UDC: 63 ISSN 1450-8109

# JOURNAL OF AGRICULTURAL SCIENCES BELGRADE

**Vol. 66, No. 4, 2021**

**Published by University of Belgrade**
**Faculty of Agriculture**
**Republic of Serbia**

UDC: 63 ISSN 1450-8109

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Journal of Agricultural Sciences (Belgrade) www.joas.agrif.bg.ac.rs
J. Agric. Sci (Bg).



PUBLISHED BY

University of Belgrade-Faculty of Agriculture
11081 Belgrade-Zemun, Nemanjina 6, PO Box 14, Serbia
Tel: + 381 11 4413-555/467; Fax: + 381 11 2193-659; E-mail: redakcija@agrif.bg.ac.rs
URL: http://www.agrif.bg.ac.rs/

DTP Service: Snežana Spirić

Printed by the Faculty of Agriculture, Belgrade-Zemun
Circulation: 100

Publishing is supported by the Ministry of Education, Science and Technological Development
of the Republic of Serbia, Belgrade

According to the opinion of the Ministry of Science of the Republic of Serbia
No. 413-00-1928/2001-01 dated November 6, 2001 this
Journal is exempt from general tax liability

Frequency: Four times per year

Abstracting and Indexing
CAB Abstracts, AGRICOLA, SCIndeks, EBSCO, Scopus, DOAJ

Number of institutions the Journal is exchanged with: 80

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE
Vol. 66, No. 4, 2021

C O N T E N T S

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE
Vol. 66, No. 4, 2021

S A D R Ž A J

Journal of Agricultural Sciences (Belgrade)
Vol. 66, No. 4, 2021
Pages 309-319

https://doi.org/10.2298/JAS2104309M
UDC: 633.31/.37:631.547.1
Original scientific paper

# THE INFLUENCE OF ELECTRIC VOLTAGE ON THE GERMINATION OF LEGUMINOUS SEEDS

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**Abstract:** The aim of this paper was to examine the influence of the electrostatic field on seeds of soybean (*Glycine max* L), vetch (Vicia spp. L.), pea (*Pisum sativum* L.), grass pea (*Lathyrus sativus* L.) and chickpea (*Cicer arietinum* L.) which were selected at the Institute of Field and Vegetable Crops in Novi Sad. The DC voltage of 9V was used. The durations of seed treatment were 0 (control), 1, 2 and 3 minutes. The trial was set up as a randomized block design with four replications. 4x100 seeds of each variant and control (untreated seed) were treated. After treatment, seed quality was examined using a germination test (optimal temperature) and a cold test (low temperature). The results of the study showed that the effect of the electrostatic field on seed quality depended on the plant species, the time of seed treatment and the temperature conditions in which the seed germinated after treatment. The increase in germination energy ranged up to 18.18% in vetch, and a decrease of up to 12% was observed in chickpea. The application of the electrostatic field had a significantly greater impact on seeds that were exposed to low temperatures in the germination process after treatment. The increase in seed germination ranged up to 82.35% in chickpeas, and the decrease amounted to 92.68% in peas. In addition, the obtained results indicate that it is not possible to talk about the universal application of a certain duration of seed treatment.

**Key words:** electrostatic field, cold test, germination, legumes.

## Introduction

The use of high-quality seeds is one of the most important elements in increasing agricultural production in any farming system. This element has become more crucial than ever for providing enough food for the rising number of people

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in the world, which is expected to exceed nine billion by the year of 2050 (Sabry, 2018). Every year, more than 2/3 of the total workable land in the world is sown with seeds, of which about 90% of humanity's needs in the food and agro-processing industry are met (Miric and Brkic, 2002). One of the basic conditions for achieving high yields is sowing quality seed (Milosevic et al., 1996). The quality of seeds is measured in many ways, including genetic and physical purity, germination, vigor, uniformity in sizes, resistance from seed-borne diseases, and any other factors that may affect seed performance in the field (Sabry, 2018). Success in producing quality seed of a particular crop in one area and failure in another area illustrates the importance of the environmental influence on seed development and maturation (Vujakovic et al., 2015). During seed development and maturation, environmental conditions, including temperature, water stress or excessive rain, lack of nutrients, disease infestation, and insect pressure, influence seed quality (Miladinov et al., 2020a). During prolonged and less than optimum storage conditions, physiological, biochemical, and cytological changes occur in soybean seeds, leading to the deterioration of their quality (Mbofung, 2012). The extent of seed deterioration depends on species, storage environment, length of the storage period, and the initial quality of the stored seeds. The seed germination was used as the basic quality indicator in the international seed trade. Different results of seed germination obtained under laboratory conditions, and field emergence have encouraged the development of the concept of seed vigor (Vujakovic et al., 2011). Seed viability or vigor presents a set of traits that affect seed germination and the formation of strong and healthy seedlings under different environmental conditions (ISTA, 2019).

In seed production, different treatments are designed to improve the quality of the seeds (Miladinov et al., 2020b; Nabi et al., 2020). Physical treatments belong to the oldest known seed treatments. Physical methods include different lasers, ultraviolet radiation (Hernández Aguilar et al., 2009), magnetic induction and electromagnetic waves (Djukic et al., 2017), etc. In recent years, there has been an increased interest in the application of a particular voltage and current as alternate physical dimensions for seed treatment (Cvijanovic and Djukic, 2020). In many studies, the exposure to electric, magnetic and electromagnetic waves showed positive or negative effects, influencing germination rate, germination percentage, seed weight, plant height, protein content, productivity, leaf surface, fruit weight and yield. Research results depend on the frequency, duration of exposure, seed traits and plant species. Proper combinations are necessary to achieve positive effects (Parsi, 2007).

The aim of this study was to investigate the influence of the electrostatic field on germination parameters (germination energy, germination percentage, atypical seedling, dead seed). Tests were performed on soybean seed, peas, beans, grass pea and chickpea using the standard and cold tests.

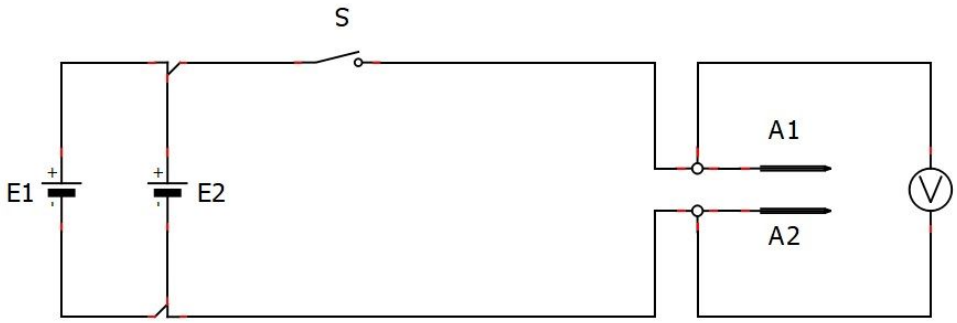
## Material and Methods

Plant materials

The experiment was performed on seeds of soybean (*Glycine max* L), vetch (*Vicia spp*. L.), pea (*Pisum sativum* L.), grass pea (*Lathyrus sativus* L.) and chickpea (*Cicer arietinum* L.), which were selected at the Institute of Field and Vegetable Crops in Novi Sad. Seeds were selected on the basis of having no visible deformities. Seed used in this experiment were sown in 2020 on the experimental field of the Institute of Field and Vegetable Crops. After harvesting, the seeds were stored in paper bags at a temperature of 10–15°C.

Electric field treatments

Electrodes with an area of 10 cm × 10 cm were made of aluminium foil. Each of the electrodes was connected to one of the terminals of the DC batteries (Picture 1).




Picture 1. The representation of electrical wiring (E1, E2 – batteries, S – electrical switch, A1, A2 – aluminium electrodes, V – voltmeter).

The DC voltage of 9 volts was used. The durations of seed treatment were 0 (control), 1, 2 and 3 minutes. A switch was placed between one electrode and the battery terminal so that the circuit between the mentioned electrodes can be interrupted at any moment. Seeds, which were subjected to treatments, were placed between the electrodes. The distance between the electrodes was 1 cm, and it was fixed during the experiment to preserve that the field was evenly distributed between the electrodes. A voltmeter was placed at the end of the electrode to measure the presence of electrical voltage. During the experiment, the voltage values were monitored during the complete experiment to determine whether the

electrostatic field between the electrodes during the experiment was constant, i.e. invariable over time.

Seed testing

The working sample consisted of 4 × 100 seeds for each plant species, applied treatment and test used. Sterile sand was used as the substrate in the germination test. Sand sterility was achieved by sand washing and drying at high temperatures. Seeds were incubated in the germination chamber at 25°C (soybean) and 20°C (pea, chickpea, grass pea, vetch). The final germination was determined after 8 days (soybean, pea, chickpea), 14 days (vetch, grass pea) (ISTA, 2019). The cold test simulates early spring field conditions by germinating the seeds in wet soils (70% water holding capacity) and incubating them at 5–10°C for a specified period. At the end of the cold period, the test is transferred to a favorable temperature for germination. The samples were exposed to 7°C for seven days and afterwards placed in the germination chamber at 25°C (soybean) or 20°C (other species) for six days. The percentage of normal seedlings is considered as an indication of seed vigor. Vigorous seeds germinate better under cold environments (Hampton and TeKrony, 1995).

Statistical analysis

The data were analyzed using statistical software 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, SAD). A two-way ANOVA was used to test the effect of legumes and exposure time. When the ANOVA test produced significant results, the Duncan's test was used to separate means in different groups ($p \leq 0.05$, and $p \leq 0.01$). The AMMI model was used to analyze the interactions. AMMI analysis of variance and AMMI1 biplot were done using the GenStat software (VSN International, UK).

## Results and Discussion

In addition to the positive impact, the application of the electrostatic field also had a negative impact. The results on chickpea seeds showed that the application of the electrostatic field led to a decrease in germination energy by exposing the seeds to electrostatic fields during treatments of 1 and 3 minutes. Germination energy was reduced by 8% and 12%, respectively. Ghodbane et al. (2013) point out that the results depend on the characteristics of the seed, the type of plant, the frequency and duration of seed stimulation. Miladinov et al. (2018) came to similar results in the case of soybean seeds. The authors point out that the effect of pre-sowing treatment depends not only on applied treatments but also on the genotype. Some genotypes show a positive response to pre-sowing treatment and improve seed

germination, as well as germination rate parameters, MGT and T50, a measure of the rate and time-spread of germination, while others have a significant inhibitory effect. In two genotypes, a decrease in seed germination was observed by as much as 11% (Miladinov et al., 2018) (Figure 1).

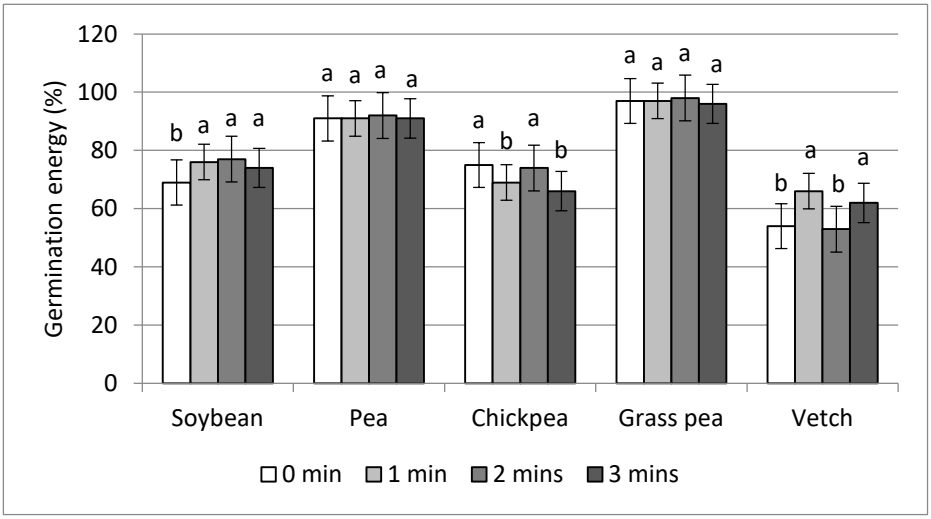



Figure 1. The influence of electric voltage on the germination energy of leguminous seeds (germination test).

Germination (using the germination test)

Within the physical treatment, which includes the use of an electrostatic field, the energy introduced into the cells creates the conditions for molecular transformations, and as a result, the necessary substances are created for the cells (Atak et al., 2007). Researches indicate that electric fields affect biological process including free radicals, excite the activity of proteins and enzymes to increase seed vigor (Morar et al., 1999). Results showed that the application of an electrostatic field could significantly increase the germination of beans, depending on the duration of treatment. The 1-minute treatment increased germination by 7.06%, while the 2- and 3-minute treatments had no significant effect. Lynikiene et al. (2006) reported that the electric field not only increased the rate of germination but also increased the germination percentage of carrot by 24%, that of radish and beetroot – by 12%, beet seeds – by 7%, and barley seeds – by 9%. Moon and Chang (2000) reported that electric fields increased the rate and percentage of germination in treated seeds during short time periods. In comparison with control seeds, the germination rate of treated seeds increased up to 1.2 to 2.8 times (Figure 2).

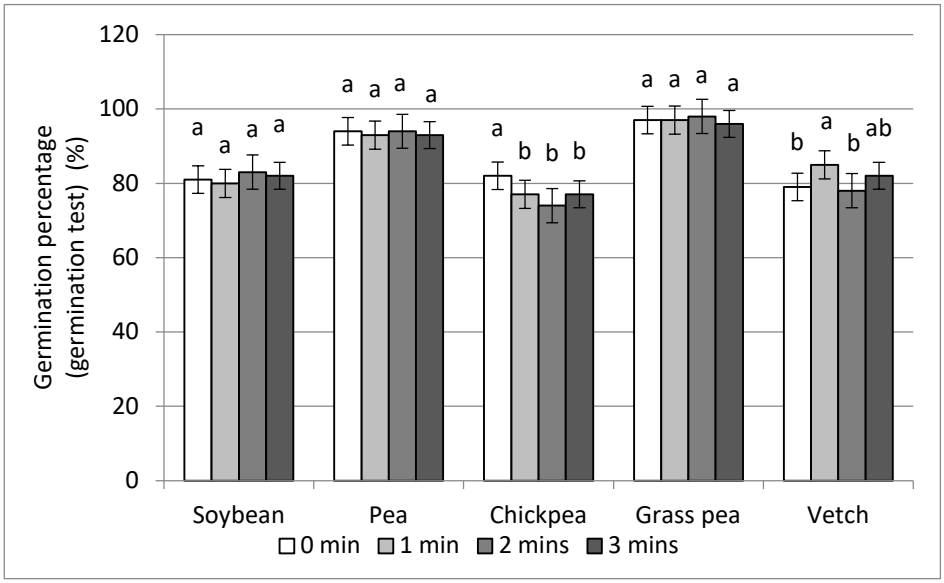



Figure 2. The influence of electric voltage on the germination percentage of leguminous seeds using the germination test.

Cai and Wang (2003) found that the electrostatic field can be an effective method for increasing the germination of seeds of many crop and tree species, when properly treating seeds, otherwise, there is a decrease in seed germination. The germination of chickpea seeds decreased by 6.10% and 9.76%, respectively, by applying 3- and 1-minute treatments. The electrostatic field had no effect on the germination of soybean seed, pea and grass pea. Mericle et al. (1964) reported no difference between germination percentages of treated and untreated barley seeds. Moreover, no significant difference has been reported regarding the seedling growth of treated and untreated seeds. Miladinov et al. (2020c) found that the effect on soybean seeds depended on the strength of the electrostatic field. The application of an electrostatic field of 9 V had no effect on seed germination, while the use of 6 V increased germination by 5%. The same authors determined that a better result was achieved with seeds that were not immersed in water before the application of the electrostatic field.

Germination percentage (using the cold test)

Testing the viability of soybean seeds in optimal conditions provides information on the maximum possible germination and initial growth. As field conditions are rarely optimal, it is necessary to perform testing under conditions that are close to environmental conditions to obtain a more reliable forecast of crop

behavior in the initial stages of growth and development (Srebric et al., 2010). Regarding beans, the application of the cold test had a positive effect on seed germination, but only in 2- and 3-minute treatments. Germination improved by 8.57% and 17.95%, respectively. The results are partly consistent with research conducted by Kerdonfag et al. (2002). They point out that the exposure of rice seeds to the electric field during different time durations had positive effects on germination. Soybean seeds also have improved seed germination, but only in a 2-minute treatment. Germination increased by 38.30%. Within the increased treatment time or the 3-minute treatment, germination reduced to 34.48% in soybean. In chickpea, the application of the electrostatic field had a negative effect. However, at low temperatures, an increase in germination was achieved. Regardless of the time of exposure to the electrostatic field, germination increased from 36.84% to 82.3% in chickpea. The results showed that the applied treatments had the ability to improve seed quality (Figure 3).

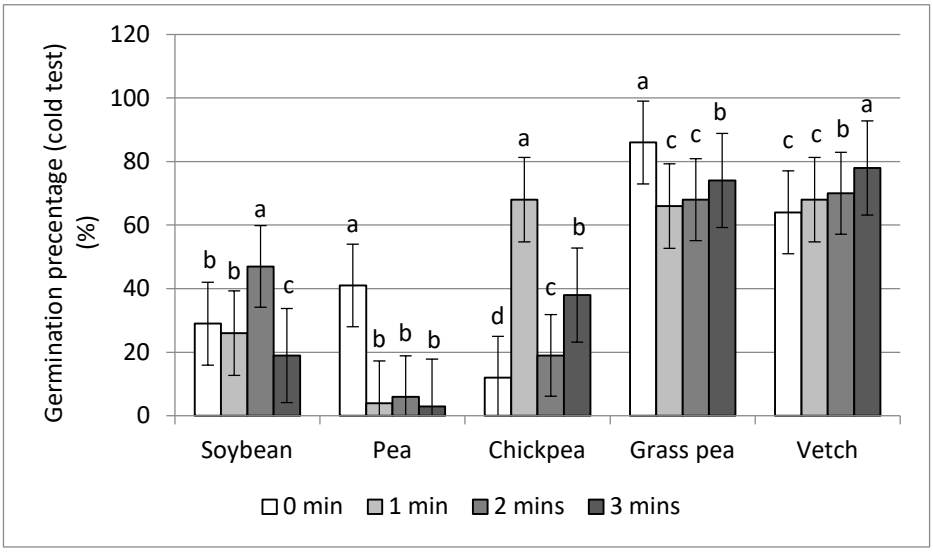



Figure 3. The influence of the electric voltage on the germination of leguminous seeds using the cold test.

The electrostatic field is a special case arising from d-c potential differences between conducting electrodes, and the direction and magnitude of the electric field do not change during the exposure. Seed vigor was apparently changed by the electrostatic field force. The germination percentage of seeds was accelerated by an electrostatic field because each seed has some electrical nature with electric potential differences existing in all tissue cells (Morar et al., 1999). As in optimal temperature conditions, the application of an electrostatic field at a lower

temperature had a negative impact on seed germination. In peas and grass pea, germination in grass pea seeds was reduced from 13.95% to 22.26%. However, a significantly higher reduction was found in pea seeds, from 85.37% to 92.68%. Observing the influence of the electrostatic field on seed germination under lower temperature conditions, a significant difference was noticed in most plant species in relation to the optimal conditions for germination. Bean is the only plant species whose seeds reacted positively to the use of the electrostatic field, regardless of temperature conditions during germination, while the 2- and 3-minute treatments did not give significant effects. However, at a lower temperature, there was no effect in the 1-minute treatment while the 2- and 3-minute treatments increased germination. Analyzing each test, i.e., temperature conditions during germination, it can be noticed that at a lower temperature, the influence of the electrostatic field on the germination of vetch seeds was greater. In chickpea, the application of electrostatic field had a negative effect in optimal temperature conditions, while at lower temperatures, an increase in germination was achieved regardless of the duration of treatment. The electrostatic field had no effect on the germination of soybean, pea and grass pea seeds in optimal temperature conditions. However, at low temperatures, there was a significant reduction in the germination of peas and grass pea, while in soybean seeds, the effect depended on the time of seed treatment.

## Conclusion

The influence of the electrostatic field on the seed germination energy depended on the plant species and exposure time. Also, the influence of the electrostatic field depended on the applied tests (germination and cold tests).

