phytic phosphorus content in the grain. The content of phytic phosphorus was determined using UV / VIS spectrophotometry. Observed results showed the highest content of phytic phosphorus for untreated control for all tested hybrids, compared to pesticide treatments. The content of Pphy in the tested maize kernel samples ranged from 2.12 to 3.40 mg g⁻¹. A significant positive correlation between attacks of larvae and Pphy content could indicate activation of its antioxidative function as a response to damage caused by ECB attack.

Key words: European corn borer, phosphorus content, grain, *Zea mays* L.

Introduction

Maize (*Zea mays* L.) is an important cereal crop used for human consumption, animal feed, and industrial applications. It is a source of many phytochemicals, such as fatty acids, provitamin A and vitamin E, and minerals, starch, proteins, oils, and secondary metabolites. Secondary metabolites and their antioxidant properties in maize grain and leaves have been described in the past ten years. Both abiotic and biotic stress, as well as growing conditions, can impact the synthesis of antioxidants in the whole plant.

Phytic acid (PA) has antinutritive and antioxidant functions and contains about 80% of maize grain phosphorus (phytic phosphorus) located mostly in the endosperm. The main

role of phosphorus is the regulation of biochemical processes in plant cells, it is a structural element of essential biomolecules (ATP and NADPH), nucleic acids (DNA and RNA), and lipids in cell membranes (de Bang et al., 2021). In the light reaction of photosynthesis, P absorbs light in the thylakoid membrane to generate ATP and NADPH which in the Calvin-Benson cycle convert CO₂ to carbohydrates in the chloroplast stroma. On the other hand, monogastric animals and humans cannot metabolize phytic acid due to the sufficient level of phytate-degrading enzyme activity in their digestive tract (Singh et al., 2011). Consuming foods rich in phosphorus can lead to mineral deficiencies as phosphorus binds to

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micronutrients. To improve seed nutritional quality and availability of micronutrients the reduction of PA in cereal seeds has become a significant challenge in breeding programs. Molecular breeding methods were used to create low phytic acid (*lpa*) mutants in maize (Raboy et al., 2020; Colombo et al., 2022). Also, to explore and identify low phytic acid genotypes diverse sets of maize landraces, inbred lines, and hybrids were analyzed for phytic acid content (Drinić Mladenović et al., 2009; Dragičević et al., 2013; Yathish et al., 2021; Kahriman et al., 2021). Brankov et al. (2020) tested herbicides and foliar fertilizer mixtures on grain composition in maize inbred lines and found a significant environmental impact on phytic acid content in maize.

Agro-technical measures such as pesticide treatments are factors that affect the quality of agricultural products by controlling different pests (Gošić-Dondo et al., 2015). On the other hand, the use of pesticides presents a signifi-

cant danger to both target and non-target individuals, creating a dual risk (Sruthi et al., 2016). European corn borer (ECB) (*Ostrinia nubilalis* Hübn.) may attack the maize plants causing damage of above-ground parts of plants (reducing plant growth) and ear damage, and leading to yield loss (Bohn et al., 2000). Chemical control of ECB attacks in maize fields can be challenging because of the overlapping of the *O.nubilalis* generations, a long period of egg-laying and short-term exposure of larvae to pesticides. Further research is necessary to gain a complete understanding of the potential oxidative damage that insecticides may inflict on maize. The aim of this work was to 1) examine the influence of different pesticide treatments for ECB attack and on phytic phosphorus (Pphy) content in maize grain of three maize hybrids and 2) to check the relationship between Pphy content in grain and damage caused by ECB attack.