In soybeans and vetch, the application of electrostatic field led to an increase in germination energy, then in chickpeas, to a decrease, while in pea and bean seeds, there was no effect. The largest increase of 18.18% was found in vetch, while the largest decrease of 12% was found in chickpeas.

After using the germination test, the increase in germination ranged up to 7.06% in vetch, and a decrease of up to 9.76% was observed in chickpea. There was no effect in soybean, peas and grass pea.

After using a cold test, the effect was significantly greater. The increase ranged up to 82.35% in chickpeas, and the decrease amounted to 92.68% in peas. The obtained results indicate that it is not possible to talk about the universal application of a certain duration of seed treatment because it can happen that it does not correspond to a certain plant species, so the quality of seeds can deteriorate.

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Received: March 13, 2021
Accepted: November 1, 2021

# UTICAJ ELEKTRIČNOG NAPONA NA KLIJAVOST SEMENA LEGUMINOZA

**Zlatica J. Mamlić[1*], Zorica T. Nikolić[1], Goran S. Mamlić[2], Gordana D. Tamindžić[1], Sanja Lj. Vasiljević[1], Snežana M. Katanski[1] i Ana L. Uhlarik[1]**

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R e z i m e

Cilj rada je bio ispitivanje uticaja elektrostatičkog polja na seme soje (*Glycine max* L.), grahorice (*Vicia spp*. L.), graška (*Pisum sativum* L.), sastrice (*Lathyrus sativus* L.) i nauta (*Cicer arietinum* L.) koji su selekcionisani na Institutu za ratarstvo i povrtarstvo u Novom Sadu. Za tretiranje je korišćen jednosmerni napon jačine 9 V. Vreme tretiranja semena iznosilo je 0 (kontrola), 1, 2 i 3 minuta. Nakon tretiranja, ispitan je kvalitet semena primenom standardnog testa klijavosti (optimalna temperatura) i hladnog testa (niska temperatura). Rezultati istraživanja su pokazali da je efekat elektrostatičkog polja na kvalitet semena zavisio od biljne vrste, vremena tretiranja semena i temperaturnih uslova u kojima je seme klijalo nakon tretiranja. Povećanje energije klijanja se kretalo do 18,18% kod grahorice, a smanjenje do 12% kod nauta. Značajno veći uticaj primena elektrostatičkog polja imala je na semenu koje je posle tretiranja izloženo niskoj temperaturi u procesu klijanja. Povećanje klijavosti semena se kretalo do 82,35% kod nauta, a smanjenje do 92,68% kod graška. Takođe, dobijeni rezultati ukazuju na to da se ne može govoriti o univerzalnoj primeni određenog vremena tretiranja, jer se može dogoditi da ono ne odgovara određenoj biljnoj vrsti, pa može doći do pogoršanja kvaliteta semena.

**Ključne reči:** elektrostatičko polje, klijavost, hladni test, leguminoze.

Primljeno: 13. marta 2021.
Odobreno: 1. novembra 2021.

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Journal of Agricultural Sciences
Vol. 66, No. 4, 2021
Pages 321-334

https://doi.org/10.2298/JAS2104321B
UDC: 633.34:631.547.1
Original scientific paper

# EFEKAT TRETMANA SEMENA ELEKTROMAGNETNIM POLJEM NISKIH FREKVENCIJA NA PRODUKTIVNOST SOJE

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**Sažetak:** Razvoj nauke i tehnologije ide u pravcu introdukcije novih pristupa u oplemenjivanju biljaka i različitih metoda za povećanja produktivnosti biljaka. Jedna od najnovijih metoda je implementacija ekološki prihvatljive tehnike korišćenja pulsirajućeg elektromagnetnog polja niskih frekvencija (PEMP). U radu su prikazani rezultati uticaja elektromagnetne stimulacije semena soje na masu zrna po biljci, masu 1.000 zrna i prinos zrna u različitim agroekološkim uslovima. U trogodišnjem istraživanju, u periodu od 2013. do 2015. godine, korišćena je sorta soje Valjevka, gajena pri različitim količinama đubriva (kontrola – bez đubrenja, 750 kg/ha i 1300 kg/ha). Pre setve, seme je bilo podvrgnuto stimulaciji sa PEMP u varijantama: kontrola – bez stimulacije i stimulacija naizmeničnim magnetnim poljem sa indukcijom od 30 mT i vreme ekspozicije od 15 minuta. Prosečna masa zrna po biljci pri stimulaciji semena bila je za 11,53% (12,09) viša nego bez PEMP (10,84). Masa 1.000 zrna sa PEMP je iznosila 155,99 g, što je bilo više za 2,06% u odnosu na varijantu bez PEMP (152,83 g). Prosečan prinos zrna soje za sve tri godine istraživanja sa stimulacijom semena je bio za 4,85% veći (3481,25 kg/ha) nego bez PEMP (3320,14 kg/ha). Stimulacija semena sa PEMP ima ekonomsku opravdanost, obzirom na rast cena soje na svetskoj berzi. Rezultati pokazuju da PEMP tretman semena soje može značajno uticati na prinos zrna soje i da se suprotstavi neželjenim efektima poput suše i nedostatka đubriva.

**Ključne reči:** soja, pulsirajuće elektromagnetno polje, masa 1.000 zrna, prinos.

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## Uvod

Biljka soje (*Glycine max* (L.) Merr.) postaje sve interesantnija na svetskom tržištu. Razlog tome se ogleda u njenom privrednom i ekonomskom značaju. Visoka hranljiva vrednost zrna, sa 40% proteina i 20% ulja, predstavlja značajan izvor biljnih ulja, stočne hrane i hrane za ljudsku ishranu (Young, 1991). Dorff (2007) opisuje upotrebu soje, gde se posebne sorte soje mogu koristiti u svežem stanju u ishrani životinja, a suvo zrelo seme za ishranu životinja i ljudi. Posebno se izdvaja upotreba zrna soje u različitim granama industrije. Soja predstavlja tipičan primer biljke koja je među prvima korišćena u genetskom inženjeringu (Nikolić et al., 2019), potom u oplemenjivanju biljaka (Lewandowska i Kozak, 2017), kao i u mnogim istraživanjima koja se vrše radi povećanja prinosa i hemijskog sastava zrna. Posebna pažnja je usmerena na različite tehnologije gajenja soje obzirom na njen pozitivan uticaj na fizička, hemijska i biološka svojstva zemljišta (Đukić et al., 2017). Uspostavljanje simbioze između korena soje i *Bradyrhizobium japonicum*, dovodi do formiranja kvržica i vezivanja azota iz vazduha, čime se prevodi u oblik koji je pogodan biljkama. Soja, takođe, ima veliku ulogu u plodoredu. Činjenica da svetska populacija konstantno raste uz sve brži razvoj nauke i tehnologije, te da je samim tim potrebno sve više hrane, sojin značaj raste kako u prerađivačkoj industriji, tako i u međunarodnoj trgovini i transportu.

Geomagnetno polje (GMF) je prirodna komponenta životne sredine, koja tokom procesa evolucije stabilno deluje na žive sisteme, i na mnoge biološke procese (Islam et al., 2020; Shabrangy et al., 2021). Električna i magnetna polja su prisutna na površini Zemlje u svakom trenutku. Prema Maffei (2014), prirodno magnetno polje Zemlje varira od 30 do 70 mT. Prva istraživanja stimulacije različitih nivoa magnetnih, električnih, elektromagnetnih, laserskih i ostalih talasa/polja obavljena su u medicini, za lečenje životinja i ljudi.

Poslednjih godina, razvijene su mnoge hipoteze koje pokušavaju da objasne uticaj električnog, magnetnog i elektromagnentog polja na biljke (Lewandowska et al., 2019), iz razloga što su biljke odlični modeli za izvođenje eksperimenata. Pietruszewski et al. (2009) navode da su za ispitivanje uticaja elektromagnetnih polja (EPM) na biološke sisteme specijalizovane dve discipline: magnetobiologija i elektrobiologija. Isti autori smatraju da postoje tri glavna pravca eksperimenata u vezi sa ispitivanjem uticaja EMP na biljke: istraživanje uticaja izuzetno jakih polja (Sarraf et al., 2021), ispitivanje rasta biljaka u odsustvu magnetnog polja Zemlje (Negishi et al., 1999), i treća i najviše zastupljena istraživanja se odnose na uticaj EMP niskih frekvencija (Sujak et al., 2013; Lewandowska et al., 2019; Sarraf et al., 2021). Raznolikost efekata električnih i magnetnih polja na biološke organizme izvode se istraživanjima obavljenim i na nivou ćelije i na organizmima (Kataria et al., 2017; Nair et. al., 2018). Postoji veliki broj istraživanja koji ukazuju na specifične promene i efikasno delovanje EMP na različite biohemijske procese

ćelija kod biljaka. Uvođenjem energije u ćelije stvaraju se molekularne transformacije, koje mogu dovesti do povećanja elektropotencijala membrana (Vasilevski, 2003), do aktiviranja jona i polarizacije dipola u živim ćelijama (Moon i Chung, 2000), kao i do procesa koji uključuju slobodne radikale tako što stimulišu aktivnost proteina i enzima (Jarayam et al., 1991). Abdel Latef et al. (2020) navode i pozitivne promene u procesu fotosinteze i sadržaj pigmenata, dok Radhakrishnan (2019) napominje prisustvo pojačane deobe ćelija, a samim tim i brži unos vode i hranljivih materija koje su potrebne biljkama.

Drugi istraživači ukazuju na promene u funkcionisanju celih proteinskih struktura, pa čak i tkiva (Broszkiewicz et al., 2018). U svojim istraživanjima koja su sproveli, Radhakrishnan i Kumari Ranjitha (2012) navode uticaj stimulacije semena soje na povećanu aktivnost enzima kao što su b-amilaze, kisele fosfataze, polifenol oksidaze i katalaze. S druge strane, postoje pozitivni efekti tretiranja vode sa EMP, koji su doveli do promene fizičko-hemijskih svojstava molekula vode, a zalivanjem biljaka, došlo je do određenih biohemijskih reakcija (Moussa, 2011; Grewal i Maheshwari, 2011).

Najveći broj istraživanja se odnosi na izlaganje električnim, magnetnim i elektromagnetnim poljima koji su dali pozitivne ili negativne efekte na intenzitet klijanja, prinos, visinu biljke, sadržaj proteina, produktivnost, veličinu lista, masu ploda, broj plodova itd. Generalno, dobijeni rezultati ne zavise samo od biljnih vrsta (stopa rasta, period skladištenja), nego i od klimatskih uslova (temperature, vlažnost vazduha i zemljišta), kao i od vremena izloženosti, intenziteta i prirode polja. Doza izlaganja zavisi od dva parametra: gustine magnetnog polja i vremena izlaganja. Međutim, potrebna su dodatna sveobuhvatna istraživanja kako bi se razumeo i objasnio mehanizam uticaja EMP i biljaka, kao i različite interakcije koje nastaju.

Sve veći apel za očuvanje životne sredine i proizvodnju zdravstveno bezbedne hrane nameće ozbiljan zadatak poljoprivrdnim stručnjacima za iznalaženje novih tehnologija proizvodnje. U tom pravcu, Cakmak et al. (2012), Shine et al. (2012) i mnogi drugi autori govore o blagotvornim efektima pulsirajućeg elektromagnetnog polja (PEMP) na klijavost semena i parametre rasta ispitivanih biljnih vrsta, te se upotreba pulsirajućeg magnetnog polja uvodi kao jedna od ekološki prihvatljivih tehnika koje ispunjavaju zahteve organske poljoprivrede. S druge strane, globalne klimatske promene koje su sve učestalije, nepredvidive i dovode do ekstremnih uslova predstavljaju limitirajući faktor za ostvarivanje visokih prinosa i stabilne proizvodnje. Mnogi istraživači su ukazali na pozitivne promene na biljkama prilikom nepovoljnih spoljnih uslova, poput suše (Anand et al., 2012; Karimi et al., 2012; Sen i Alikamanoglu, 2014), saliniteta (Radhakrishnan i Ranjitha-Kumari, 2013), poplava, preventivnih i kurativnih mera zaštite bilja od korova (Stanković et al., 2016), bolesti i štetočina (Galland i Pazur 2005; Abdollahi et al., 2012).

Radhakrishnan (2019) objašnjava uticaj magnentog polja na biljke i odličan potencijal u promeni konvencionalnih sistema biljne proizvodnje, radi eliminacije hemijskih inputa i toksičnih rezidua, što je u pozitivnoj korelaciji sa novim svetskim trendovima u cilju zaštite životne sredine u proizvodnji bezbedne hrane. Prednost ima korišćenje elektromagnetnih tretmana kao biostimulatora u odnosu na tradicionalne hemijske metode, jer ne ostavlja nikakav toksični efekat. Takođe, Cvijanović i Đukić (2020) navode prednosti primene ovih tretmana jer su ekološki i ekonomski prihvatljive. Osim toga, da istraživanja sa elektromagnetnim tretmanima pred setvu budu još preciznija i uspešnija, poželjno bi bilo prikupljanje podataka o prisutnim statičkim magnetnim poljima, kojima je zemlja konstantno izložena, a ti podaci često nedostaju u literaturi (Maffei, 2014), kao i podataka o istraživanjima koja se vrše na otvorenom polju pod različitim klimatskim uticajima (Đukić et al., 2017).

Različite frekvencije i vreme trajanja ekspozicije su korišćeni za različite eksperimente. Većina naučnih istraživanja zaključuje da MP, EMP i pulsirajuće EMP (PEMP) pozitivno utiču na bolju klijavost semena i rast biljaka, što rezultira povećanjem prinosa (Bilalis et al., 2013; da Silva i Dobránszki, 2016; Lewandowska et al., 2019; Sukhov et al., 2021). Međutim, najveći problem ovih istraživanja je što se obično svode na ekonomsku računicu.

Stoga je cilj ovog istraživanja ispitivanje stimulacije semena soje pre setve sa pulsirajućim elektromagnetnim poljem niske frekvencije na masu zrna po biljci, masu 1.000 zrna i prinos zrna u različitim agroekološkim uslovima.

## Materijal i metode

Poljski ogled je izveden po tzv. „split plot" sistemu, odnosno sistemu podeljenih parcela u 4 ponavljanja sa randomiziranim dizajnom na zemljištu tipa karbonatni černozem, površine (53 m x 23 m = 1.219 $m^2$) u Institutu za ratarstvo i povrtarstvo u Novom Sadu, region Vojvodina – Srbija. Istraživanja su obavljena u trogodišnjem periodu (2013–2015). Svake godine je korišćena selekcija NS sorta soje Valjevka, 0 grupe zrenja, dužine vegetacionog perioda do 120 dana, genetskog potencijala za prinos iznad 4500 kg/ha. Ukupna površina parcele za izvođenje ogleda iznosila je 1.219 $m^2$ (53 m x 23 m). Dužina redova u ponavljanjima osnovnih parcela je 11 m, u okviru kojih je dužina redova potparcela 5 m. Za svaku potparcelu pripremljeno je 500 zrna koja su stimulisana puslirajućim elektromagnetnim poljem.

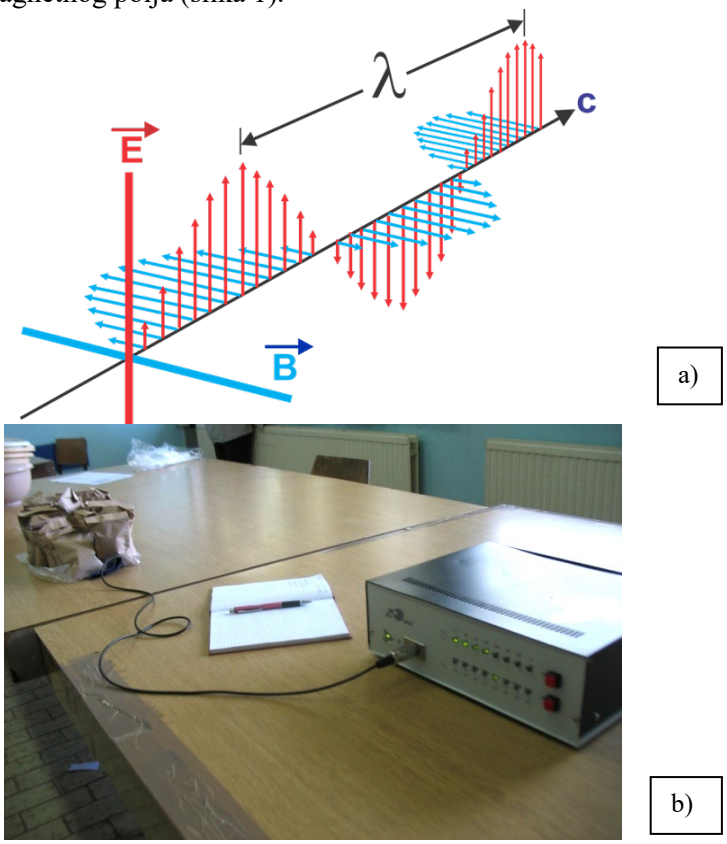
Ovim istraživanjima su obuhvaćna tri faktora (tretmana) i to:

A) godina, jer klimatski uslovi utiču na vegetativan i generativan porast soje;

B) količina organskog granulisanog živinskog đubriva (formulacije 4:4:4): Ø (kontrola), 750 kg/ha и 1300 kg/ha;

C) stimulacija semena PEMP – frekvencije 30 mT i ekspozicije 30 minuta, koja je urađena neposredno pred setvu.

Stimulacija semena soje je izvedena pomoću generatora impulsa i trakastog aplikatora (maksimalne frekvencije do 100 Hz i maksimalnog vremena trajanja do 60 minuta), preko koga se odvija proces pulsirajućeg naizmeničnog kretanja električnog i magnetnog polja (slika 1).




Slika 1. Elektromagnetni talas i priprema faktora C (a) Šema elektromagnetnog ravnog talasa koji prikazuje poprečno i u prostoru promenljivo električno ($E$) i magnetno polje ($B$). Talasna dužina ($\lambda$) je rastojanje između dva grebena. Pravac talasa je označen sa slovom c; b) Aparat specifičnog spektralnog sadražaja u toku stimulacije semena neposredno pred setvu.

Prilikom aktiviranja električnog polja dolazi do ubrzavanja kretanja elektrona uz pomoć koga se formiraju oscilirajući električni i magnetni poremećaji što implicira stvaranje elektromagentog polja, odnosno talasa koji emituje određeni oblik energije. Tako stvoreni talasi imaju određene karakteristike, poput talasne

dužine i frekvencije. Talasna dužina predstavlja rastojanje između dva uzastopna vrha talasa. Frekvencija je broj ciklusa u određenom vremenskom periodu. Ona se meri kao broj talasnih ciklusa u sekundi i označava se jedinicom zvanom Herc (Hz).

Na kraju vegetacionog perioda u fazi tehnološke zrelosti biljaka obeleženi su i uzeti uzorci od po 30 biljaka, metodom slučajnog uzorka sa sva 4 ponavljanja i svih varijanti za analizu sledećih svojstava: masa zrna po biljci i masa 1.000 zrna, dok je nakon žetve izmeren prinos u kg/ha sveden na vlagu od 14%.

Rezultati istraživanja obrađeni su statistički analizom varijanse trofaktorijalnog ogleda, a statistička značajnost razlika testirana LSD testom na 5% i 1% (Statistički program „Statistica 10.0"). Rezultati su prikazani tabelarno.

Sve prisutnija promena klime na ovim prostorima utiče na neravnomerno raspoređene padavine u toku godine, odnosno vegetacionog perioda, kao i pojavu suše usled povišenih temperatura, te je proizvodnja soje vrlo nestabilna. Cvijanović et al. (2019) ukazuju da ranija setva i stimulacija semena koja utiče na brže klijanje i porast biljaka predstavljaju bitne mere adaptacije na nastale klimatske promene. Podaci o temperaturi i padavinama prikazani u tabeli 1, prikupljeni su na meteorološkoj stanici Rimski Šančevi, nadomak Novog Sada. Suma padavina u vegetacionom periodu u sve tri ispitivane godine (2013 – 448,2 mm, 2014 – 595,6 mm, 2015 – 389,0 mm) bile su više od višegodišnje prosečne sume padavina za period od 1964. do 2015. (375,1 mm). Takođe, pored sume padavina veoma je bitan i raspored padavina, jer najveće količine vode su potrebne u fazama cvetanja, formiranja mahuna i nalivanja zrna soje (jun–avgust). Prosečna temperatura vazduha višegodišnjeg perioda iznosila je 18,0°C, što je niže u odnosu na sve tri ispitivane godine (2013 – 18,7°C; 2014 – 18,3°C; 2015 – 19,8°C). Ovi podaci ukazuju na brzu i nepredvidivu promenu klimatskih uslova na našim područjima.

Tabela 1. Prosečne sume padavina i temperature vazduha za vegetacioni period soje u trogodišnjem periodu (2013–2015) i višegodišnjem proseku (Rimski Šančevi).

| | Suma padavina (mm) | | | | Prosečne temperature (°C ) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 1964–2015 | 2013 | 2014 | 2015 | 1964–2015 |
| April | 35,8 | 51,2 | 15,0 | 46,9 | 13,4 | 13,2 | 11,8 | 11,7 |
| Maj | 118,1 | 202,1 | 192,0 | 67,1 | 17,4 | 16,3 | 17,8 | 17,0 |
| Jun | 125,7 | 38,2 | 28,0 | 86,6 | 20,2 | 20,5 | 20,5 | 20,0 |
| Jul | 34,1 | 141,1 | 2,0 | 67,4 | 22,3 | 21,9 | 24,5 | 21,7 |
| Avgust | 26,7 | 78,7 | 99,0 | 59,3 | 22,9 | 20,9 | 24,4 | 21,2 |
| Septembar | 107,8 | 84,3 | 53,0 | 47,8 | 15,7 | 17,2 | 19,9 | 16,9 |
| Prosek | 448,2 | 595,6 | 389,0 | 375,1 | 18,6 | 18,3 | 19,8 | 18,0 |

## Rezultati i diskusija

Produktivne osobine biljaka poput mase zrna po biljci i masa 1.000 zrna mogu imati značajan uticaj na visinu prinosa, a da pri tome i same zavise od različitih uticaja, kao što su uslovi spoljne sredine, đubrenje i drugi parametri proizvodnje, kao i odabir tehnologije po kojoj se proizvodnja izvodi. Za ostvarivanje visokog i stabilnog prinosa, soji je potrebno oko 300–350 mm vodenog taloga, od toga oko 80% je potrebno u reproduktivnim fazama, od kraja juna do septembra. Nedostatak padavina, visoke temperature i pojava sušnih perioda u navedenim fazama dovode do smanjenja prinosa, a uzrok tome je redukcija broja zrna po biljci i/ili mase hiljadu zrna. Kobraee i Shamsi (2012) navode da se smanjenje prinosa u zavisnosti od spoljnih uslova kreće od 25% do 55%.

Prosečna vrednost mase zrna po biljci (11,47 g) statistički se vrlo značajno razlikovala ($p<0,01$) u toku tri godine istraživanja po svim nivoima ispitivanih faktora (tabela 2), izuzev đubrenja i interakcije godina-đubrenja koji su bili na nivou od 5%.

Tabela 2. Srednje vrednosti mase zrna po biljci (g) za ispitivane faktore.

| Godina (A) | Đubrenje (kg/ha) (B) | Stimulacija semena (C) | | AB | A | |
|---|---|---|---|---|---|---|
| | | Bez PEMP | Sa PEMP | | | |
| 2013 | 0 | 9,21 | 15,03 | 12,12 | 11,72 | |
| | 750 | 9,37 | 13,77 | 11,57 | | |
| | 1300 | 10,84 | 12,09 | 11,47 | | |
| | AC | 9,81 | 13,63 | | | |
| 2014 | 0 | 9,90 | 15,10 | 12,50 | 12,14 | |
| | 750 | 10,58 | 13,43 | 12,01 | | |
| | 1300 | 11,04 | 12,82 | 11,93 | | |
| | AC | 10,50 | 13,78 | | | |
| 2015 | 0 | 3,87 | 7,50 | 5,68 | 5,86 | |
| | 750 | 4,60 | 7,10 | 5,85 | | |
| | 1300 | 5,80 | 6,28 | 6,04 | | |
| | AC | 4,75 | 6,96 | | | |
| BC | 0 | 8,35 | 11,46 | 9,91 | | |
| | 750 | 9,21 | 15,03 | 12,12 | | |
| | 1300 | 9,37 | 13,77 | 11,57 | | |
| | C | 10,84 | 12,09 | | | |
| Prosek 2013–2015 | | | | 11,47 | | |
| | A** | B* | C** | AB* | AC** | BC** | ABC** |
| LSD 0,05 | 0,49 | 0,24 | 0,17 | 0,41 | 0,30 | 0,30 | 0,51 |
| LSD 0,01 | 0,58 | 0,32 | 0,23 | 0,56 | 0,40 | 0,40 | 0,60 |

Najveća masa zrna po biljci je utvrđena u 2014. godini – 12,14 g, što je bilo za 3,58% više nego u 2013. godini (11,72 g), dok je u 2015. godini bila dvostruko

manja i iznosila je samo 5,86 g. Razlog tome je nedostatak vlage u periodu koji je veoma bitan za formiranje prinosa zrna po biljci. S druge strane, visoke temperature i dugi sušni periodi su uticali na smanjenje mase zrna, što je u saglasnosti sa istraživanjima Kobraee i Shamsi (2012). Različite varijante đubrenja uticale su na varijabilnost mase zrna po biljci na nivou statističke značajnosti $p<0,05$, gde je najveća masa zrna po biljci bila pri đubrenju sa 750 kg $ha^{-1}$ (12,12 g), što je za 4,75% (11,57 g) više u odnosu na 2015. godinu i za 22,30 % u odnosu na kontrolu (9,91 g). Stimulacija semena sa PEMP bila je veoma izražena na nivou statističke značajnosti $p<0,01$ (12,09 g) u odnosu na varijantu bez stimulacije semena (10,84 g). Pri stimulaciji semena, masa zrna po biljci je bila viša za 11,53% u odnosu na kontrolu. Đukić et al. (2017) ističu da frekvencija – 16 Hz i trajanje tretmana semena 30 minuta daju najbolji efekat na različite morfološke karakteristike soje. Radhakrishnan i Ranjitha-Kumari (2012) konstatuju da stimulacija semena sa PEMP igra važnu ulogu u poboljšanju stanja useva i produktivnosti soje kroz bržu akumulaciju minerala i enzimske aktivnosti, brže usvajanje vode, klijanje i nicanje, što na kraju dovodi do povećanja prinosa.

Đukić et al. (2011) navode da je masa 1.000 zrna visoko nasledna osobina, te zavisi od sorte soje odnosno genetskiog faktora, agrometeoroloških uslova u proizvodnoj godini, tehnologije proizvodnje i plodnosti zemljište (tabela 3).

Tabela 3. Srednje vrednosti mase 1.000 zrna (g) za ispitivane tretmane.

| Godina (A) | Đubrenje (kg/ha) (B) | Stimulacija semena (C) | | AB | A |
|---|---|---|---|---|---|
| | | Bez PEMP | Sa PEMP | | |
| 2013 | 0 | 128,19 | 131,00 | 129,59 | 129,33 |
| | 750 | 128,69 | 130,13 | 129,41 | |
| | 1300 | 128,94 | 129,06 | 129,00 | |
| | AC | 128,60 | 130,06 | | |
| 2014 | 0 | 187,75 | 191,65 | 189,70 | 189,99 |
| | 750 | 188,60 | 192,30 | 190,45 | |
| | 1300 | 187,90 | 192,85 | 190,38 | |
| | AC | 188,08 | 191,90 | | |
| 2015 | 0 | 143,05 | 146,20 | 144,63 | 143,72 |
| | 750 | 139,25 | 145,65 | 142,45 | |
| | 1300 | 143,05 | 145,10 | 144,08 | |
| | AC | 141,78 | 154,65 | | |
| BC | 0 | 153,00 | 156,20 | 154,64 | |
| | 750 | 152,18 | 156,03 | 154,10 | |
| | 1300 | 153,30 | 155,67 | 154,48 | |
| | C | 152,83 | 155,87 | | |
| Prosek 2013–2015 | | | | 154,41 | |
| | A** | $B^{ns}$ | C** | $AB^{ns}$ | $AC^{ns}$ | $BC^{ns}$ | $ABC^{ns}$ |
| LSD 0,05 | 1,23 | 3,38 | 1,64 | 5,86 | 2,84 | 2,84 | 4,91 |
| LSD 0,01 | 1,45 | 4,64 | 2,21 | 8,03 | 3,83 | 3,83 | 6,64 |

Dobijeni rezultati pokazuju da je masa 1.000 zrna pokazala visoko signifikantne razlike između godina i pri stimulaciji semena, dok su ostali ispitivani parametri bili bez statističke značajnosti. Najveća masa 1.000 zrna bila je u najpovoljnijoj proizvodnoj 2014. godini (189,99 g), za razliku od druge dve godine. Istraživanje Toleikiene et al. (2021) ukazuje da su nepovoljni uslovi tokom faze reproduktivnog rasta značajno uticali na smanjenje mase 1.000 zrna. Stimulacija semena sa PEMP je pozitivno uticala na povećanje mase 1.000 zrna (155,87 g) u odnosu na varijantu bez stimulacije (152,83) na nivou značajnosti od $p<0,01$. Milošev i Šeremešić (2005) su utvrdili da je intezitet PEMP od 30Hz i 30 minuta značajno povećao masu 1.000 zrna ozime pšenice. Brojna istraživanja pokazuju da tretman semena stimuliše rastenje i prinos najvažnijih ratarskih biljaka, s tim da zavisi od jačine frekvencije i vremena izloženosti (Sujak et al., 2013; Maffei, 2014; Sarraf et al., 2021) u opsegu od 30 do 250 mT na fiziološke efekte, uključujući klijanje, rast, razvoj, fotosintezu i redoks status kod širokog spektra biljaka poput soje (Đukić et al., 2017), kukuruza (Florez et al., 2007), pšenice (Payez et al., 2013; Araujo Sde et al., 2016).

Na osnovu rezultata istraživanja utvrđeno je da su godina i stimulacija semena sa PEMP kao ispitivani faktori statistički vrlo značajno uticali na prinos zrna soje (tabela 4).

Tabela 4. Srednje vrednosti prinosa zrna soje (kg/ha) za ispitivane faktore.

| Godina (A) | Đubrenje (kg/ha) (B) | Stimulacija semena (C) | | AB | A |
|---|---|---|---|---|---|
| | | Bez PEMP | Sa PEMP | | |
| 2013 | 0 | 2.878,63 | 3.063,33 | 2.970,98 | 2.989,68 |
| | 750 | 2.925,15 | 3.059,99 | 2.992,57 | |
| | 1300 | 2.978,01 | 3.032,99 | 3.005,50 | |
| | AC | 2.927,26 | 3.052,10 | | |
| 2014 | 0 | 4.984,51 | 5.302,28 | 5.143,39 | 5.145,80 |
| | 750 | 5.065,34 | 5.222,99 | 5.144,16 | |
| | 1300 | 5.087,80 | 5.211,92 | 5.149,86 | |
| | AC | 5.045,88 | 5.245,73 | | |
| 2015 | 0 | 1.961,99 | 2.174,59 | 2.068,29 | 2.066,60 |
| | 750 | 1.961,15 | 2.144,48 | 2.052,82 | |
| | 1300 | 2.038,72 | 2.118,66 | 2.078,69 | |
| | AC | 1.987,29 | 2.145,92 | | |
| BC | 0 | 3.275,04 | 3.513,40 | 3.394,22 | |
| | 750 | 3.317,21 | 3.475,82 | 3.396,52 | |
| | 1300 | 3.368,18 | 3.454,53 | 3.411,35 | |
| | C | 3.320,14 | 3.481,25 | | |
| Prosek 2013–2015 | | | | 3400,70 | |
| | A** | B$^{ns}$ | C** | AB$^{ns}$ | AC$^{ns}$ | BC** | ABC$^{ns}$ |
| LSD 0,05 | 51,75 | 34,81 | 25,99 | 60,30 | 45,01 | 45,01 | 77,97 |
| LSD 0,01 | 60,88 | 47,70 | 35,09 | 82,62 | 60,79 | 60,79 | 105,28 |

Prosečan prinos zrna soje iznosio je 3400,70 kg/ha. Najviši prinos utvrđen je u 2014. godini (5.145,80 kg/ha). U aridnoj 2015. godini utvrđen je najmanji prinos, svega 2.066,60 kg/ha, što je manje za 48,99% u odnosu na 2014. godinu i za 44,66% u odnosu na 2013. godinu (2.989,68 kg/ha). Brojne studije pokazuju jak uticaj faktora spoljne sredine na masu 1.000 zrna i prinos zrna soje (Đukić et al., 2011). Isti autori navode da visoke temperature u početnim fazama rasta i nedostatak padavina dovode do isušivanja površinskog sloja zemljišta, što negativno utiče na nicanje i porast biljke. Đubrenjem sa 1.300 kg/ha ostvaren je najveći prinos zrna soje (3.411,35 kg/ha), ali bez statističke značajnosti. Stimulacija semena je statistički značajno uticala na povećanje prinosa i to za 4,85% u odnosu na kontrolnu varijantu.

Vršena su brojna istraživanja u kojima se ističe pozitivan efekat upotrebe elektromagnetnog polja, različitih frekvencija i vremena trajanja, na organizme i na ćelijskom nivou (Payez et al., 2013; Nair et al., 2018; Radhakrishnan, 2019; Shabrangy et al., 2021; Sarraf et al., 2021). Na osnovu rezultata istraživanja, Đukić et al. (2017) ukazuju na povećanje prinosa za 7,25% stimulacijom semena sa PEMP sa frekvencijom 72 Hz i ekspozicije 60 minuta u odnosu na kontrolu, kao i povećanje prinosa za 8% sa frekvencijom 16 Hz u trajanju 30 minuta. Parsi (2007) izveštava o povećanju dužine korena soje za 49% u odnosu na kontrolu pri frekvenciji 0,88 T u trajanju od 40 minuta.

## Zaključak

Na osnovu dobijenih rezultata izvode se sledeći zaključci: smanjenje mase zrna, mase 1.000 zrna kao i prinosa zrna soje bilo je izraženije u godinama sa nepovoljnim klimatskim uslovima i karakterističnim periodom suše. Masa zrna po biljci i masa 1.000 zrna su pokazatelji agroklimatskih i proizvodnih uslova u određenim godinama. Đubrenjem sa 750 kg/ha dobijene su najveće vrednosti ispitivanih parametara. Uvođenje novih tehnologija poput biostimulacije semena sa PEMP može uticati na stvaranje visokih i stabilnih prinosa, dodatno pri nepovoljnim agrometeorološkim uslovima, kao što je suša. Takođe, primena ovih tretmana se smatra ekološkom, jeftinom i neinvazivnom tehnikom.

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Primljeno: 19. avgusta 2021.
Odobreno: 24. novembra 2021.

# EFFECT OF SEED TREATMENT BY LOW-FREQUENCY ELECTROMAGNETIC FIELD ON SOYBEAN PRODUCTIVITY

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A b s t r a c t

The development of science and technology introduces new approaches in plant breeding and various methods to increase plant productivity. One of the latest methods is the implementation of an environmentally friendly technique of using a pulsed low-frequency electromagnetic field (PEMP). The paper presents the results of the influence of the electromagnetic stimulation of soybean seeds on grain weight per plant, weight of 1,000 grains and grain yield in different agroecological conditions. In the three-year research, in the period from 2013 to 2015, the soybean variety Valjevka was used, grown with different amounts of fertilizers (control – without fertilization, 750 kg/ha and 1300 kg/ha). Before sowing, the seed was subjected to PEMP stimulation in variants: control – without stimulation and alternating magnetic field stimulation (PEMP) with induction of 30 mT and exposure time of 15 minutes. The average grain weight per plant during seed stimulation was 11.53% (12.09) higher than without PEMP (10.84). The weight of 1,000 grains with PEMP was 155.99 g, which was 2.06% higher than the weight of 1,000 grains of the variant without PEMP (152.83 g). The average soybean grain yield for all three years of research with seed stimulation was 4.85% higher (3,481.25 kg/ha) than without PEMP (3,320.14 kg/ha). Stimulation of seeds with PEMP has economic justification given the growth of soybean prices on the world stock market. The results show that the PEMP treatment of soybean seeds can significantly affect soybean grain yield and counteract side effects such as drought and lack of fertilizers.

**Key words:** soybeans, pulsed electromagnetic field, weight of 1,000 grains, yield.

Received: August 19, 2021
Accepted: November 24, 2021

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Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2104335E
Vol. 66, No. 4, 2021 UDC: 632.954(669)
Pages 335-350 Original scientific paper

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# ENHANCING THE WEED CONTROL EFFICACY OF TWO PRE-EMERGENCE HERBICIDES IN MAIZE/JACK BEAN AND MAIZE/GROUNDNUT INTERCROPS IN DERIVED SAVANNA

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**Abstract:** The inability of pre-emergence herbicides alone to give season-long weed control in maize production justifies the need for supplementary weed control. Field trials were conducted to evaluate intercropped groundnut and jack bean for enhanced weed control efficacy of two pre-emergence herbicides in maize production in 2013/2014 and 2015 cropping seasons at the Federal University of Agriculture, Abeokuta, Nigeria. The experiment was arranged in split-plot fitted in a randomized complete block design with three replications. Main plots consisted of eight weed control treatments of a commercially formulated mixture of prometryne + metolachlor (Probaben$^R$); prometryne + acetochlor (Super Union$^R$) each at 2.4 kg a.i ha$^{-1}$, 1.6 kg a.i ha$^{-1}$ with and without supplementary hoe-weeding, two hoe-weedings and a weedy check. Sub-plot treatments consisted of eight cropping patterns (sole maize, maize-jack bean and maize-groundnut intercrops, each at two spacings [100 x 37.5 cm and 75 x 50 cm] and sole crops of jack bean and groundnut). All the weed control methods significantly reduced ($p<0.05$) weed dry matter compared to the weedy check by 10–81%. Maize grain yields (1.4–5.2 t ha$^{-1}$) were similar for all plots with the weed control methods, except in the 2015 late season, but significantly depressed (21–48%) on those weed-infested throughout. Intercropped jack bean and groundnut with maize significantly suppressed weed growth relative to sole crops of maize, jack bean or groundnut by 20–67%. Therefore, the efficacy of pre-emergence herbicides in this study was enhanced by intercropping of maize with jack bean and groundnut at the spacings of 75 x 50 cm and 100 x 37.5 cm in the early seasons.

**Key words:** intercropping, jack bean, pre-emergence herbicide, weed control.

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## Introduction

Maize (*Zea mays L.*) ranks third after wheat and rice among the world's most traded cereal crops and first in Africa, but more maize is currently produced annually than any other crop (FAOSTAT, 2014). Nigeria is the largest producer of maize in Africa, with 7.2 million tonnes (USDA, 2017). Virtually all parts of maize are utilized for various purposes ranging from direct human consumption and animal feed to raw materials in industries (IITA, 2009; Nuss and Tanumihardjo, 2010; Sule-Enyisi et al., 2014).

In spite of the great potentials of maize, both as a consumer crop and an industrial crop, its production in Nigeria is constrained by several factors, which include the use of low-yielding varieties, problems of low soil fertility, drought, diseases, pests and weed infestation, price fluctuation and inadequate storage facilities (IITA, 2000; Ojo, 2000). In 2019, the average yield of maize in Nigeria was 1.69 tonnes per ha (Knoema.com, 2019) compared with the global average yield of 4.3 t $ha^{-1}$ and 8.6 t $ha^{-1}$ of the United States.

Weeds appear to have the most deleterious effects on maize of all the constraints militating against its production and causing 69% to 92% loss in grain yield in the savanna agro-ecological zones of Nigeria (Adigun and Lagoke, 2003; Eni et al., 2012; Lagoke et al., 2014).

The use of a single method of weed control usually has several limitations (Jhala et al., 2014). It has been observed by several authors (Akobundu, 1987; Badmus et al., 2006; Eni et al., 2012; Lagoke et al., 2014; Adeyemi et al., 2019) that the use of one method of weed control was unable to give season-long control of weeds, hence the use of supplementary hoe-weeding, post-emergence herbicide application or/and cover crops to pre-emergence herbicide application is justified. The use of low growing legume cover crops to supplement other control measures for season-long weed control has been suggested by various workers (Akobundu, 1987; Yenish et al., 1996; Udensi et al., 1999; Badmus et al., 2006; Ojelade, 2004; Giwa, 2007).

The use of jack bean to suppress weed growth in maize production is currently under-exploited. The complementary use of the spatial arrangement of maize could be considered as it would affect cover crop spread and total light transmission to the weeds and consequently their smothering. Therefore, there is the need to determine the most appropriate combinations of maize spacing, cover crop and weed control treatment for effective season-long weed control and acceptable maize growth and productivity.