Material and methods

Plant materials and field trials

The field trial was conducted in the experimental field of the Maize Research Institute “Zemun Polje” at low-carbonated Chernozem in the 2021 growing season. Sowing of the maize hybrids (ZP 457- FAO 400, ZP 5601-FAO 500 and ZP 606-FAO 600) was performed during the first half of May, using the randomized complete block design (RCBD) in three replications, with an elementary plot of 10.5 m², including three rows of 5 m length with 0.7 m inter-row distance and 0.25 m between plants in row, making a set of 57,143 plants per ha. The experiment was set up according to a random block system with seven treatments (two seed treatments and 5 foliar treatments) and control in three repetitions. Before sowing, the seeds were treated with concentrated

seed treatment suspensions ST1 - Sonido (insecticide) and ST2- Maxim (fungicide). Foliar insecticides (FT1 - Decis, FT2 - Coragen, FT3 - Avaunt, and FT4 - Ampligo) and bioinsecticide (FT5 - Lepinox) were applied twice with a motor atomizer 15 days after the maximum flight of the first generation (June 30, 2021) and 15 days after the maximum flight of the second generation (July 30, 2021). All treatments were compared with the untreated control (C). The list of applied pesticide treatments is given in Table 1. In assessing pest tolerance, attention is given to the percentage of corn borer attacks, taking into account the damage to all above-ground parts: leaves, tassel, stem and corn cobs. The percentage of attacked plants is presented as a ratio between the total number of plants and the number of damaged plants.

Table 1. List of applied pesticides, method of application, active ingredients and concentrations
 Tabela 1. Spisak primenjenih pesticida, način primene, njihove aktivne supstance i koncentracija

Commercial name	Method of application	Active ingredients	Concentration
Decis 2.5 EC	FT1- foliar treatments	Deltamethrin	400 ml ha ⁻¹
Coragen 20 SC	FT2- foliar treatments	Chlorantranilprole	100 ml ha ⁻¹
Avaunt 15 EC	FT3- foliar treatments	Indoxacarb	250 ml ha ⁻¹
Ampligo 150 ZC	FT4- foliar treatments	Chlorantranilprole + Lambda-cyhalothrin	200 ml ha ⁻¹
Lepinox plus	FT5- foliar treatments	<i>Bacillus thuringiensis</i> ssp. <i>Kurstaki</i>	750 kg ha ⁻¹
Sonido	ST1- seed treatment	Thiacloprid	62.5 ml/25000 seeds
Maxim XL035 FS	ST2- seed treatment	Fludioxonil metalaksil M	100ml/100kg seeds
Control	C-untreated	-	-

Sample preparation and biochemical analysis

In the experimental field, ten maize ears from the inner row were manually sampled in the full physiological maturity stage and dried at a moisture content of 14% for the determination of changes in Pphy content in the grain. Grain samples were ground in a Perten (Instruments, Hägersten, Sweden) mill for fine sample preparation, with particle size <500 µm. Sample extraction was done with 5% trichloroacetic acid after which samples were

centrifuged at 12,000 rpm for 15 min (Eppendorf centrifuge 5471r, Hamburg, Germany) at 4 °C. Absorbance was measured at 500 nm using a spectrophotometer (UV-1601 Shimadzu, UV-VIS Spectrophotometer, Japan). The Pphy concentration was determined based on the pink colour formed upon the reaction between ferric ion and sulfosalicylic acid from the Wade reagent (Dragičević et al., 2011). The content of Pphy was presented as mg g⁻¹.

Statistical Analysis

All data analysis were done in MSTAT-C software using two-factor ANOVA with an LSD (Least Significant Difference) test level of 0.05 used for ranking the tested varieties. In-

terrelations of analyzed Pphy content and ECB damage to the plant were presented as Pearson's correlation coefficients and regression analysis.

Results and discussion

Effective crop-pest management in maize production can preserve yield stability and quality. Besides the alternatives such as the use of bioinsecticides, the use of chemical insecticides is the most commonly used pest control strategy for yield stability (Abhilash and Singh, 2009). On the other hand, the use of conventional insecticides in ECB attack control can induce abiotic stress through the phytotoxic effect in plants and have drawbacks to agriculture. In addition to the abiotic stress

caused by different active ingredients, ECB attacks can cause biotic stress in plants. During vegetative growth maize plants can be exposed to both stresses, which induce a higher level of non-enzymatic antioxidative compounds (Shakir et al., 2018; Lei et al., 2020).

Activation of metabolic changes and induction of antioxidant defence system occur in plants as a reaction to stresses. The increase of secondary metabolites in plants triggered by stress is described in many works. Some results

indicate that maize genotypes with low phytic acid are correlated to a high degree of oxidative stress during maturation (Doria et al., 2009). Banothu and Uma (2021) showed that stress conditions are associated with changes in various phenolic compounds. In the work of Brankov et al (2020), the content of phenolic, proteins, total carotenoid content, total chlorophyll, and Pphy, was diverse in maize leaves treated with different herbicide doses. Foster and Brust (1995) noticed that the insecticide carbofuran may be acting as a growth stimu-

lant, as well as an insecticide on watermelon. Some other studies confirm that indiscriminate use of chemicals affects increased enzyme activity and reduction of plant yield or quality (García-Hernández et al., 2000).

To understand the phytotoxicity of pesticides, maize plants were treated with pesticides with different active ingredients. Alterations of Pphy contents in maize grain of the three analyzed maize hybrids, with seven different pesticide treatments for pest control and untreated control were presented in Figure 1.

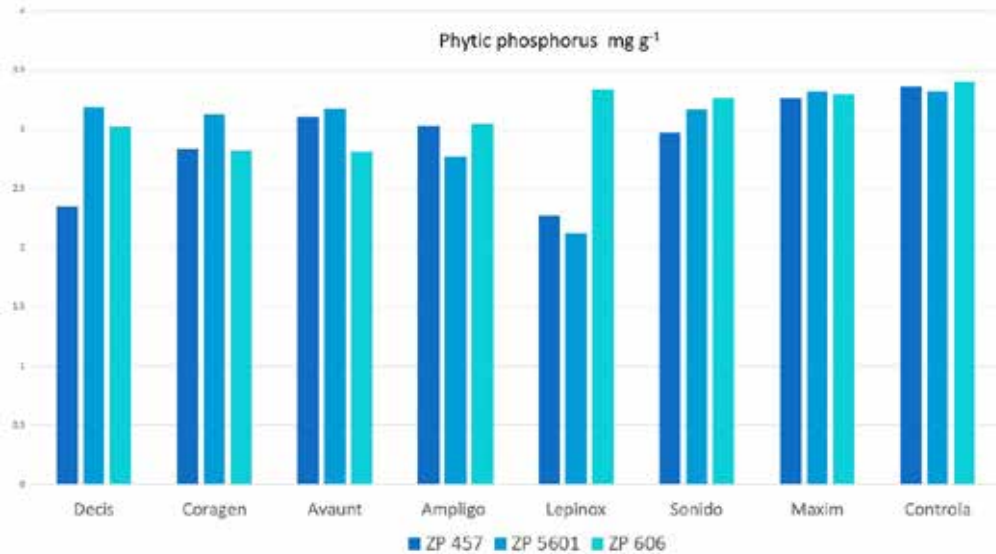


Figure 1. Alterations of phytic phosphorus in maize hybrids grain for different treatments
 Grafikon 1. Sadržaj fitinskog fosfora u zrnju ispitivanih hibrida kukuruza po tretmanima

The total Pphy content varied greatly among the tested maize grain samples, ranging from 2.12 to 3.40 mg g⁻¹ (Table 2). All tested hybrids showed the highest Pphy content in the untreated control, where total ECB attack was also high. Hybrid ZP 606 showed the highest Pphy content (3.40 mg g⁻¹), following ZP 457 and ZP 5601 with 3.36 and 3.32 mg g⁻¹, respectively. No statistically significant difference existed between untreated control and seed treatments, except for hybrid ZP 457 treated with Sonido. Additionally, all tested hybrids showed the highest Pphy content for Maxim among pesticide treatments. This could be explained by the fact that Maxim has a fungicide effect, and the total ECB attack was

also high, which may increase the level of the non-enzymatic oxidative compounds in grain as Pphy. On the contrary, the lowest level of Pphy was detected with the bioinsecticide treatment for ZP 457 and ZP 5601. This can be attributed to the fact that *B. thuringiensis ssp. Kurstaki* has a relatively low toxicity impact on plant cells, although it may not have the lowest percentage of ECB attacks. It is known that maize hybrids with higher FAO groups have intensive vegetative growth suitable for ECB infestation (Lemic et al., 2019). There was a statistically significant difference between the hybrids belonging to FAO 400 and FAO 500 treated with bioinsecticide and the rest of the treatments.