The use of a single herbicide and mixtures of herbicides like atrazine, prometryne, diuron, metolachlor, alachlor and pendimethalin has been reported in maize production in Nigeria (Akinyemiju, 1993; Osikanlu, 1996; Adigun and Lagoke, 1999). However, there is a paucity of information on the enhancement of

the effectiveness of some commercially formulated mixtures of pre-emergence herbicides with cover crops, especially groundnut and jack bean, to substitute supplementary hoe-weeding. Therefore, the objective of this study was to evaluate the weed control efficacy of pre-emergence herbicide mixtures of prometryne, metolachlor and acetochlor in maize production as influenced by intercropped groundnut and jack bean as legume cover crops and row spacing.

## Material and Methods

Field trials were conducted to evaluate intercropped groundnut and jack bean for enhanced weed control efficacy of pre-emergence herbicide use in maize production in the late seasons of 2013 and 2015 and early seasons of 2014 and 2015 at the Federal University of Agriculture, Abeokuta. Details of the physico-chemical properties and weed flora are contained in Tables 1 and 2. In all the trials, the land was ploughed and harrowed at the two-week interval. The experiment was arranged in split-plot fitted in a randomized complete block design with three replications. Main plots consisted of eight weed control methods (WCMs): Probaben$^{R}$ (prometryne + metolachlor, 200+200 g of a.i. per L) and Super Union$^{R}$ (prometryne+acetochlor, 130+380 g of a.i. per L) each applied at 2.4 kg a.i ha, 1.6 kg a.i/ha and each at 1.6 kg a.i/ha followed by supplementary hoe-weeding at 6 WAP, two hoe-weedings at 3 and 6 WAP and a weedy check. Sub-plot treatments consisted of eight cropping patterns (CPs) – sole maize, maize-jack bean and maize-groundnut intercrops, each at two spacings (100 x 37.5 cm and 75 x 50 cm) and sole crops of jack bean and groundnut.

Table 1. Soil physico-chemical characteristics at the experimental sites at Alabata Road, Abeokuta, Nigeria.

| Soil composition | 2013L | 2014E | 2015E | 2015 L |
|---|---|---|---|---|
| Particle size analysis | | | | |
| Sand (%) | 83.8 | 82.8 | 70.8 | 82.8 |
| Silt (%) | 8.5 | 4.4 | 17.4 | 4.4 |
| Clay (%) | 9.2 | 12.8 | 11.8 | 12.4 |
| Textural class | sandy-loam | sandy-loam | sandy-loam | sandy-loam |
| Chemical composition | | | | |
| Organic carbon (%) | 1.89 | 4.19 | 3.83 | 2.99 |
| Available P (mg/kg) | 1.90 ppm | 40.89 | 41.26 | 41.92 |
| Total N (%) | 1.12 | 0.14 | 0.15 | 0.16 |
| Exchangeable K (Cmol/g) | 0.74 | 0.54 | 0.62 | 0.6 |

E = Early season, L= Late season.

Three seeds of maize were planted per hill at the spacing indicated according to the treatment structure and thinned to 2 plants per stand three weeks later to give a total population of 53,333 plants/ha. The gross plot size was 6 m x 3 m while 4.5 m x 2.0 m and 4 m x 2.25 m were the net plot sizes for maize spaced at 75 x 50 cm and 100 x 37.5 cm, respectively. Maize genotype Oba Super 2, a single cross hybrid, groundnut local variety *Jawanda* and an unnamed variety of jack bean sourced from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were planted.

Table 2. Common weed flora composition and levels of weed species occurrence before soil cultivation at the experimental sites at Alabata Road, Abeokuta, Nigeria.

| Weed species | Level of infestation | | | |
|---|---|---|---|---|
| Broadleaves | 2013L | 2015L | 2014E | 2015E |
| *Mucuna utilis* (Wight) Burck. | + | - | ++ | - |
| *Meremia aegyptia* L. | ++ | ++ | +++ | + |
| *Talinum triangulare* (Jacq.) Willd. | ++ | + | + | - |
| *Tridax procumbens* L. | ++ | ++ | +++ | + |
| *Ageratum conyzoides* | _ | - | ++ | + |
| *Euphorbia heterophylla* L. | +++ | +++ | + | ++ |
| *Euphorbia hirta* L. | ++ | + | + | - |
| *Aspilia africana* (Pers.) C.D. Adams. | + | + | + | + |
| *Centrosema pubescens Benth.* | + | + | - | + |
| Unknown pulse | + | + | ++ | + |
| Grasses | | | | |
| *Andropogun gayanus* (Kunth.) | + | + | + | ++ |
| *Panicum maximum* Jacq. | +++ | +++ | ++ | +++ |
| *Rottboellia cochinchinensis* (Lour.) W.D. Clayton | + | + | + | + |
| *Eleusine indica* (Gaertn.) | + | + | - | + |
| *Digitaria horizontalis* (Willd) | + | + | + | + |
| *Physalis angulata* L. | + | - | + | - |
| Sedges | | | | |
| *Cyperus esculentus* L. | + | + | - | + |
| *Cyperus rotundus* L. | + | + | - | + |
| Spiderwort | | | | |
| *Commelina bengalensis* L. | ++ | + | + | - |

E = Early season; L = Late season; +++ = High infestation (60–90% occurrence); ++ = Moderate infestation (30–59% occurrence); + = Low infestation (1–29% occurrence).

Inorganic fertilizer was applied to maize at the rate of 120 kg N, 60 kg $P_2O_5$ and 60 kg $K_2O$ per hectare. All herbicide treatments were applied pre-emergence one day after planting in a spray volume of 230 l/ha using a CP3 knapsack sprayer with a green deflector nozzle at a pressure of 2.1kg/cm$^2$. Hoe-weeding was carried out on appropriate plots at 3 and 6 weeks after planting (WAP) using a West

African hand hoe. The weeding operation was proceeded by weed cover score assessment and sampling. Data collected included the grain yield of maize, weed cover score and weed dry matter production. All data collected were subjected to analysis of variance, and treatment means were compared using Duncan Multiple Range Test ($p<0.05$).

The statistical package used was GENSTAT 12th Edition, following the procedure of www.vsn.co/software/genstat/htmhelp/server/HCITEGEN.

## Results and Discussion

The cropping patterns (CPs) affected weed infestation in this study (Tables 3 to 7). The groundnut and jack bean components in a mixture with maize reduced weed infestation compared to sole crops of maize, groundnut and jack bean. Weed infestation at 9 WAP, as reflected in weed cover score and broadleaf weed dry matter production, was, in most cases, lower in the intercrops at both spacings compared to the corresponding sole maize, sole jack bean and sole groundnut. Moreover, at 12 WAP, the weed cover score was generally lower on plots of groundnut intercropped with maize at both spacings compared with the sole maize in 2015 early and late wet seasons (Table 3). In all cases, plots of maize intercropped with groundnut consistently produced lower broadleaf weed dry matter compared with the respective maximum in each trial. Total weed infestation at harvest was also lower in the maize/groundnut mixture than the corresponding sole maize and sole groundnut (Tables 4 to 6).

The additional shade caused by maize stands in the mixture, which enhanced the smothering effect of jack bean and groundnut canopies, was probably responsible for better suppression of weeds in the mixture compared with the sole crops. The results corroborate those of Badmus et al. (2006), Lagoke et al. (2014), Adebomi (2008) and Williams (2010), who reported reduced weed infestation in maize–groundnut mixtures compared with the sole maize crop. Bilalis et al. (2010) also reported reduced weed infestation in intercrops compared with their sole crops.

However, at 12 WAP and harvest, weed infestation was only higher on the plots with sole maize at 100 x 37.5 cm spacing than the corresponding intercrop with jack bean. The higher weed infestation in sole maize than in the intercrop with jack bean at 100 x 37.5 cm can be attributed to the wider inter-row spacing than at 75 x 50 cm, which allowed for more light transmission to lower strata for weed growth. Maize in narrow rows was reported to shade soil surface and consequently smother weeds beneath the canopy earlier than those in wider rows (Jhala et al., 2014; Simić et al., 2020). Weed infestation was, however, observed to be consistently similar on plots of jack bean intercropped with maize at the two spacings, indicating that both intercrop combinations controlled weed similarly, probably due to the greater role of the cover crop in suppressing weeds than maize.

Table 3. Effects of weed control treatments and cropping patterns on weed cover score* at 9 and 12 WAP at Abeokuta.

| Treatment | 9 WAP | | | | 12 WAP | | | |
|---|---|---|---|---|---|---|---|---|
| | 2013L | 2014E | 2015E[1] | 2015L[2] | 2013L | 2014E | 2015E | 2015L |
| Weed Control Method (WCM) | | | | | | | | |
| Probaben[R] at 2.4 kg a.i $ha^{-1}$ | 3.20bc | 4.10b | 2.10b | 5.40b | 5.80bc | 4.10b | 3.40bc | 6.10b |
| Probaben[R] at 1.6 kg a.i $ha^{-1}$ | 3.70b | 3.90bc | 1.90bc | 5.50b | 6.70ab | 3.80bc | 3.60bc | 6.20b |
| Probaben[R] at 1.6 kg a.i $ha^{-1}$ fb[3] SHW[4] | 2.10cd | 2.20e | 1.10d | 1.50c | 4.80cd | 2.70de | 1.80e | 2.10c |
| Super Union[R] at 2.4 kg a.i $ha^{-1}$ | 3.40b | 3.20cd | 1.80bcd | 5.60b | 6.90ab | 3.40bcd | 2.90cd | 6.30b |
| Super Union[R] 1.6 kg a.i $ha^{-1}$ | 4.10b | 3.10cd | 2. 20b | 5.20b | 6.80ab | 3.40bcd | 3.90b | 6.30b |
| Super Union[R] 1.6 kg a.i $ha^{-1}$ fb[3] SHW[4] | 1.90d | 1.70e | 1.20d | 1.60c | 4.50cd | 2.40e | 2.10de | 1.90c |
| Hoe-weeding at 3 and 6 WAP[5] | 1.60d | 2.30de | 1.30cd | 1.40c | 4.30d | 2.90cde | 2.00de | 1.80c |
| Weedy check | 6.10a | 7.20a | 6.10a | 7.30a | 8.10a | 7.60a | 7.80a | 7.50a |
| SE ± (WCM) | 0.38 | 0.28 | 0.23 | 0.34 | 0.43 | 0.29 | 0.32 | 0.26 |
| Cropping Pattern (CP) | | | | | | | | |
| 75 x 50 cm Sole maize | 4.20a | 4.30a | 2.50a | 4.30bcd | 7.70a | 4.50a | 3.60bc | 4.70bcd |
| 75 x 50 cm Maize + Jack bean | 3.00b | 2.30d | 2.10bc | 3.50de | 4.50e | 2.60c | 3.10d | 4.2cd |
| 75 x 50 cm Maize + Groundnut | 2.80b | 2.80cd | 1.80c | 3.80cde | 5.70cd | 3.40b | 2.80d | 4.40bcd |
| Sole jack bean | 2.90b | 3.00bc | 2.50a | 4.20bcd | 4.90de | 3.30b | 3.80ab | 4.90bc |
| Sole groundnut | 3.40b | 4.20a | 1.90c | 5.20a | 5.90c | 5.10a | 3.00d | 6.10a |
| 100 x 37.5 cm Maize + Jack bean | 3.20b | 2.90bc | 2.50ab | 3.20e | 5.70cd | 3.10bc | 3.60bc | 4.10d |
| 100 x 37.5 cm Maize + Groundnut | 3.30b | 3.50b | 1.80c | 4.50abc | 6.50bc | 3.60b | 3.10cd | 4.90bc |
| 100 x 37.5 cm Sole maize | 3.30b | 4.80a | 2.70a | 4.90ab | 7.00ab | 4.80a | 4.30a | 4.90b |
| SE ± (CP) | 0.19 | 0.19 | 0.12 | 0.29 | 0.29 | 0.20 | 0.18 | 0.23 |
| SE ± Interaction (WCM x CP) | 0.62 | 0.59 | 0.40 | 0.84 | 0.88 | 0.60 | 0.57 | 0.66 |

[1]E = Early season; [2]L = Late season; [3]fb = Followed by; [4]SHW = Supplementary hoe-weeding; [5]WAP = Weeks after planting; [6]Means followed by the same letter(s) within the same column and treatments are not significantly different at the 5% level of probability using Duncan's New Multiple Range Test (DNMRT); [7]Probaben[R] = Prometryne (20%) + Metolachlor (20%); [8]Super Union[R] = Prometryne (13%) + Acetochlor (38%); *Weed cover score was based on visual observations using a scale of 1–10, where 1 = no weed cover and 10 = complete weed cover.

In this study, weed infestation was observed to be consistently similar on plots of maize/groundnut and maize/jack bean intercrops at both spacings, indicating that all the intercrop combinations similarly suppressed weeds.

The weed control methods (WCMs) also affected the level of weed infestation in this study (Tables 3 to 7). All the weed control methods generally reduced weed infestation, especially at the early stage of crop growth compared with the weedy check. Weed cover scores at 9 and 12 WAP were consistently lower in the supplementary hoe-weeded plots and those hoe-weeded twice than those with the application of herbicides alone, indicating that additional hoe-weeding at 6 WAP to the application of pre-emergence herbicides was still required to give satisfactory season-long weed control (Table 3). Various authors have earlier reported the inability of pre-emergence herbicides alone to give season-long weed control in maize production in various agroecological zones of Nigeria (Bakut, 1985; Akobundu, 1987; Adigun and Lagoke, 2003; Williams, 2010; Lagoke et al., 2014; Adeyemi et al., 2019). Akobundu (1987) indicated that pre-emergence herbicide application gave early weed control of emerging weed seedlings but lost effectiveness later due to depletion in the soil, thereby allowing late-emerging weeds to re-infest plots.

In this study, the herbicide treatments also reduced broadleaf weed dry matter production compared to the weedy check at 9 WAP with the exception of the pre-emergence application of Super union$^{R}$ alone in 2013 late and 2015 early seasons (Table 4). This confirms that the weed control methods gave various levels of early weed control until 9 WAP. Furthermore, broadleaf weed dry matter production at 12 WAP was generally lower with the similar values of the supplementary hoe-weeded plots and those hoe-weeded twice, compared to the other weed control treatments. It is, therefore, apparent that the late supplementary hoe-weeding at 6 WAP to either pre-emergence herbicides or one hoe-weeding at 3 WAP is critical for improved season-long weed control in maize production despite intercropped legume cover crops.

The pre-emergence application of both Probaben$^{R}$ and Super Union$^{R}$ alone was observed to give moderate weed control, which was, however, lower than the maximum obtained from the two hoe-weedings in this study. These results suggest that the pre-emergence application of the herbicides alone was also effective in controlling weeds, especially more in the early than in the late season. These results also suggest that the application of pre-emergence herbicides could be as effective as hoe-weeding in the management of weeds in maize production as earlier indicated by Adigun and Lagoke (2003), Chikoye et al. (2005), Olatunji et al. (2016).

At equal rates, without supplementary hoe-weeding, the two herbicide treatments consistently resulted in similar weed infestation as reflected in weed cover scores, broadleaf and grass weed dry matter production, as well as total weed dry matter production at harvest when compared with each other. This also translated into similar crop yield parameters in the two herbicide treatments.

Table 4. Effects of weed control treatments and cropping patterns on broadleaf weed dry matter production (kg ha$^{-1}$) at 9 and 12 WAP at Abeokuta.

| Treatments | 9 WAP | | | | 12 WAP | | | |
|---|---|---|---|---|---|---|---|---|
| | 2013L | 2014E | 2015E[1] | 2015L[2] | 2013L | 2014E | 2015E | 2015L |
| Weed Control Method (WCM) | | | | | | | | |
| Probaben$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 464c | 727a | 295bc | 591bc | 762 | 514a | 268abc | 513b |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ | 549bc | 404b | 296bc | 750b | 511 | 328ab | 324abc | 661ab |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 372c | 225b | 206c | 626bc | 439 | 350ab | 221bc | 216c |
| Super Union$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 550bc | 272b | 247c | 787b | 714 | 219b | 391ab | 779ab |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ | 844ab | 319b | 447ab | 651bc | 576 | 304ab | 416abc | 527b |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 537bc | 123b | 241c | 624bc | 460 | 285b | 273abc | 215c |
| Hoe-weeding at 3 and 6 WAP[5] | 342c | 239b | 215c | 234bc | 429 | 406ab | 176c | 178c |
| Weedy check | 954a | 662a | 608a | 1256a | 999 | 308ab | 456a | 885a |
| SE ± (WCM) | 99.2 | 83.5 | 56.9 | 136.6 | 127.9ns | 70.5 | 71.1 | 92.2 |
| Cropping Pattern (CP) | | | | | | | | |
| 75 x 50 cm Sole maize | 1070a | 330c | 305bc | 835ab | 677 | 305bc | 330b | 449bc |
| 75 x 50 cm Maize + Jack bean | 356b | 381bc | 238bc | 444c | 305 | 154c | 297b | 358c |
| 75 x 50 cm Maize + Groundnut | 452b | 171c | 163c | 396c | 513 | 292bc | 191b | 429bc |
| Sole jack bean | 546b | 644a | 368b | 956a | 596 | 229bc | 493a | 720a |
| Sole groundnut | 633b | 590ab | 243bc | 820ab | 702 | 793a | 226b | 689a |
| 100 x 37.5 cm Maize + Jack bean | 420b | 220c | 333bc | 668abc | 600 | 272bc | 229b | 371c |
| 100 x 37.5 cm Maize + Groundnut | 530b | 287c | 218bc | 581bc | 568 | 280bc | 256b | 346c |
| 100 x 37.5 cm Sole maize | 607b | 347c | 688a | 819ab | 925 | 391b | 502a | 609ab |
| SE ± (CP) | 90.7 | 75.9 | 62.4 | 95.5 | 108.1ns | 58.4 | 53.3 | 76.7 |
| SE ± Interaction (WCM x CP) | 259.6ns | 217.4ns | 174.5ns | 287.2ns | 313.3ns | 169.8ns | 157.9ns | 222.9ns |

[1]E = Early season; [2]L = Late season; [3]fb = Followed by; [4]SHW = Supplementary hoe-weeding; [5]WAP = Weeks after planting; [6]Means followed by the same letter(s) within the same column and treatments are not significantly different at the 5% level of probability using Duncan's New Multiple Range Test (DNMRT); [7]Probaben$^{R}$ = Prometryne (20%) + Metolachlor (20%); [8]Super Union$^{R}$ = Prometryne (13%) + Acetochlor (38%).

Grass weed dry matter production was similarly controlled by the weed control methods at 9 WAP but not at 12 WAP in most cases, indicating the weed control methods reduced grass weed infestation more at 9 WAP compared to 12 WAP (Table 5). This may be attributed to the weed control treatments losing their efficacy on grasses over time. Metolachlor and acetochlor have been reported to

lose their persistence in the soil and efficacy over time, depending on environmental factors such as soil moisture content (Zimdahl and Clark, 1982; Thomas et al., 1999). Even at harvest, the plots treated with various weed control methods still produced lower weed dry matter than those left weed-infested throughout, confirming their effectiveness in ensuring various levels of control during the life cycle of the crops.