Table 2. The percentage of second generations of ECB attack and total attack, and Pphy content of hybrids under different pesticide treatments

Tabela 2. Procenat napada druge generacije i totalnog napada kukuruznog moljca, i sadržaj Pphy u zrnu hibrida kod različitih tretmana pesticidima

Treatments	Hybrid	IIA (%)	ΣA (%)	Pphy (mg g ⁻¹)
FT1 - Decis	ZP 457/1	56.52 ^{de}	76.53 ^{de}	2.34 ^j
	ZP 5601/1	51.69 ^{fg}	74.6 ^e	3.18 ^{bcde}
	ZP 606/1	51.76 ^{fg}	75.38 ^e	3.02 ^{efg}
FT2 - Coragen	ZP 457/2	46 ^{hi}	69.04 ^f	2.83 ^{ghi}
	ZP 5601/2	39.61 ^k	66.67 ^f	3.12 ^{cdef}
	ZP 606/2	43.14 ^{ijk}	67.65 ^f	2.82 ^{ghi}
FT 3 - Avanut	ZP 457/3	41.17 ^{jk}	67.21 ^f	3.1 ^{def}
	ZP 5601/3	45.7 ^{hi}	68.03 ^f	3.17 ^{bcdef}
	ZP 606/3	43.81 ^{ij}	68.58 ^f	2.81 ^{hi}
FT4 - Ampligo	ZP 457/4	49.51 ^{gh}	69.5 ^f	3.01 ^{efgh}
	ZP 5601/4	45.92 ^{hi}	69.39 ^f	2.77 ⁱ
	ZP 606/4	46.64 ^{hi}	68.96 ^f	3.05 ^{ef}
FT5 - Lepinox	ZP 457/5	52.31 ^{fg}	74.33 ^e	2.27 ^{jk}
	ZP 5601/5	54.86 ^{def}	80.78 ^{bc}	2.12 ^k
	ZP 606/5	52.92 ^{efg}	77.1 ^{de}	3.33 ^{ab}
ST1 - Sonido	ZP 457/6	60.96 ^{bc}	82.74 ^b	2.97 ^{fghi}
	ZP 5601/6	52.28 ^{fg}	79 ^{cd}	3.16 ^{bcdef}
	ZP 606/6	57.95 ^{cd}	81.32 ^{bc}	3.26 ^{abcd}
ST2 - Maxim	ZP 457/7	51.92 ^{fg}	82.9 ^b	3.27 ^{abcd}
	ZP 5601/7	54.47 ^{def}	82.84 ^b	3.32 ^{abc}
	ZP 606/7	53.91 ^{ef}	82.74 ^b	3.29 ^{abcd}
C - Controla	ZP 457/k	63.61 ^{ab}	89.65 ^a	3.36 ^{ab}
	ZP 5601/k	61.82 ^{abc}	88.07 ^a	3.32 ^{abc}
	ZP 606/k	64.87 ^a	89.54 ^a	3.4 ^a
LSD 0.05		3.875	3.388	0.2069

IIA - second generation attack; ΣA - total attack; Pphy - phytic P

Hybrid ZP 457 treated with Decis showed a statistically significant difference compared with the rest of the foliar treatments. The level of Pphy in maize grain did not show higher content with pesticide treatments, on the contrary Pphy was lower in all analyzed seed samples treated with all pesticides. This could be explained by the low phytotoxicity of applied pesticides, so there is no accumulation of antioxidative compounds in plants. Additionally, compared with the control, lower values for

Pphy could be due to a lower percentage of attacks in treated samples. In the work of Noori (2021), an increase in Pphy content in wheat grain was observed during drought stress in wheat grain. On the contrary, various herbicide applications on maize inbred lines decrease Pphy content (Dragičević et al., 2010). Nam et al. (2018) found that the amount of phytic acid was higher in rice grains in stressed plants, implying that plants might tolerate oxidative stress by increasing its content.

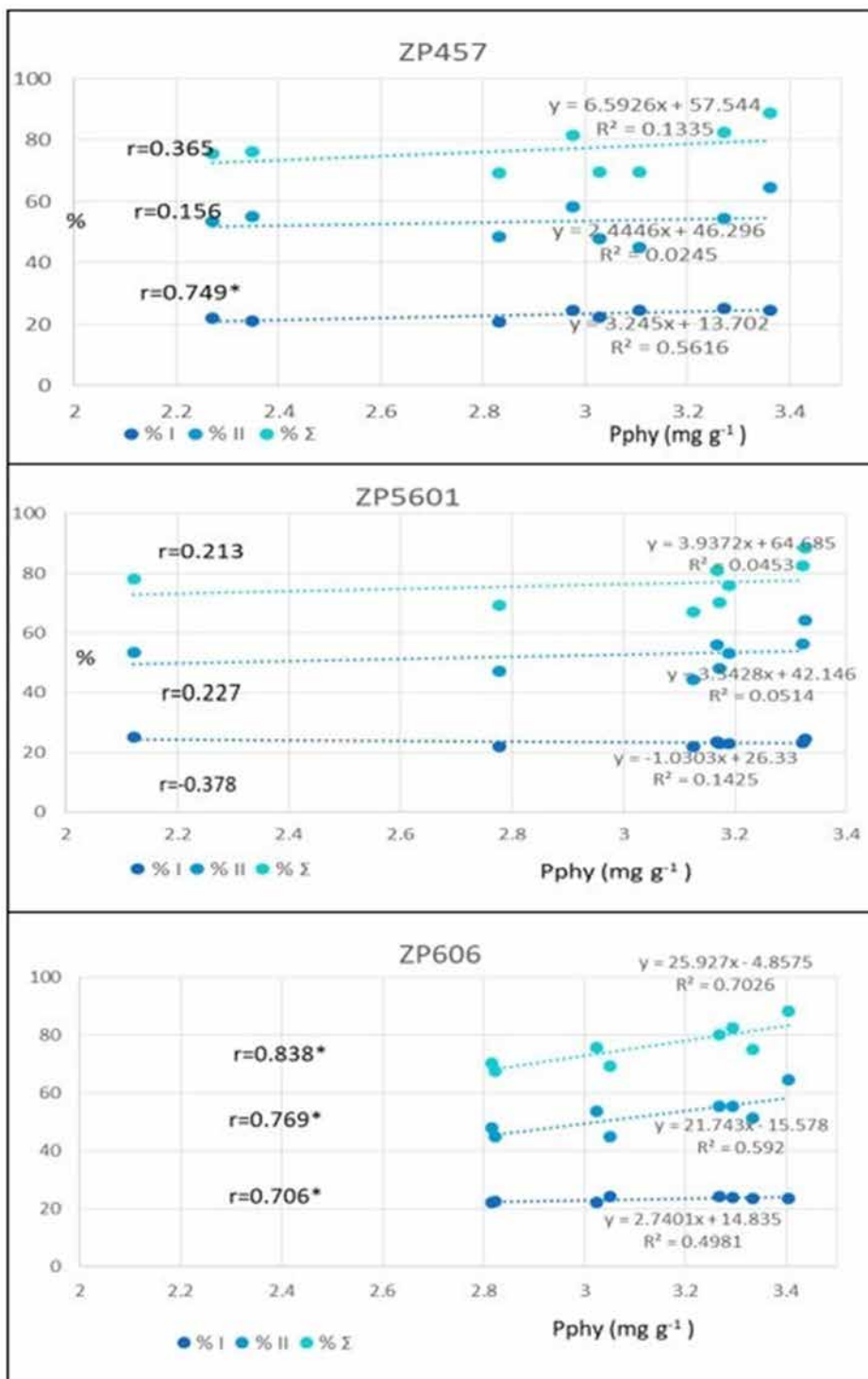


Figure 2. Regression analysis and the correlation coefficients of Pphy content in seeds of hybrids: ZP 457, ZP 5601 and ZP 606