Table 5. Effects of weed control treatments and cropping patterns on grass dry matter production (kg ha$^{-1}$) at 9 and 12 WAP at Abeokuta.

| Treatments | 9 WAP | | | | 12 WAP | | | |
|---|---|---|---|---|---|---|---|---|
| | 2013L | 2014E | 2015E[1] | 2015L[2] | 2013L | 2014E | 2015E | 2015L |
| Weed Control Methods (WCM) | | | | | | | | |
| Probaben$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 160b | 200bc | 55.0b | 93.0 | 252 | 05.0b | 64.0 | 228 |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ | 85b | 223bc | 123b | 243 | 69.0 | 96.0b | 181 | 101 |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 63b | 59.0c | 80.0b | 65.0 | 185 | 35.0b | 77.0 | 39.0 |
| Super Union$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 96b | 485b | 166b | 75.0 | 395 | 40.0b | 118 | 317 |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ | 141b | 224bc | 162b | 143 | 146 | 76.0b | 138 | 198 |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 96b | 121bc | 31.0b | 20.0 | 84.0 | 19.0b | 24.0 | 47.0 |
| Hoe-weeding at 3 and 6 WAP[5] | 66b | 92bc | 110b | 98.0 | 194 | 41.0b | 92.0 | 103 |
| Weedy check | 719a | 1135a | 558a | 282 | 823 | 358a | 660 | 461 |
| SE ± (WCM) | 104.7 | 119.0 | 75.8 | 66.5ns | 155.0ns | 59.5 | 137.6ns | 130.5ns |
| Cropping Pattern (CP) | | | | | | | | |
| 75 x 50 cm Sole maize | 327 | 327 | 226 | 08.0 | 300 | 85.0 | 86.0 | 05.0 |
| 75 x 50 cm Maize + Jack bean | 175 | 148 | 138 | 16.0 | 366 | 84.0 | 166 | 25.0 |
| 75 x 50 cm Maize + Groundnut | 129 | 405 | 72.0 | 261 | 151 | 130 | 145 | 293 |
| Sole Jack bean | 116 | 203 | 186 | 107 | 214 | 35.0 | 262 | 311 |
| Sole groundnut | 148 | 365 | 169 | 194 | 239 | 192 | 138 | 280 |
| 100 x 37.5 cm Maize + Jack bean | 109 | 244 | 191 | 114 | 307 | 39.0 | 236 | 292 |
| 100 x 37.5 cm Maize + Groundnut | 299 | 477 | 124 | 76.0 | 391 | 48.0 | 107 | 98 |
| 100 x 37.5 cm Sole maize | 124 | 367 | 177 | 242 | 179 | 54.0 | 212 | 188 |
| SE ± (CP) | 99.9ns | 101.9ns | 69.9ns | 77.6ns | 113.5ns | 39.8ns | 55.2ns | 94.1ns |
| SE ± Interaction (WCM x CP) | 284.2ns | 294.7ns | 199.9ns | 215.7ns | 337.9ns | 121.0ns | 200.7ns | 281.1ns |

[1]E = Early season; [2]L = Late season; [3]fb = Followed by; [4] SHW = Supplementary hoe-weeding; [5]WAP = Weeks after planting; [6]Means followed by the same letter(s) within the same column and treatments are not significantly different at the 5% level of probability using Duncan's New Multiple Range Test (DNMRT); [7]Probaben$^{R}$ = Prometryne (20%) + Metolachlor (20%); [8]Super Union$^{R}$ = Prometryne (13%) + Acetochlor (38%).

Table 6. Effects of weed control treatments and cropping patterns on weed dry matter production at harvest (kg ha$^{-1}$) at Abeokuta.

| Treatments | Weed dry matter production at harvest (kg ha$^{-1}$) | | |
|---|---|---|---|
| Weed Control Method (WCM) | 2014L | 2015E[1] | 2015L[2] |
| Probaben$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 795b | 768b | 3299ab |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ | 583bc | 840b | 3293ab |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 370c | 461b | 1075c |
| Super Union$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 559bc | 789b | 2828b |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ | 509bc | 868b | 3156b |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 300c | 659b | 662c |
| Hoe-weeding at 3 and 6 WAP[5] | 423c | 741b | 1086c |
| Weedy check | 1356 | 525a | 3722a |
| SE ± (WCM) | 94.2 | 153.6 | 156.5 |
| Cropping Pattern (CP) | | | |
| 75 x 50 cm Sole maize | 574b | 622c | 2340bc |
| 75 x 50 cm Maize + Jack bean | 421b | 602c | 2033 |
| 75 x 50 cm Maize + Groundnut | 548b | 948ab | 1734d |
| Sole jack bean | 613b | 1106a | 2813b |
| Sole groundnut | 1069a | 1039a | 3628a |
| 100 x 37.5 cm Maize + Jack bean | 429b | 447c | 1733d |
| 100 x 37.5 cm Maize + Groundnut | 600b | 718bc | 2324bc |
| 100 x 37.5 cm Sole maize | 642b | 1168a | 2514bc |
| SE ± (CP) | 75.3 | 104.3 | 188.9 |
| SE ± Interaction (WCM x CP) | 220.5ns | 315.7ns | 523.7 |

[1]E = Early season; [2]L = Late season; [3]fb = Followed by; [4] SHW = Supplementary hoe-weeding; [5]WAP = Weeks after planting; [6]Means followed by the same letter(s) within the same column and treatments are not significantly different at the 5% level of probability using Duncan's New Multiple Range Test (DNMRT); [7]Probaben$^{R}$ = Prometryne (20%) + Metolachlor (20%); [8]Super Union$^{R}$ = Prometryne (13%) + Acetochlor (38%).

The interaction of weed control treatments and the cropping pattern was significant on total weed dry matter production at harvest in the 2015 late season (Table 7). The sole groundnut plots treated with Probaben$^{R}$ at both rates produced the maximum weed dry matter, which was significantly higher than all the intercropped plots and sole maize treated with Probaben$^{R}$ and Super Union$^{R}$ each at 1.6 kg a.i/ha fb SHW and those hoe-weeded twice. The plots with maize at the 75 x 50 cm spacing intercropped with groundnut and at the 100 x 37.5 cm spacing intercropped with jack bean treated with the two herbicides each at 1.6 kg a.i/ha alone also produced significantly lower weed dry matter than the maximum of plots with sole groundnut given Probaben$^{R}$ at both rates and comparable to the minimum.

Table 7. The interaction of weed control treatments and the cropping pattern on weed dry matter production at harvest (kg ha$^{-1}$) in the 2015 late season at Abeokuta.

| Weed Control Methods | Cropping Pattern | | | |
|---|---|---|---|---|
| | 75 cm Sole maize | 75 cm Maize + Jack bean | 75 cm Maize + Groundnut | Sole jackbean |
| Probaben$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 2612.0d-n | 3291.0b-j | 2662.0d-m | 4908.0ab |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ | 3275.0b-j | 3579.0b-g | 1895.0f-r | 3891.0b-e |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$fb$^{3}$ SHW$^{4}$ | 1058.0 l-r | 541.7qr | 658.3o-r | 891.7m-r |
| Super Union$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 3512.0b-g | 2316.0e-q | 2391.0e-p | 2675.0d-m |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ | 2475.0e-o | 2875.0d-l | 1025.0m-r | 4366.0a-d |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$fb$^{3}$ SHW$^{4}$ | 987.0m-r$^{6}$ | 154.2r | 1137.0k-r | 454.2r |
| Hoe-weeding at 3 and 6 WAP$^{5}$ | 866.7m-r | 587.5pqr | 795.8n-r | 954.2m-r |
| Weedy check | 3937.0b-e | 2925.0d-k | 3308.0b-j | 4366.0a-d |
| Weed Control Methods | Sole groundnut | 100 cm Maize + Jack bean | 100 cm Maize + Groundnut | 100 cm Sole maize |
| Probaben$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 5754.0a | 1650h-r | 2325.0e-q | 3187.0b-j |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ | 5683.0a | 1554j-r | 3450.0b-i | 3016.0c-j |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$fb$^{3}$ SHW$^{4}$ | 1875.0f-r | 895.8m-r | 683.3o-r | 1995.0f-r |
| Super Union$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 3483.0b-h | 1637.5i-r | 4125.0a-e | 2487.0e-o |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ | 4804.0abc | 3241.0b-j | 2491.0e-o | 3970.0b-e |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$fb$^{3}$ SHW$^{4}$ | 941.0m-r | 529.1qr | 258.3r | 837.5m-r |
| Hoe-weeding at 3 and 6 WAP$^{5}$ | 1491.0j-r | 1750.0g-r | 1566.0j-r | 675.0o-r |
| Weedy check | 4995.0ab | 2608.0d-n | 3695.0b-f | 3941.0b-e |
| SE ± 523.7 | | | | |

$^{1}$E = Early season; $^{2}$L = Late season; $^{3}$fb = Followed by; $^{4}$SHW = Supplementary hoe-weeding; $^{5}$WAP = Weeks after planting; $^{6}$Means followed by the same letter(s) within the same column and treatments are not significantly different at the 5% level of probability using Duncan's New Multiple Range Test (DNMRT); $^{7}$Probaben$^{R}$ = Prometryne (20%) + Metolachlor (20%); $^{8}$Super Union$^{R}$ = Prometryne (13%) + Acetochlor.

Maize grain yields (1.4–5.2 t ha$^{-1}$) were similar for all WCM, except in the 2015 late season, but significantly depressed (21–48%) in those weed-infested throughout. Maize intercropped with groundnut and jack bean produced similar

cob and grain yields compared to corresponding sole maize in the early seasons. However, lower yields were recorded in the intercrop than in the corresponding sole crop in the late seasons (Table 8). This was probably because the adequate growth resources of the early season ameliorated the effect of interspecific competition.

Table 8. Effects of weed control treatments and the cropping pattern on maize cob and grain yields at Abeokuta.

| Treatments | Cob yield (kg ha$^{-1}$) | | | | Grain yield (kg ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | 2013L | 2014E[1] | 2015E | 2015L[2] | 2013L | 2014E | 2015E | 2015L |
| Weed Control Methods (WCMs) | | | | | | | | |
| Probaben$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 2524abc | 5556a | 4624bc | 892cd | 2052ab | 4141a | 3742bc | 657cd |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ | 2428abc | 5685a | 5707ab | 1096bc | 1931abc | 4128a | 4263ab | 822bc |
| Probaben$^{R}$ at 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 2750ab | 5611a | 5787ab | 1927a | 2205ab | 4298a | 4634ab | 1439a |
| Super Union$^{R}$ at 2.4 kg a.i ha$^{-1}$ | 1776cd | 4408ab | 4842bc | 1220bc | 1386cd | 3239ab | 3865bc | 950c |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ | 2282bc | 3685bc | 5041bc | 1132bc | 1817bc | 2708bc | 4011ab | 862c |
| Super Union$^{R}$ 1.6 kg a.i ha$^{-1}$ fb[3] SHW[4] | 2652ab | 5000ab | 53594ab | 1853a | 2216ab | 3716ab | 4412ab | 1496a |
| Hoe-eeding at 3 and 6 WAP[5] | 3086a[6] | 5070a | 6549a | 1471ab | 2567a | 3869a | 5236a | 1112ab |
| Weedy Check | 1337d | 2630c | 3654c | 4896d | 1072d | 1988c | 2709c | 349d |
| SE ± (WCM) | 228.4 | 417.6 | 432.6 | 147.1 | 191.2 | 341.7 | 393.0 | 129.5 |
| Cropping Patterns (CPs) | | | | | | | | |
| 75 x 50 cm Sole maize | 2761a | 4646ab | 591 | 1823a | 2227a | 3549ab | 4395 | 1406 a |
| 75 x 50 cm Maize + Jack bean | 1957b | 4837ab | 5336 | 1031bc | 1564b | 3516ab | 4256 | 796bc |
| 75 x 50 cm Maize + Groundnut | 1975b | 5445a | 5006 | 927c | 1587b | 3924a | 4026 | 673c |
| 100 x 37.5 cm Maize + Jack bean | 2257ab | 5174a | 5046 | 900c | 1817ab | 4050a | 4067 | 703c |
| 100 x 37.5 cm Maize + Groundnut | 267a | 3972b | 4763 | 1381abc | 2127a | 3011b | 3501 | 1043abc |
| 100 x 37.5 cm Sole maize | 2504a | 4160b | 5458 | 1499ab | 2037a | 3016b | 4409 | 1144ab |
| SE ± (CP) | 176.8 | 295.6 | 297.2 | 181.5 | 148.9 | 239.7 | 245.9ns | 141.7 |
| SE ± Interaction (WCM x CP) | 510.5 | 869.9 | 880.8 | 491.2 | 429.3 | 706.9 | 746.3ns | 388.1 |

[1]E = Early season; [2]L = Late season; [3]fb = Followed by; [4]SHW = Supplementary hoe-weeding; [5]WAP = Weeks after planting; [6]Means followed by the same letter(s) within the same column and treatments are not significantly different at the 5% level of probability using Duncan's New Multiple Range Test (DNMRT); [7]Probaben$^{R}$ = Prometryne (20%) + Metolachlor (20%); [8]Super Union$^{R}$ = Prometryne (13%) + Acetochlor (38%).

Maize cob and grain yields were higher in maize/groundnut intercrop at 75 x 50 cm than at 100 x 37.5 cm in the early seasons, while the reverse was the case in the late seasons. This could be attributed to the limited available resources and interspecific competition in the late season, which probably could not sustain the higher plant density of maize at the 75 x 50 cm intercrop since the closer inter-row spacing had higher maize and groundnut plant densities per unit area. Maize yield is known to be influenced by plant densities (Fanadzo, 2007; Vega et al., 2001; Olaniyan and Lucas, 2002; Shrestha et al., 2018; Simić et al., 2012). The meteorological records during the trial periods suggest that the late-season maize, especially in 2015, might have experienced some drought during the period of grain filling.

## Conclusion

Jack bean and groundnut intercropped with maize significantly suppressed weed growth compared to sole crops of maize, consequently maize intercropped with groundnut and jack bean produced similar cob and grain yields compared to corresponding sole maize in the early seasons. However, the weed-suppressive effect of intercropped jack bean/groundnut did not reflect in the cob and grain yields of maize in the late wet season because of the limited resources in the late season.

Intercropping of maize with either jack bean or groundnut at 75 x 50 and 100 x 37.5 cm spacings enhanced the efficacy of prometryne pre-emergence herbicides when combined with either metolachlor or acetochlor and increased maize cob and grain yields, hence, it can be recommended for optimal yield.

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Received: May 28, 2020
Accepted: July 12, 2021

# POVEĆANJE EFIKASNOSTI SUZBIJANJA KOROVA DVA HERBICIDA PRIMENJENIH PRE NICANJA KOD ZDRUŽENIH USEVA KUKURUZA/PASULJA I KUKURUZA/KIKIRIKIJA U PRELAZNOM POJASU SAVANE

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R e z i m e

Nemogućnost herbicida, u ranoj primeni pre nicanja glavnog useva, da sami obezbede suzbijanje korova tokom cele sezone u proizvodnji kukuruza opravdava potrebu za dodatnim suzbijanjem korova. Sprovedena su ispitivanja metodom poljskog ogleda da bi se procenila združena setva kikirikija i pasulja radi poboljšane efikasnosti suzbijanja korova dva herbicida pre nicanja u proizvodnji kukuruza tokom vegetacione sezone 2013/2014. i 2015. na Federalnom poljoprivrednom univerzitetu u Abeokuti (Nigerija). Eksperiment je postavljen kao podeljena parcela uklopljena u randomizirani potpuni blok dizajn sa tri ponavljanja. Glavne parcele su se sastojale od osam tretmana za suzbijanje korova komercijalno formulisaneherbicidne preparate na generičkoj osnovi prometrina + metalohlora (Probaben$^{R}$); prometrina + acetohlora (Super Union$^{R}$) svaki po 2,4 kg a.s ha$^{-1}$, 1,6 kg a.s ha$^{-1}$ sa i bez dodatnog okopavanja, dva okopavanja i apsolutne kontrole bez uklanjanja korova. Tretmani potparcela sastojali su se od osam sistema gajenja (kukuruz kao jedini usev, združena setva kukuruza i pasulja, kukuruza i kikirikija, svaki sa dva međuredna rastojanja [100 x 37,5 cm i 75 x 50 cm] i usevi pasulja i kikirikija kao jedinih useva bez združivanja). Sve metode suzbijanja korova značajno su smanjile ($p<0,05$) suvu materiju korova u poređenju sa kontrolom bez uklanjanja korova za 10–81%. Prinosi zrna kukuruza (1,4–5,2 t ha$^{-1}$) bili su slični na svim parcelama sa metodama suzbijanja korova, osim u kasnoj vegetacionoj sezoni 2015. godine, ali su značajno smanjeni (21–48%) u poredjenju sa kontrolom. Združena setva pasulja i kikirikija sa kukuruzom značajno (20–67%) je smanjila zakorovljenost u odnosu na useve kukuruza, pasulja ili kikirikija gajenih bez združivanja. Efikasnost herbicida primenjenih posle setve, a pre nicanja u ovom istraživanju je, stoga, poboljšana združenom setvom kukuruza sa pasuljem i kikirikijem na rastojanjima od 75 x 50 cm i 100 x 37,5 cm u ranim sezonama.

**Ključne reči:** združena setva, pasulj, herbicidi pre nicanja glavnog useva, suzbijanje korova.

Primljeno: 28. maja 2020.
Odobreno: 12. jula 2021.

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Journal of Agricultural Sciences (Belgrade)
Vol. 66, No. 4, 2021
Pages 351-358

https://doi.org/10.2298/JAS2104351D
UDC: 634.23:581.46]:632.111.5
Original scientific paper

# THE INFLUENCE OF ROOTSTOCKS ON THE SENSITIVITY OF FLOWER BUDS TO FROST AND THE MAIN PROPERTIES OF THE 'CARMEN' SWEET CHERRY CULTIVAR

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**Abstract:** The study examined the influence of five vegetative rootstocks on the flower bud sensitivity to frost during the period of ecological dormancy and the most important biological and pomological properties of the 'Carmen' sweet cherry cultivar. The 'Carmen' cultivar was grafted on the following rootstocks: 'Colt', 'Gisela 5', 'Gisela 6', 'MaxMa 14' and 'Oblačinska cherry'. Winter frosts occurred during the ecological dormancy of sweet cherry trees. The intensity of frost was between –5°C and –7°C. The percentage of damaged and non-damaged flower buds per fruiting branch was determined by counting (50 flower buds per tree were taken from every part of the canopy and fruiting branches). The highest rate of damage of flower buds was in trees grafted on 'Oblačinska cherry', an average of 77.2% of flower buds, while the lowest was observed in trees grafted on 'MaxMa 14', an average of 24.3% of total flower buds. The significantly higher sensitivity of flower buds was found in spur fruiting branches compared to lateral fruiting branches. The fruits of the 'Carmen' cultivar had the earliest ripening on rootstocks 'Gisela 5' and 'Oblačinska cherry'. The 'Carmen' cultivar had the highest yield per tree on the 'MaxMa 14' rootstock, while the lowest yield rate was observed in 'Oblačinska cherry', 5.4 kg and 1.9 kg, respectively. The highest mass of fruits was noticed in trees grafted on 'Gisela 6', an average of 11.6 g, while the smallest was recorded in trees grafted on 'Gisela 5'– an average of 9.4 g.

**Key words:** ecological dormancy, flower buds, frost, percentage of damage, yield, fruit quality.

## Introduction

Sweet cherries are among the most popular summer fruits in Europe, and in most cherry growing countries, their production has an increasing trend (Deķena et al., 2020). Flowering is the crucial process affecting the possibility of obtaining a

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satisfactory fruit crop in sweet cherry (Dziedzic et al., 2019). Many authors studied the problems of sweet cherry flowering and related it with flower structures, especially generative organs, pollen-pistil interaction and the rootstock effect on fruit setting (Zhang et al., 2015; Radunić et al., 2017).

The suitability of cultivar-rootstock combinations to local climate conditions, together with soil properties and growing technologies, is one of the key factors in sweet cherry production (Pal et al., 2017). In the last two decades, the new dwarfing and semi-dwarfing cherry rootstocks have allowed obtaining high fruit yields of satisfactory quality. In many of the studies, authors have examined the following rootstocks: 'Weiroot 72', 'Weiroot 158', 'Weiroot 13', 'Gisela 5', 'Gisela 6', 'Edabriz', 'Pi-Ku 4.20', 'MaxMa 14' and 'F 12/1' (Gratacos et al., 2008; Usenik et al., 2008; Bielicki and Rozpara, 2010). The sweet cherry is a strong growing tree and requires the use of poorly growing rootstocks in contemporary production. Vegetative rootstocks are mostly used in intensive cherry production, of which the most important is 'Gisela 5' (Milatović et al., 2013). A powerful effect of poorly growing rootstocks was observed in the growth and yielding of sweet cherry cultivars (Usenik et al., 2008). Also, the rootstocks affect the intensity of flowering (Gratacos et al., 2008), the flowering time (Blažkova et al., 2010), as well as fruit quality (Balmer, 2008) in sweet cherry. Nevertheless, growers prefer dwarfing rootstocks for producing hand-picked cherries for fresh markets, which allow planting densities of up to 1.000–5.000 trees per hectare (Musacchi et al., 2015).