Grafikon 2. Regresiona analiza i koeficijenti korelacije fitinskog P u zrnu hibrida: ZP 457, ZP 5601 and ZP 606

To determine the connection between changes in Pphy and resistance of maize genotypes, regression and correlation analysis were done between first and second-generation attacks and total attack and amount of Pphy (Figure 2). Regression analysis showed that there is a linear increase in Pphy content in grain with an increase in the percentage of plants infected with the first and second generation of ECB larvae, as well as with total attack in ZP 457 and ZP 606 hybrids. Opposite, Hybrid ZP 5601 showed a linear decrease of Pphy amount in grain with the increase in the percentage of plants infected with the first generation of ECB larvae. On the contrary, there was a linear increase of Pphy with the increasing second generation of ECB larvae and total attack.

According to our previous report (Gošić et al., 2023), a linear decrease in the percent-

age of plants infected with second-generation ECB larvae with increasing Pphy content in the seed was observed, as well as a linear increase in first-generation attacks and an increase in Pphy content. Correlation analysis showed a trend of positive correlation (0.749*) of the amount of Pphy in the seed with the attack of the first generation for hybrid ZP 457. Furthermore, a trend of positive correlation (0.706*; 0.769* and 0.838*) was observed for hybrid ZP 606 in the amount of Pphy in seed and attack of the first and second generation and total attack. In hybrid ZP 5601 correlation values were low i.e. insignificant. A significant positive correlation between attacks of larvae and Pphy content could indicate activation of its antioxidative function as a response to damage caused by ECB attack.

Conclusion

Secondary metabolic changes in maize kernel are a response to chemical stress conditions and attacks caused by ECB, ensuring better competitiveness and protection from biotic and abiotic stress. The content of Pphy in the tested maize kernel samples ranged from 2.12 to 3.40 mg g⁻¹. The results of this study showed that pesticides had a positive impact on the

average changes in the maize kernel, demonstrating that adequate and suitable use could improve grain quality. Further experiments should be conducted to investigate the relationship between Pphy and yield performance under various pesticide treatments, as well as to test their correlation with the percentage of ECB attacks.

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UTICAJ RAZLIČITIH PESTICIDA I NAPADA KUKURUZNOG PLAMENCA NA SADRŽAJ FITINSKOG FOSFORA U ZRNU KUKURUZA

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Sažetak

Cilj ovog rada je procena promene u sadržaju fitinskog fosfora kao odgovora tri hibrida kukuruza (ZP 457, ZP 5601 i ZP 606) na različite tretmane pesticidima protiv napada kukuruznog plamenca, kao i određivanje korelacije između oštećenja biljke izazvane napadom larvi i sadržaja fitinskog fosfora. U oglednom polju uzorkovani su klipovi kukuruza radi određivanja promene sadržaja fitinskog fosfora u zrnu UV/VIS spektrofotometrijom. Dobijeni rezultati su pokazali najveći sadržaj fitinskog fosfora za netretiranu kontrolu kod svih ispitivanih hibrida u poređenju sa tretmanima pesticidima. Sadržaj fitinskog fosfora u ispitivanim uzorcima zrna kukuruza kretao se od 2,12 do 3,40 mg g⁻¹. Uočena značajna pozitivna korelacija između napada larvi i sadržaja fitinskog P može ukazivati na aktivaciju njegove antioksidativne funkcije kao odgovor na oštećenja izazvana napadom kukuruznog plamenca.

Ključne reči: kukuruzni plamenac, sadržaj P, zrno, *Zea mays* L.

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