Also, the influence of rootstocks on grafted cultivar properties is expressed as the difference in the efficiency of water usage, advance or delay of flowering and harvesting, survival and yield efficiency (Cantin et al., 2010). The rootstocks also influence the response of grafted cultivars to cold temperatures (Hrotkó, et al., 2009). There were significant differences observed between rootstocks while assessing tree viability and winter damages. The trees on the P. *mahaleb* and 'Gisela 5' rootstocks were in the best condition (Deķena et al., 2020). Nevertheless, regarding dwarfing rootstocks, smaller tree canopies are manifested by shorter shoots and less leaf area, decreasing overall carbohydrate production for growth and storage. Stored carbohydrates are critical for allocation to new vegetative and reproductive structures in spring and are associated with negative effects on stored carbohydrates and fruit quality (Olmstead et al., 2010). Moghadam et al. (2015) recorded a direct relationship between the increased resistance of tree plants to frosts and carbohydrate content. The damage percentage is not only influenced by the temperature but also by the phenological stage.

Sweet cherry production in Serbia has a long tradition. Unfortunately, the quality of production and the quality of fruit are still at a low level. In addition, in most sweet cherry orchards, trees are grown on vigorous seedling rootstocks (P. *avium* L. and P. *mahaleb* L.) in low-density planting. The prerequisites for the

successful production of sweet cherries are increased fruit quality and yields per tree and planting area. These goals can be achieved by testing new cultivars and rootstocks in different climatic and soil conditions before cultivating in a large planting area.

The study aimed to examine the influence of different vegetative rootstocks for cold hardiness in the ecological dormancy of the 'Carmen' sweet cherry cultivar in Belgrade's environmental conditions. Also, the influences of those rootstocks on the fruit quality and yield of trees were examined.

## Material and Methods

### Plant material and growing conditions

The study was carried out at a commercial orchard located at the Faculty of Agriculture experimental estate 'Radmilovac', Belgrade, in the fifth growing year of the orchard (2020). The area has a temperate continental climate with an average annual rainfall of 650 mm. The orchard was established in the spring of 2016 with high-quality 1-year-old nursery trees. The 'Carmen' sweet cherry cultivar was grafted on five vegetative rootstocks ('Colt', 'Gisela 5', 'Gisela 6', 'MaxMa 14' and 'Oblačinska cherry'). Trees were planted at a distance of 3.5×1.6 m (1,750 trees/ha).

The study examined the influence of winter frost on flower bud damages of the 'Carmen' cultivar grafted on different vegetative rootstocks. Winter frosts occurred during the ecological dormancy of sweet cherry (code 01 on the BBCH scale, according to Meier, 2018). During the early morning hours of March 14 and March 15, the temperatures were -5°C and -7°C, respectively.

### Flowers sensitivity, phenological and biological properties

Sensitivity of flowers was represented as the percentage of damaged and non-damaged flowers per spur flowering shoots (SFB) and lateral fruiting shoots (LFB – shoots longer than 7 cm) separately and in total. Also, the study researched the following properties: the number of flowers per flower bud on short and long fruiting branches, time of blooming and harvesting, trunk cross-sectional area (TCSA), yield per tree and yield efficiency, the mass of fruit, a diameter of fruit, soluble solid contents (SSC), total sugars (TS) and total acids (TA).

The experiment was conducted on a random field with four repetitions (50 flower buds per tree were taken from every part of the canopy and fruiting branches, five trees were taken for a repeat). The physical properties of fruits were determined with five repetitions, and each repetition included 25 fruits. The chemical properties were determined with three repetitions. Physical properties

were determined using standard morphometrics methods. SSC was determined by refractometer (Atago, pocket PAL-1. Kyoto, Japan). Titratable acidity (TA) was determined by titrating 25 g of fruits with 0.1N NaOH up to pH 7.0 and expressed in %. Total sugars (TS) were determined by the Luff-Schoorl method and expressed in %. Analysis of variance was done using the STATISTICA 9 software package. The significant differences between means were determined at $P<0.05$, measured by the LSD test.

## Results and Discussion

The highest percentage of damaged flowers was recorded in trees of the 'Carmen' cultivar grafted on 'Oblačinska cherry' (77.2%), while trees grafted on 'MaxMa 14' (24.3%) had the lowest percentage of damaged flowers (Table 1). Trees grafted on 'Colt' and 'Gisela 5' had over 50% of damaged flowers. Milatović et al. (2013) recorded significantly sensitive flower buds in winter frost in many sweet cherry cultivars grafted on 'Gisela 5'. All trees had a higher percentage of damaged flowers on spur fruiting branches (SFB) compared to lateral fruiting branches (LFB). The percentages of damaged flowers on SFB were between 35.2% and 80.2%, while on LFB they ranged from 18.2% to 65.2%.

Table 1.The percentage of damage and the number of flowers per bud in different fruiting branches of the 'Carmen' sweet cherry cultivar grafted on vegetative rootstocks.

| Rootstocks | Percentage of damaged flower buds | | | Flowers per bud | |
|---|---|---|---|---|---|
| | SFB | LFB | TOTAL | SFB | LFB |
| 'Colt' | 73.2ab | 58.2ab | 62.3ab | 2.1c | 2.5b |
| 'Gisela 5' | 56.1ab | 44.6b | 52.2b | 2.3bc | 2.5b |
| 'Gisela 6' | 44.2b | 36.9bc | 41.1bc | 2.4bc | 2.4b |
| 'MaxMa 14' | 35.2b | 18.2c | 24.3c | 2.7a | 3.1a |
| 'Oblačinska cherry' | 80.2a | 65.2a | 77.2a | 2.4b | 2.5b |
| LSD | 27.2 | 14.5 | 19.6 | 0.2 | 0.4 |

SFB – spur fruiting branch; LFB – lateral fruiting branch.

The rootstocks had a significant influence on bud differentiation. The highest number of flowers per bud was found in trees grafted on 'MaxMa 14', while the lowest number of flowers per bud was recorded in trees grafted on 'Colt' (Table 1). All trees had a higher number of flowers in LFB compared to SFB. In SFB, the number of flowers per bud was between 2.1 ('Colt') and 2.7 ('MaxMa 14'), while in LFB, the number of flowers was between 2.4 ('Gisela 6') and 3.1 ('MaxMa 14').

The earliest blooming happened in the trees grafted on 'Oblačinska cherry', while trees grafted on 'Colt' and 'MaxMa 14' had the latest flowering

time (Table 2). Also, trees grafted on the 'Oblačinska cherry' rootstock had the longest blooming period (18 days), while trees grafted on 'Colt' had the shortest blooming period (11 days). The earliest full blooming stage was observed in trees grafted on 'Gisela 5' and 'Gisela 6'. Fruits of the 'Carmen' cultivar had the earliest ripening on 'Gisela 5' and 'Oblačinska cherry' rootstocks, while the trees grafted on 'MaxMa 14' had the latest ripening of fruits.

The trees grafted on 'Colt' had significantly higher values of TCSA compared to other rootstocks. Hrotkó et al. (2009) recorded significantly higher values of TCSA of 'Carmen' trees grafted on 'Colt' compared to the 'MaxMa 14' rootstock. The 'Carmen' cultivar had a higher yield per tree in 'MaxMa 14' (5.4 kg) and 'Gisela 6' (3.6 kg), while the yield had significantly lower values in 'Oblačinska cherry' (1.4 kg) and 'Colt' (2.5 kg). The obtained result could be due to a higher percentage of damaged flowers on trees grafted on 'Oblačinska cherry'. Also, trees grafted on very dwarf or vigorous rootstocks in early bearing periods had significantly lower yield, which is consistent with the results of other authors (Whiting et al., 2005; Sitarek et al., 2008; Bielicki and Rozpara, 2010).

Table 2. Phenological properties and yield of the 'Carmen' sweet cherry cultivar grafted on vegetative rootstocks.

| Rootstocks | Start of blooming | Full blooming | End of blooming | Bloom longevity (days) | Time of harvest | Yield (kg/tree) | TCSA ($cm^2$) | Yield efficiency ($kg/cm^2$) |
|---|---|---|---|---|---|---|---|---|
| 'Colt' | March 30 | April 05 | April 10 | 11 | May 28 | 2.5bc | 23.9a | 0.11c |
| 'Gisela 5' | March 28 | April 04 | April 12 | 15 | May 24 | 3.4b | 13.8bc | 0.25ab |
| 'Gisela 6' | March 28 | April 04 | April 13 | 16 | May 26 | 3.8ab | 15.9bc | 0.24b |
| 'MaxMa 14' | March 30 | April 07 | April 13 | 14 | June 01 | 5.4a | 18.1b | 0.30a |
| 'Oblacinska cherry' | March 27 | April 07 | April 14. | 18 | May 24 | 1.9c | 11.3c | 0.16c |
| LSD | | | | | | 1.6 | 5.4 | 0.05 |

The highest yield efficiency was found in trees grafted on 'MaxMa 14' (0.30 $kg/cm^2$), while significantly lower yield efficiency was observed in trees grafted on 'Colt' (0.11 $kg/cm^2$). Similar results were obtained by Rubauskis et al. (2014). Also, according to Hrotkó et al. (2009), trees with higher values of TCSA had significantly lower values of yield efficiency, which was confirmed in our research. The highest fruit mass was noticed in trees grafted on 'Gisela 6' (11.6 g), while the smallest mass was recorded in trees grafted on 'Gisela 5' (9.4 g). Also, the highest diameter of fruits was found in trees grafted on 'Gisela 6' (Table 3).

Trees grafted on 'Gisela 6' and 'MaxMa 14' had marketable fruits whose diameters were higher than 28 mm. Significantly higher contents of soluble solids and total sugars were determined in fruits of the 'Carmen' cultivar grafted on 'Oblačinska

cherry' (19.2% and 18.1%, respectively), while fruits of trees grafted on 'Gisela 5' had the lowest contents of those parameters (17.2% and 15.3%, respectively).

Table 3. Physical and chemical properties of fruits of the 'Carmen' sweet cherry cultivar grafted on vegetative rootstocks.

| Rootstocks | Mass of fruit (g) | Diameter of fruits (mm) | Soluble solids (%) | Total sugars (%) | Total acids (%) |
|---|---|---|---|---|---|
| 'Colt' | 9.8b | 26.8b | 18.2b | 16.5b | 0.9a |
| 'Gisela 5' | 9.4b | 25.7b | 17.2c | 15.3c | 0.8ab |
| 'Gisela 6' | 11.6a | 30.2a | 17.5bc | 15.5bc | 0.9a |
| 'MaxMa 14' | 10.2b | 28.9ab | 18.1b | 17.1ab | 0.8ab |
| 'Oblacinska cherry' | 9.5b | 25.9b | 19.2a | 18.1a | 0.7b |
| LSD | 1.2 | 1.4 | 0.7 | 1.1 | 0.1 |

Compared to the results of other authors (Hrotkó et al., 2009; Cantin et al., 2010; Rubauskis et al., 2014), higher values of SSC and TS in fruits of the 'Carmen' cultivar on all examined rootstocks were obtained. Higher values might be due to more favourable agro-ecological conditions for growing.

## Conclusion

Considering the importance of sweet cherries for fruit growers, determining the influence of vegetative rootstocks on biological and pomological properties of cultivars is very important. The results obtained in this study indicated that the 'Carmen' cultivar grafted on 'MaxMa 14' and 'Gisela 6' had the lowest percentage of damaged flower buds (less than 50%). Also, trees grafted on these rootstocks had a higher yield and better physical properties of fruits. According to recorded results, a growing of the 'Carmen' cultivar on 'MaxMa 14' and 'Gisela 6' rootstocks could be recommended to producers of sweet cherries in similar agro-ecological conditions. Also, to obtain more complete results of rootstock effects on cultivars, it is necessary to continue with further examinations.

## Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia and the Faculty of Agriculture, e.n. 451-03-68/2020-14/200116.

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Received: May 19, 2021
Accepted: November 10, 2021

# UTICAJ RAZLIČITIH PODLOGA NA OSETLJIVOST CVETNIH PUPOLJAKA NA MRAZ I NAJZNAČAJNIJE OSOBINE SORTE TREŠNJE KARMEN

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R e z i m e

U radu je ispitivan uticaj pet vegetativnih podloga na osetljivost cvetnih pupoljaka na mraz i proizvodna svojstava sorte trešnje Karmen. Istraživanje je sprovedeno u komercijalnom voćnjaku na OFD Radmilovac, Beograd. Sorta Karmen okalemljena je na sledeće vegetativne podloge: Colt, Gisela 5, Gisela 6, MaxMa 14 i Oblačinska višnja. Zimski mrazevi su se desili tokom ekološkog mirovanja stabala, u ranim jutarnjim časovima 14. i 15. marta. Intenzitet mraza bio je od -5°C do -7°C. Procenat oštećenih i neoštećenih cvetova na rodnim granama utvrđen je brojanjem (po 50 cvetnih pupoljaka po drvetu, sa svakog dela krošnje i rodne grane). Najveće oštećenje cvetova imala su stabla okalemljena na Oblačinskoj višnji, u proseku 77,2%, dok su najmanja oštećenja zabeležena na stablima koja su kalemljena na MaxMa 14, u proseku 24,3% od ukupnog broja cvetova. Znatno veći stepen oštećenja cvetnih pupoljaka utvrđen je na majskim buketićima u odnosu na mešovite grane. Plodovi sorte Karmen najranije su sazreli na podlogama Gizela 5 i Oblačinska višnja, dok su najpoznije sazrevali na stablima gajenim na podlozi MaxMa 14. Sorta Karmen imala je najveći prinos po stablu na podlozi MaxMa 14 (5,4 kg), dok je najmanji prinos zabeležen na podlozi Oblačinska višnja (1,9 kg). Visokom produktivnošću su se odlikovala i stabla kalemljena na podlozi Gizela 6. Najveću masu plodova imala su stabla na podlozi Gizela 6 (11,6 g), a najmanju na podlozi Gizela 5 (9,4 g).

**Ključne reči:** ekološko mirovanje, cvetni pupoljci, procenat oštećenja, prinos, kvalitet plodova.

Primljeno: 19. maja 2021.
Odobreno: 10. novembra 2021.

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Journal of Agricultural Sciences (Belgrade)
Vol. 66, No. 4, 2021
Pages 359-369

https://doi.org/10.2298/JAS2104359S
UDC: 639.3.043:597.552.56]:546.56
Original scientific paper

# EFFECTS OF COPPER MICROPARTICLES ON THE GROWTH AND SURVIVAL OF JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WAL.)

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**Abstract:** During the farming of juvenile rainbow trout (*Oncorhynchus mykiss*), losses can be very high. In aquaculture, various agents are used, such as copper, for the purpose of preventive action and prevention of the appearance of pathogens (ectoparasites, etc.). The aim of this study was to determine the effects of substrate-fixed copper microparticles on the growth and survival of juvenile rainbow trout (*Oncorhynchus mykiss*) up to the age of 5 months. The experiment was conducted in the Laboratory for Aquaculture of the Faculty of Agriculture, University of Banja Luka, for 142 days in flow aquariums with a 65 l/aquarium volume. The experiment was set up in two groups (150 units/group) with three replicates (50 units/replicate), a control group without copper microparticles (K) and a group with copper microparticles (Cu). The initial weight and total body length (mean ± SD) of rainbow trout in group K were 0.199 ± 0.008 g and 2.876 ± 0.036 cm, and in group Cu 0.197 ± 0.009 g and 2.893 ± 0.038 cm. The average total length and body weight of individuals from the K and Cu groups were similar ($p > 0.05$). The differences in FCR, CF, SGR and TGC between group K and group Cu were not statistically significant ($p > 0.05$). Survival of juvenile rainbow trout was high and it was 97.33% in group K and 96.00% in group Cu, and no statistically significant difference was found ($p > 0.05$). The growth and survival of juvenile rainbow trout in the K and Cu groups were similar.

**Key words:** microparticles, copper, growth, survival, juvenile, rainbow trout.

## Introduction

The breeding of the youngest age categories of rainbow trout (*Oncorhynchus mykiss*) deserves special attention, as it is the most sensitive stage of cultivation. Taking into account the size of the fry after swimming and the high stocking

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density per unit of production volume, it is necessary to pay special attention to maintaining good conditions of the breeding environment in order to provide optimal conditions for good growth, health and survival of juvenile rainbow trout, especially when it comes to the initial diet of trout, where a significant part of the high-protein feed used in the diet falls to the bottom, which is a good basis for the development of various organisms that can have a negative effect on the water quality and can also lead to diseases in farmed fish. Therefore, in aquaculture, in order to preserve the quality of the breeding environment and good health status of farmed fish, the prescribed technology of breeding different age categories and different assets are applied for the purpose of of preventive action and reducing the possibility of pathogens. Heavy metals are toxic and can be very harmful to organisms (Majzlik et al., 2011), but they are still used in a certain form and concentration in aquaculture. Copper is a significant additive in aquaculture for controlling the growth of algae and ectoparasites, and can also affect the microbiological degradation of organic matter, depending on its availability (Noga, 2010; Tom-Petersen et al., 2011). A number of studies have been conducted to investigate the effects of different forms of copper in water on the growth, survival and body organs of rainbow trout. Copper is used in various forms for specific purposes, e.g. copper sulphate is used to control the growth of algae in lakes and ponds, while copper nanoparticles have promising applications, but the availability and toxicity of copper in the form of nanoparticles are largely unknown (Malhotra et al., 2020). Ruparelia et al. (2008) state that the application of copper nanoparticles for use in antimicrobial materials is still being investigated, while Majzlik et al. (2011) state that copper nanoparticles show very good results as antibacterial and antimicrobial agents.

The effects of copper in water on cultivated rainbow trout depend on the form and concentration of copper in the water. The growth rate of rainbow trout larvae reacts sensitively to the chronic toxicity of copper ions, i.e. by increasing the content of copper ions in water, the growth of rainbow trout larvae decreases (Mickėnienė et al., 2007). Marr et al. (1996) state that exposure to copper concentrations in water of 4.6 ng $l^{-1}$ resulted in reduced growth of juvenile rainbow trout in the experimental group of about 4.5% compared to the control group, with individuals not recovering and achieving a growth rate of individuals from control groups during the exposure period, i.e. the duration of the experiment. Shaw et al. (2012) state that the mortality of juvenile rainbow trout at exposure to copper sulfate concentration in water of 100 µg/l on the fourth day was recorded at 85%, while at exposure to copper nanoparticles at 100 µg/l, mortality was significantly lower and was 14%.

In this study, copper microparticles fixed on a substrate placed on the bottom of the aquarium were used, with direct contact with the culture medium, feces and feed deposited on the bottom of the aquarium. The subject of this study was

juvenile rainbow trout from the moment of transition to exogenous feeding with compound feed until five months of age.

The aim of this study was to determine the effects of substrate-fixed copper microparticles on the growth and survival of juvenile rainbow trout (*Oncorhynchus mykiss*).

## Material and Methods

An experiment to examine the effects of copper microparticles on the growth and survival of juvenile rainbow trout (*Oncorhynchus mykiss*) was conducted in the Aquaculture Laboratory of the Faculty of Agriculture, University of Banja Luka. The experiment was carried out during 142 days in flow-through aquariums with an individual volume of 65 l. The experiment was set up in two groups with three replicates, a control group without copper microparticles (K) and a group with copper microparticles (Cu). At the beginning of the experiment, there were a total of 300 individuals (150 individuals/group, 50 individuals/replicate) of rainbow trout. In the group with copper microparticles (Cu), glass (55 x 30 cm) coated with araldit was placed on the bottom of the aquarium, which was used to fix copper microparticles, and the other group was the control (K), without copper microparticles. After coating the entire surface of the glass with araldit, 10 g of copper microparticles with an average size of 60 μm were evenly distributed over the coating. The OMAX A35140U microscope (China) was used to observe copper microparticles, and the size of copper microparticles was determined using the processing and measurement software. Substrates with copper microparticles were placed in three aquariums of the Cu group. The aquarium was cleaned of accumulated feed and feces once a week.

During the experiment, the control of body growth and body length was performed periodically (0, 35, 73, 113 and 142 days). One day before the measurement of body weight and body length, the individuals were not fed. All fish were measured during the checkups, and before the measurement, fish were anesthetized with the anesthetic 2-phenoxyethanol. The initial weight and total body length (mean ± SD) of juvenile rainbow trout in the control group (K) were 0.199 ± 0.008 g and 2.876 ± 0.036 cm, and in the group with copper microparticles (Cu) – 0.197 ± 0.009 g and 2.893 ± 0.038 cm. Mortality was registered continuously. During the experiment, the photoperiod was 8:16 (L:D).

Based on the obtained measurement results (body weight and length, water temperature), the feed conversion ratio (FCR) and growth characteristics were determined: condition factor (CF), specific growth rate (SGR), thermal unit growth coefficient (TGC), using the following formula:

$$FCR = F/G \quad (1)$$

FCR – feed conversion ratio; F – feed consumption (kg); G – weight gain (kg).

$$CF = (BW/L^3) \times 100 \quad (2)$$

CF – condition factor; BW – body weight (g); L – total body length (cm).

$$SGR = [(\ln FBW - \ln IBW)/D] \times 100 \quad (3)$$

SGR – specific growth rate; FBW – final body weight (g); IBW – initial body weight (g); ln – natural logarithm (e = 2.7183); D – number of days.

$$TGC = [FBW^{1/3} - IBW^{1/3}]/\Sigma[TxD] \times 100 \quad (4)$$

TGC – thermal unit growth coefficient; FBW – final body weight (g); IBW – initial body weight (g); T – water temperature (°C); D – days.

The following formula was used to determine the survival of juvenile rainbow trout (%):

$$Survival\ (\%) = (Nt/N0) \times 100 \quad (5)$$

Nt – number of fish at the end of the experiment (n); $N_0$ – number of fish at the beginning of the experiment (n).

Water temperature (°C) and dissolved oxygen content (mg/l) in water were measured with a digital oximeter WTW (Oxi 330i/SET 2B20 - 0011), and water pH was measured with a digital pH meter WTW (pH 330i/SET 2A20 - 1011). Body weight was measured with a Denver DL - 501 scale, load capacity 0.5 kg (accuracy 0.1 g), and body length was measured with an ichthyometer (accuracy 0.1 cm).

Rainbow trout were fed the same amount and type of feed in all treatments. As the age of juvenile rainbow trout increased, the size and composition of the feed used changed (Table 1).

Table 1. The size and composition of the feed used in the experiment.

| Feed composition | Feed size (mm) | | | |
|---|---|---|---|---|
| | 0.5 mm | 0.7 mm | 1.5 mm | 2.0 mm |
| Crude protein (%) | 55.0 | 55.0 | 52.0 | 48.00 |
| Crude fat (%) | 18.0 | 18.0 | 20.0 | 22.00 |
| NFE (%) | 8.0 | 8.0 | 10.5 | - |
| Ash (%) | 10.5 | 10.5 | 9.0 | 7.70 |
| Fiber (%) | 0.5 | 0.5 | 0.5 | 0.90 |
| Phosphorus (%) | 1.7 | 1.7 | 1.5 | 1.29 |
| Digestible energy (MJ/kg) | 19.7 | 19.7 | 20.2 | - |

The obtained data were processed statistically using the statistical program SPSS17 and MS Excel 2016.

## Results and discussion

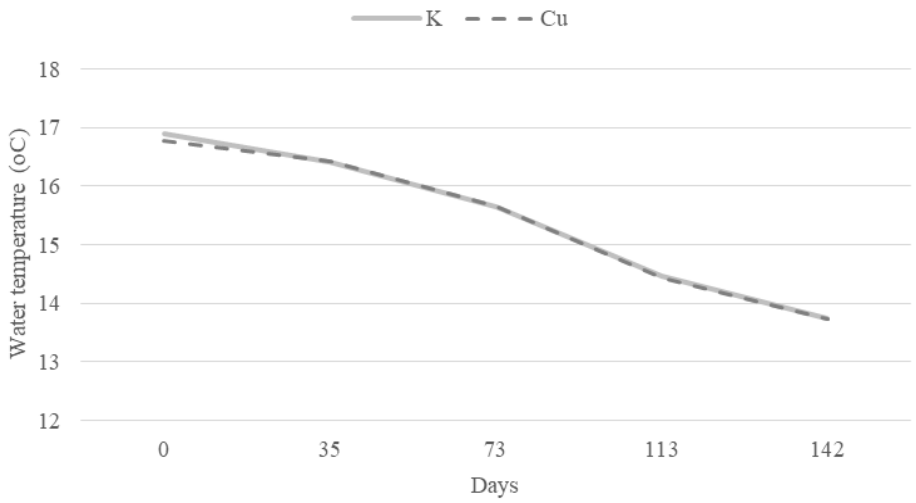
The basic parameters of water quality (Table 2) during the experiment in the observed groups K and Cu were similar.

Table 2. Average values (± SD) and CV of analyzed physical and chemical characteristics of water during the experiment.

| Parameter | K | | Cu | |
|---|---|---|---|---|
| | Mean ± SD | CV | Mean ± SD | CV |
| Water temperature (°C) | 15.11 ± 0.02 | 0.15 | 15.11 ± 0.01 | 0.07 |
| Dissolved oxygen, $O_2$ (mg/l) | 8.16 ± 0.08 | 1.01 | 8.15 ± 0.04 | 0.44 |
| Water saturation $O_2$ (%) | 82.17 ± 0.88 | 1.08 | 82.19 ± 0.49 | 0.59 |
| Water pH | 7.21 ± 0.05 | 0.66 | 7.20 ± 0.02 | 0.22 |

SD – standard deviation; CV – coefficient of variation.

The analysis using the t-test revealed no statistically significant differences ($p > 0.05$) of the observed water quality parameters between the groups. At the beginning of the experiment, the water temperature (Graph 1) was high for rainbow trout that had just swum and averaged 16.8 °C (Cu) and 16.9 °C (K), but there was no increased mortality.




Graph 1. Average water temperatures during the experiment.

From the beginning to the end of the experiment, there was a constant drop in water temperature, which, in the last period, averaged 13.7 °C in the Cu and K

groups. The water temperature was initially very high and was not in the optimal range for the swimming-up of fry rainbow trout, however, no negative effects of high temperature (growth, conversion, survival) were observed.

The average total length and body weight of individuals from the K and Cu groups (Table 3) during the experiment were similar, thus no statistically significant difference was found between the means of the average weight and total body length ($p > 0.05$).

Table 3. Average weight (g), total body length (cm) (± SD) and CV during the experiment.

| Days | Parameter | K | | Cu | |
|---|---|---|---|---|---|
| | | Mean ± SD | CV | Mean ± SD | CV |
| 0 | BW (g) | 0.199 ± 0.008 | 4.00 | 0.197 ± 0.009 | 4.38 |
| | TL (cm) | 2.876 ± 0.036 | 1.27 | 2.893 ± 0.038 | 1.30 |
| 35 | BW (g) | 0.91 ± 0.02 | 1.99 | 0.86 ± 0.03 | 3.30 |
| | TL (cm) | 4.54 ± 0.05 | 1.11 | 4.44 ± 0.06 | 1.27 |
| 73 | BW (g) | 3.97 ± 0.02 | 0.47 | 3.89 ± 0.08 | 2.17 |
| | TL (cm) | 7.32 ± 0.02 | 0.23 | 7.27 ± 0.04 | 0.53 |
| 113 | BW (g) | 10.30 ± 0.12 | 1.15 | 10.29 ± 0.43 | 4.15 |
| | TL (cm) | 9.65 ± 0.07 | 0.77 | 9.67 ± 0.12 | 1.29 |
| 142 | BW (g) | 22.17 ± 0.66 | 2.97 | 22.10 ± 1.20 | 5.44 |
| | TL (cm) | 12.39 ± 0.07 | 0.53 | 12.42 ± 0.14 | 1.14 |

SD – standard deviation; CV – coefficient of variation; BW – body weight; TL – total length.

The average body weights were similar in the K and Cu groups, but it can be seen that SD and the coefficient of variation in the Cu group increased with increasing body weight. FCR and growth characteristic parameters (± SD) by days are shown in Table 4.

The feed coefficient ratio was the lowest in the first 35 days, and then with the increase of weight, the FCR also increased, which is an indicator of good utilization of consumed feed, even with the determined increase. Condition factor (CF) tended to increase in individuals from both groups (K and Cu) from the beginning to the end of the experiment, while SGR had a declining trend (Table 4). SGR is known to decrease with an increasing body weight of fish (Bureau et al., 2000). During the first 35 days, SGR was the highest and was 4.36 ± 0.07 in group K and 4.21 ± 0.16 in group Cu. Uysal & Alpbas (2002) state that the SGR of rainbow trout during the first 30 days was 3.87%, at an average water temperature of 11.90 °C, and the faster growth found in our study can be attributed to the higher water temperature which averaged 16.42 °C during the first 35 days of the experiment in the K and Cu groups. The analysis of CF and the SGR t-test by controls did not reveal a statistically significant difference between the means ($p > 0.05$).

TGC from the beginning to the end of the experiment had a tendency to increase (Table 4) in individuals from both the control group (K) and the group treated with copper microparticles (Cu). The analysis of the TGC t-test by controls did not reveal a statistically significant difference in the mean ($p > 0.05$).

Table 4. Growth parameters of juvenile rainbow trout (mean ± SD) and CV during the experiment.

| Parameter | Days | K | | Cu | |
|---|---|---|---|---|---|
| | | Mean ± SD | CV | Mean ± SD | CV |
| CF | 0 | 0.84 ± 0.027 | 3.21 | 0.81 ± 0.006 | 0.79 |
| | 35 | 0.98 ± 0.034 | 3.46 | 0.98 ± 0.005 | 0.53 |
| | 73 | 1.01 ± 0.006 | 0.55 | 1.01 ± 0.025 | 2.43 |
| | 113 | 1.15 ± 0.030 | 2.60 | 1.14 ± 0.048 | 4.21 |
| | 142 | 1.17 ± 0.023 | 1.98 | 1.15 ± 0.027 | 2.32 |
| FCR | 35 | 0.48 ± 0.01 | 1.50 | 0.52 ± 0.02 | 3.25 |
| | 73 | 0.56 ± 0.01 | 1.64 | 0.57 ± 0.003 | 0.50 |
| | 113 | 0.62 ± 0.02 | 3.09 | 0.63 ± 0.04 | 6.95 |
| | 142 | 0.73 ± 0.02 | 2.75 | 0.68 ± 0.03 | 4.94 |
| SGR | 35 | 4.36 ± 0.07 | 1.70 | 4.21 ± 0.16 | 3.87 |
| | 73 | 3.97 ± 0.046 | 1.17 | 4.08 ± 0.043 | 1.05 |
| | 113 | 2.38 ± 0.040 | 1.70 | 2.43 ± 0.107 | 4.39 |
| | 142 | 2.55 ± 0.079 | 3.09 | 2.55 ± 0.046 | 1.80 |
| TGC | 35 | 0.067 ± 0.001 | 0.91 | 0.064 ± 0.002 | 3.78 |
| | 73 | 0.106 ± 0.001 | 1.11 | 0.107 ± 0.001 | 0.76 |
| | 113 | 0.102 ± 0.002 | 1.53 | 0.104 ± 0.005 | 5.10 |
| | 142 | 0.154 ± 0.006 | 3.61 | 0.153 ± 0.005 | 3.26 |

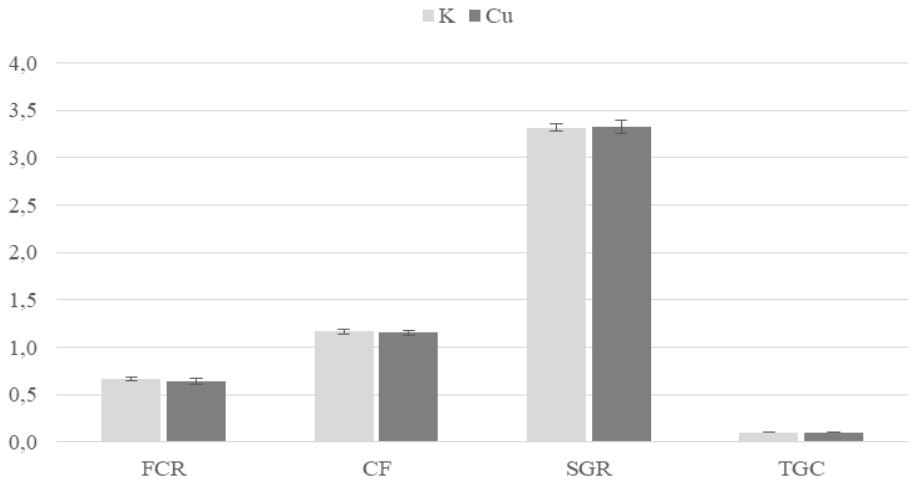
SD – standard deviation; CV – coefficient of variation.

It can be seen from Table 4 that the observed parameters of growth characteristics were similar in individuals from the K and Cu groups, which indicates that juvenile rainbow trout in treatment with copper microparticles had similar growth tendencies.

Graph 2 shows the analyzed parameters, total FCR, CF, SGR and TGC during the experiment.

The coefficient of feed conversion is one of the indicators of the success of fish farming and good breeding conditions. The analysis of the total FCR showed that it was slightly higher in group K (0.66 ± 0.021) in relation to the group Cu (0.64 ± 0.031), and the observed difference in mean was not statistically significant ($p > 0.05$; $t = 0.937$; $p = 0.402$). Shah et al. (2018) state that during the growing period of rainbow trout with an initial weight of 5 g to a final weight of 20 g grown for six months, at average water temperatures of 6 to 9 °C, the FCR was 1.9 and 3.1. Based on the total FCR, it can be seen that the feed conversion ratio in the Cu

group was slightly better, i.e. that the individuals used feed somewhat better in the treatment with copper microparticles (Cu).



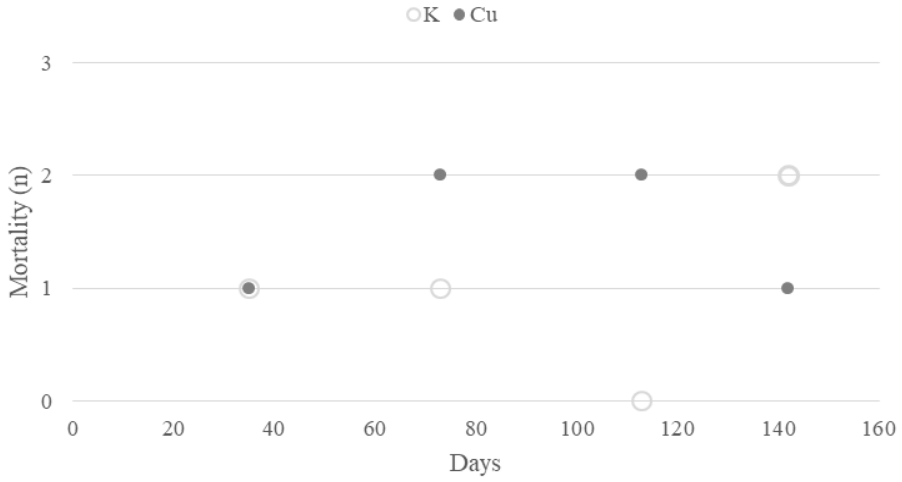

Graph 2. Total FCR, CF, SGR and TGC (± SD) juvenile rainbow trout in K and Cu groups.

During the breeding process, it is important to monitor the condition of the fish, which indicates the general condition and growth of the rainbow trout. The condition factor of rainbow trout in this experiment was slightly higher in the control group (K) and was 1.167 ± 0.03, while in the Cu group, it was 1.153 ± 0.03, and the difference in environments was not statistically significant ($t = 0.649$; $p = 0.552$). Dekić et al. (2016) state that the condition factor of fish is one of the ways to monitor the impact of the breeding environment on fish. The specific growth rate (SGR) of juvenile rainbow trout in the observed period was similar (K = 3.317 ± 0.04; Cu = 3.323 ± 0.07), and no statistically significant difference was found ($t = 0.145$; $p = 0.892$), which means that the individuals had similar growth during the observed period. The thermal unit growth coefficient (TGC) of juvenile rainbow trout was the same in both observed groups (K = 0.103 ± 0.001; Cu = 0.103 ± 0.003), and there was no statistically significant difference ($t = 0.189$; $p = 0.859$).

Observing the total values of CF, SGR and TGC, it is evident that there are slight differences (not statistically significant) between the individuals in the control (K) group and the group treated with copper microparticles (Cu). The observed indicators of the growth characteristics of juvenile rainbow trout indicate very good growth, condition and feed utilization.

Although water temperatures at the beginning of the experiment were high for rearing juvenile rainbow trout, which tended to decline steadily until the end of the

experiment, juvenile survival was high at 97.33% (K) and 96.00% (Cu). Larenas-Uría et al. (2016) report that, in the conducted experiment of growth and survival of fingerlings, using three different feeds during 8 weeks, survival was 98.5% in the control group and in the treated groups – 98.7%, 99.3% and 99.4%, which was higher compared to the results of this study. These results can be related to the duration of the study, which in our case was 20 weeks. Survival (n) of individuals in the control group averaged 48.67 ± 1.53 individuals, and in group Cu, the average survival of individuals was 48.00 ± 1.73 individuals, and no statistically significant difference ($t = 0.500$; $p = 0.643$) was found between the analyzed groups.




Graph 3. The mortality (n) of juvenile rainbow trout during the experiment.

Although the water temperature in the first half of the experiment was 17–15°C, the mortality of rainbow trout was not high (Graph 3). Mortality was similar in the examined groups (K and Cu), which means that there was no negative effect of copper microparticles on the increase in mortality.

## Conclusion

The analysis of the obtained results showed that the growth of mass and total body length, as well as FCR and the analyzed growth characteristics of CF, SGR and TGC of juvenile rainbow trout (*Oncorhynchus mykiss*) in the control group (K) and the group with copper microparticles (Cu) were similar, without statistically significant differences ($p > 0.05$). The survival of rainbow trout in the control group (K) and the group with copper microparticles (Cu) was high, i.e. mortality was low, without a statistically significant difference between the examined groups

($p > 0.05$). Based on the obtained results, it can be seen that the growth and survival of individuals in the K (without copper microparticles) and Cu (with copper microparticles) groups were similar, namely good growth and survival of juvenile rainbow trout in the K and Cu groups were achieved.

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Received: July 12, 2021
Accepted: November 16, 2021

# EFEKTI MIKROČESTICA BAKRA NA RAST I PREŽIVLJAVANJE JUVENILNE DUŽIČASTE PASTRMKE (*ONCORHYNCHUS MYKISS* WAL.)

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R e z i m e

Tokom uzgoja juvenilne dužičaste pastrmke (*Oncorhynchus mykiss*) gubici mogu biti veoma visoki. U akvakulturi se primjenjuju različita sredstva, kao što je bakar, radi preventivnog djelovanja i sprečavanja pojave uzročnika bolesti (ekto parazita i drugih). Cilj rada je bio da se utvrde efekti mikročestica bakra fiksiranih za podlogu na rast i preživljavanje juvenilne dužičaste pastrmke (*Oncorhynchus mykiss*) od proplivavanja do starosti od 5 mjeseci. Eksperiment je realizovan u Laboratoriji za akvakulturu Poljoprivrednog fakulteta Univerziteta u Banjoj Luci, tokom 142 dana u protočnim akvarijumima zapremine 65 l/akvarijum. Eksperiment je postavljen u dvije grupe (150 jedinki/grupi) sa tri ponavljanja (50 jedinki/ponavljanju), kontrolna grupa bez mikročestica bakra (K) i grupa sa mikročesticama bakra (Cu). Početna masa i totalna dužina tijela (prosjek±SD) dužičaste pastrmke u grupi K iznosila je 0,199 ± 0,008 g i 2,876 ± 0,036 cm, a u grupi Cu 0,197 ± 0,009 g i 2,893 ± 0,038 cm. Prosječna totalna dužina i masa tijela jedinki iz K i Cu grupe po kontrolama bile su slične ($p > 0.05$). Razlike između FCR, CF, SGR i TGC između grupe K i grupe Cu nisu bile statistički značajne ($p > 0.05$). Preživljavanje juvenilne dužičaste pastrmke je bilo visoko i iznosilo je u grupi K 97.33% i 96.00% u grupi Cu, te nije utvrđena statistički značajna razlika ($p > 0.05$). Rast i preživljavanje juvenilne dužičaste pastrmke u grupama K i Cu bili su slični.

**Ključne riječi:** mikročestice, bakar, rast, preživljavanje, juvenilna, dužičasta pastrmka.

Primljeno: 12. jula 2021.
Odobreno: 16. novemba 2021.

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Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2104371O
Vol. 66, No. 4, 2021 UDC: 36.52/.57.084.52(669)
Pages 371-382 Original scientific paper

# GROWTH RESPONSE, FLOCK UNIFORMITY, ORGAN WEIGHT, ABDOMINAL FAT, CARCASS YIELD AND HEPATIC HISTOLOGY OF FINISHER BROILER CHICKEN FED TOASTED PIGEON PEA SEED MEAL

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**Abstract:** Three hundred (300) 21d old (Arbor-acre) broiler chicks apportioned to five (5) dietary groups of sixty (60) birds each, further replicated six (6) times were fed graded levels of toasted pigeon pea seed meal (TPSM) to assess the performance, flock uniformity, organ weights, carcass yield and hepatic histology at the finisher phase. A maize-full-fat soybean meal diet served as the control diet (I). The TPSM was incorporated to replace full-fat soybean meal at 12.5%, 25.0%, 37.5% and 50.0% in diets II, III, IV and V, respectively. Toasting improved the protein content, ether extract, fibre content, metabolizable energy and reduced the anti-nutrients except for oxalate. The final weight, total weight change, average weight gain, feed conversion ratio, eviscerated weight, breast yield and thigh yield were significantly influenced ($P<0.05$), especially depressed at 50% replacement, unlike the average feed intake, mortality and flock uniformity ($P>0.05$). The kidney and abdominal fat were also influenced ($P<0.05$). There were varying levels of hepatic degeneration, which increased in intensity as the level of inclusion increased. They ranged from mild sinusoidal congestion and cellular infiltration to necrosis of the cells in the liver. Up to 37.5% TPSM toasted pigeon pea seed meal replacement for soybean meal supported optimum growth, breast and thigh yield, and mild to moderate hepatic disruptions.

**Key words:** broiler, *Cajanus cajan*, growth response, organ weight, carcass yield.

## Introduction

Pigeon pea is a drought-resistant, fast-growing (Bekele-Tessema, 2007), multi-purpose perennial legume shrub (Sarkar et al., 2018), adapted to tropical and sub-tropical climates of Africa, Asia and the Caribbean (Mallikarjuna et al., 2011). It is majorly planted for its seeds, especially in drought prone areas, for food

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security. Dried stems are a source of fuel in rural settings, and the leaves are a source of forage to ruminants. It is also used to reclaim unfertile lands because of its nitrogen-fixing potentials as green manure, a shade plant or a cover crop (Cook et al., 2005).

The *Cajanus cajan* seed has a low monetary value and is obtainable at Nigerian markets (Esonu, 2006). This is because of its despised food preference ascribed to lengthy cooking time, unlike alternative beans (Amaefule and Obioha, 2001) resulting in a decline in popularity, thus, on the brink of extinction in some areas. So, the alternative economic use has to be explored. The seed, according to Amaefule and Nwagbara (2004) and Esonu (2006), has crude protein ranging from 18% to 30%, while Ahmed et al. (2006) observed 7%–10% crude fiber, 61% nitrogen-free extract and an average of 1.85% ether extract. Amaefule et al. (2011) and Esonu (2006) showed that the seed is somewhat lower in methionine compared to lysine.

## Material and Methods

### The site and interval of the experiment

The feeding trial was carried out at the Broiler Unit, Ladoke Akintola University of Technology Farm, Ogbomosho, Nigeria, situated in the derived savannah zone between latitudes 8°15'N and longitude 4°16'E. The location has a mean annual temperature of about 26.20°C and a mean annual rainfall of about 1200mm, with the relative humidity ranging from 75% to 95%. The experiment lasted for four weeks (Ojedapo et al., 2009).

### The collection of test ingredients

Pigeon pea (*Cajanus cajan*) seeds were purchased from a local market. They were properly cleaned and toasted in a pan at $105^0$C for 7 minutes, and they were later hammer-milled to pass through a chick mesh at a reputable feedmill.

### Experimental birds and management

A total of three hundred (300) day-old unsexed Abor-acre broiler strain chicks were procured from a reputable hatchery. Standard management practices and routine vaccination were strictly observed. At the three-week starter phase, the chicks were placed on a commercial starter ration, after which the birds were apportioned into 5 dietary groups of 60 birds each and were further replicated six times, comprising 10 birds per replicate in a completely randomized design. Open system housing was used, and the replicate pen was $1m^2$ each.

Study diets

Five treatment diets were compounded. A maize-full-fat soybean meal diet served as the control diet (I). The milled toasted pigeon pea seed meal was incorporated to replace full-fat soybean meal at 12.5%, 25.0%, 37.5% and 50% in diets II, III, IV and V, respectively, as shown in Table 1. The feeds were isocaloric.

Table 1. The composition of experimental diets (%), 3–7 weeks.

| Parameter (%) | I (0.00%) | II (12.5%) | III (25%) | IV (37.5%) | V (50%) |
|---|---|---|---|---|---|
| Maize | 50.00 | 50.00 | 50.00 | 50.00 | 50.00 |
| Full-fat soybean meal | 27.00 | 23.62 | 20.25 | 16.87 | 13.50 |
| Pigeon pea | 0.00 | 3.38 | 6.75 | 10.13 | 13.50 |
| Fish meal (72%) | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Palm kernel cake | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Wheat offal | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Bone meal | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Limestone | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Lysine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated nutrient composition | | | | | |
| Metabolizable E (Kcal/kg) | 3110.4 | 3111.85 | 3113.85 | 3114.96 | 3116.21 |
| Crude protein | 20.20 | 19.69 | 19.19 | 18.53 | 18.18 |
| Ether extract | 7.94 | 8.30 | 8.75 | 9.18 | 9.34 |
| Crude fibre | 4.17 | 4.45 | 4.74 | 5.02 | 5.31 |
| Lysine | 1.33 | 1.22 | 1.14 | 1.04 | 0.93 |
| Methionine | 0.63 | 0.61 | 0.59 | 0.57 | 0.35 |

Data sets and analysis

Chemical and anti-nutrient composition:

Proximate composition and anti-nutrients were evaluated using the AOAC (2005) procedure while the metabolizable energy component was estimated by the formula ME (Kcal $kg^{-1}$) = 37 x % crude protein + 81.1x % ether extract + 35.0x % nitrogen free extract (Pauzenga, 1985). Trypsin inhibitors and tannin were evaluated by the procedure of Kakade et al. (1969) and Swain (1979), respectively. Total saponin and phytate were determined by the spectrophotometric method described by Hiai et al. (1989) and the procedure recommended by Maga (1983), respectively.

Growth performance:

Growth indices (feed consumed, weight change and feed conversion ratio) were monitored, and flock uniformity was calculated as described by Ojediran et al. (2017), including the average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR).

Organ weight and carcass cuts

At the termination of the experiment, 2 birds per replicate were picked, tagged, fasted overnight for 12 h and were humanely decapitated for organ and carcass examination. The internal organs were collected after evisceration prior to scalding, de-feathering and dissection for carcass cuts. Eviscerated organs and carcass cuts were measured by the use of an electronic weighing scale and were expressed in percentages relative to the live weight of the chicken.

Histological study

Samples of the liver fixed in pre-tagged bottles filled with 10% formalin for histopathological examination were transported to the laboratory. Microscopic slides were prepared by fixing portions of the liver in eosin-haematoxylin dye as described by Carson and Christa (2009). The histopathological observations were completed under light microscopes. Photomicrographs of the liver samples were snapped using Authtek Camera (L GmBh, Germany).

Experiment design and analysis

The birds were allocated to respective treatments in a completely randomized design. The various data taken were analyzed in a one-way analysis of variance using the SAS (2005) package while significant means were separated using Duncan's multiple range test.

## Results and Discussion

The chemical composition and anti-nutrients of raw and toasted *C. cajan* seed meal are shown in Table 2. The unprocessed seed had 92.77% dry matter, 21.70% crude protein, 32.74% ether extract, 5.07% crude fibre, 4.00% ash, 29.26% NFE and 4482.21 kcal/kg ME, while the toasted seed meal had 93.11% dry matter, 25.26% crude protein, 35.01% ether extract, 4.40% crude fiber, 3.10% ash, 25.28% NFE and 4663.60 kcal/kg ME. The concentration of anti-nutrients revealed that phytic acid, trypsin inhibitor, oxalate and tannin amounted to 20.02 mg/kg, 65.70 TIU/mg, 105.62 mg/kg and 28.50mg/kg in the raw pigeon pea seed meal, respectively, unlike 19.00 mg/kg, 38.25 TIU/mg, 194.10 mg/kg and 18.40 mg/kg, respectively, in the toasted seed meal.

Table 2. The chemical composition and anti-nutrient composition of raw and toasted pigeon pea seed meals.

| Parameter (%) | Raw | Toasted |
|---|---|---|
| Chemical composition | | |
| Dry matter | 92.77 | 93.11 |
| Crude protein | 21.70 | 25.26 |
| Ether extract | 32.74 | 35.07 |
| Crude fibre | 5.07 | 4.40 |
| Ash | 4.00 | 3.10 |
| Nitrogen free extract | 29.26 | 25.28 |
| Metabolizable energy (kcal/kg) | 4482.21 | 4663.60 |
| Anti-nutrients | | |
| Phytic acid (mg/kg) | 20.02 | 19.00 |
| Trypsin inhibitor (TIU/mg) | 65.70 | 38.25 |
| Oxalate (mg/kg) | 105.62 | 194.10 |
| Tannin (mg/kg) | 28.50 | 18.40 |

The growth response of finisher broilers offered graded levels of toasted pigeon pea seed meal is presented in Table 3. The final weight, total weight gain, average weight gain and FCR were significantly influenced ($P<0.05$), while average feed intake, mortality and flock uniformity ($P>0.05$) were not influenced by pigeon pea meal supplementation. A linear decrease in final weight and weight gains was observed as the toasted seed meal increased from diets I to V. Although birds fed diets I to IV were not different ($P>0.05$) from one another, they were different ($P<0.05$) from those feed diet V. However, a quadratic response ($P<0.05$) was observed for feed conversion ratio with chickens offered diet 2 having the least value (3.08) comparable to those fed diet I (3.28) and IV (3.39) while birds on diet IV had the highest value (4.17).

Table 3. The growth response and flock uniformity of finisher broiler chickens offered toasted pigeon pea seed meal.

| Parameter | I | II | III | IV | V | SEM | P-value |
|---|---|---|---|---|---|---|---|
| IW g/b | 426.77 | 435.00 | 435.00 | 436.77 | 425.00 | 2.37 | 0.430 |
| FW g/b | $1255.00^{a}$ | $1283.33^{a}$ | $1231.67^{a}$ | $1205.00^{a}$ | $1056.67^{b}$ | 24.19 | 0.003 |
| TWG g/b | $828.33^{a}$ | $843.33^{a}$ | $796.67^{a}$ | $768.33^{a}$ | $631.67^{b}$ | 23.27 | 0.003 |
| ADWG g/b/d | $29.58^{a}$ | $30.30^{a}$ | $28.45^{a}$ | $27.44^{a}$ | $22.56^{b}$ | 0.83 | 0.003 |
| ADFI g/b/d | 97.02 | 93.38 | 99.82 | 92.85 | 94.10 | 1.48 | 0.600 |
| FCR | $3.28^{bc}$ | $3.08^{c}$ | $3.53^{b}$ | $3.39^{bc}$ | $4.17^{a}$ | 0.11 | 0.001 |
| Mortality % | 3.33 | 10.00 | 3.33 | 10.00 | 0.00 | 1.92 | 0.398 |
| FU % | 82.62 | 89.32 | 88.80 | 87.06 | 85.72 | 1.06 | 0.320 |

abc = means with different superscripts in rows are significantly ($P<0.05$) different. IW = Initial weight; FW = Final weight; TWG = Total weight gain; AWG = Average weight gain; AFI = Average feed intake; FCR = Feed conversion ratio; FU = Flock uniformity.

Table 4 shows the carcass characteristics, organ weight, and abdominal fat weights of finisher broiler chickens fed toasted *C. cajanus* seed meal. The eviscerated weight, breast yield, thigh yield, kidney and abdominal fat were influenced (P<0.05) by the feeds, unlike the live weight, back, drumstick, liver, pancreas, proventriculus, heart, lungs, and empty gizzard (P>0.05). The eviscerated weight ranged from 75.98% to 80.75% in birds fed diets V to II, respectively. This trend is also noticeable in breast weight. The breast weight of birds fed diets I–IV were comparable (P>0.05), while those fed diet V had the least breast weight. The kidney of animals offered pigeon pea diets had higher weight compared to those fed diet I (P<0.05). Observation on the abdominal fat is quadratic (P<0.05): birds given diet II (2.06%) had a higher value, those offered diet III (1.58%) had the least while those fed other diets were comparable (P>0.05).

Table 4. Carcass characteristics, organ weight and abdominal fat of finisher broiler chickens fed toasted pigeon pea seed meal.

| Parameter (%) | I | II | III | IV | V | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Live weight (kg) | 1.73 | 1.85 | 1.77 | 1.80 | 1.60 | 0.39 | 0.30 |
| Eviscerated weight | 79.62$^{a}$ | 80.57$^{a}$ | 79.53$^{a}$ | 77.28$^{ab}$ | 75.98$^{b}$ | 0.59 | 0.04 |
| Back yield | 12.73 | 12.35 | 12.84 | 13.13 | 13.52 | 0.24 | 0.67 |
| Breast yield | 19.98$^{a}$ | 20.05$^{a}$ | 18.87$^{ab}$ | 17.91$^{ab}$ | 16.53$^{b}$ | 0.47 | 0.05 |
| Thigh yield | 11.45$^{b}$ | 13.02$^{a}$ | 13.14$^{a}$ | 12.82$^{ab}$ | 11.81$^{ab}$ | 0.24 | 0.04 |
| Drumstick yield | 9.77 | 10.46 | 10.41 | 10.06 | 10.21 | 0.31 | 0.47 |
| Liver | 2.15 | 2.20 | 2.22 | 2.63 | 2.74 | 0.11 | 0.34 |
| Kidney | 0.14$^{b}$ | 0.44$^{a}$ | 0.43$^{a}$ | 0.37$^{a}$ | 0.34$^{a}$ | 0.04 | 0.01 |
| Pancreas | 0.31 | 0.23 | 0.20 | 0.20 | 0.15 | 0.02 | 0.16 |
| Proventriculus | 0.49 | 0.38 | 0.36 | 0.49 | 0.40 | 0.03 | 0.41 |
| Heart | 0.42 | 0.47 | 0.42 | 0.41 | 0.42 | 0.01 | 0.81 |
| Lungs | 0.43 | 0.45 | 0.48 | 0.43 | 0.44 | 0.03 | 0.98 |
| Empty gizzard | 1.82 | 1.63 | 1.72 | 1.96 | 1.95 | 0.98 | 0.42 |
| Abdominal fat | 1.98$^{ab}$ | 2.06$^{a}$ | 1.58$^{b}$ | 1.78$^{ab}$ | 1.72$^{ab}$ | 0.07 | 0.03 |

a,b,c means with different superscripts in rows and columns are significantly (P>0.05) different.

The histological observation of the liver

Histological studies revealed some structural changes in the liver sections examined from the chickens fed pigeon pea seed meal. There were no hepato-histological alterations observed in the liver of the birds fed diet I. The arrangement of hepatocytes was normal (normal sinusoids NS, portal vein NPV and hepatocytes NH) and neat (Plate 1). The liver of birds fed diet II showed mild sinusoidal congestion (mSC) with mild diffuse necrosis (mN) of the hepatocytes (Plate 2). Moderate portal sinusoidal congestion (MSC), mild hepatic erosion (mHE) and mild necrosis (mN) of the hepatocytes were noticed in birds offered diet III (Plate 3).

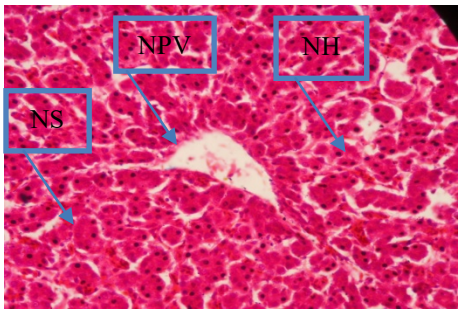


Plate 1. The micrograph of the liver of birds fed diet I (x40).

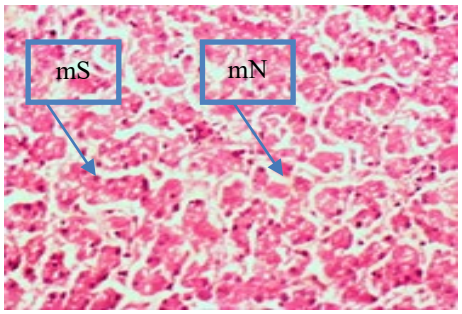


Plate 2. The micrograph showing the liver structure of birds fed diet II.

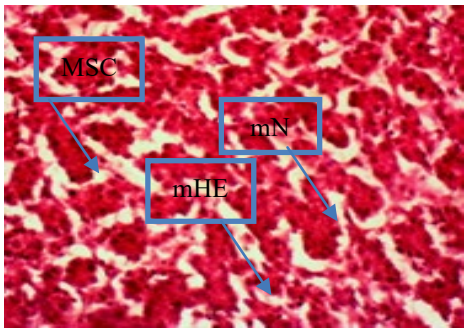


Plate 3. The micrograph showing the liver structure of birds fed diet III.

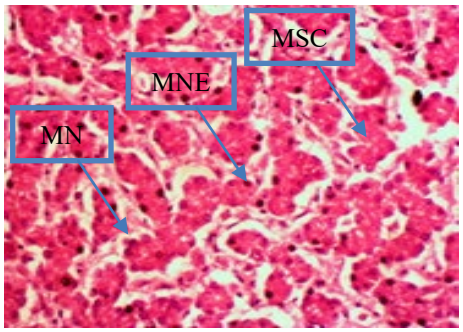


Plate 4. The micrograph showing the liver structure of birds fed diet IV.

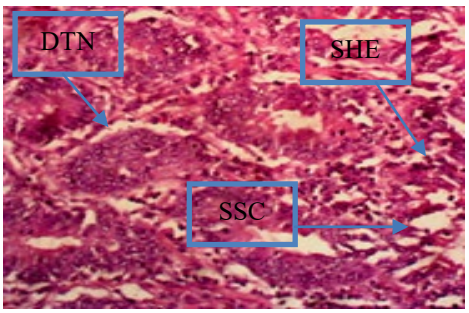


Plate 5. The micrograph showing the liver structure of birds fed diet V.

The histological changes in birds offered diet IV included moderate sinusoidal congestion (MSC), hepatic erosion (MHE) and necrosis (MN) (Plate 4). The group fed diet V (Plate 5) showed diffuse tubular necrosis (DTN) or tissue autolysis or

severe degeneration and destruction of hepatocyte architecture with severe sinusoidal congestion (SSC) and hepatic erosion (SHE).

The crude protein observed in this study fell within the range reported by Amaefule and Nwagbara (2004), and Esonu (2006), while the other proximate contents and metabolizable energy fell short. Ahmed et al. (2006) and Abioye et al. (2018) have reported that *Cajanus cajan* seed meal contains 21–28% CP, 4.57%–10% CF, 61.2–66.70% NFE, 1.7%–3.89% EE, 2.57–4.2% ash. Abioye et al. (2018) observed an improved CP, ash and NFE while the CF and EE reduced post-fermentation. However, Solomon et al. (2017) observed that toasted pigeon pea seed meal had higher crude protein and fibre content than the raw meal, which agrees with the report of this study. However, the author reported a non-significant EE as a result of toasting.

Processing of pigeon pea seed meal was reported to improve the growth performance of broiler chickens, unlike the unprocessed one. However, the processing method employed determined the level of residual anti-nutrients in the seed meal and subsequently the performance of the birds with higher effects in starter broilers than in finishers (Ani and Okeke, 2011; Igene et al., 2012). Oso et al. (2012) had earlier observed depressed performance in broilers fed pigeon pea seed meal, which corroborates the findings of this study at 50% replacement for soybean meal. The feed intake in this study was not influenced, similarly to the report of Tangtaweewipat and Elliot (1989), suggesting that palatability problems were not induced. However, the feed conversion ratio was degraded beyond 37.50% replacement for full-fat soybean in this study translating to 10.13% inclusion. Nevertheless, toasted pigeon pea was reported to support performance up to 27% in finisher broilers (Ani and Okeke, 2011). Amaefule et al. (2011) and Babiker et al. (2006) have observed that methionine addition to pigeon pea diet improves performance, indicating that amino acid balance could play an important role in a pigeon pea diet apart from the anti-nutrients.

Carcass output is a crucial factor in the poultry subsector. Carcass quality could affect consumer perception because of health advantages in low cholesterol-lean meat (Attia et al., 2001) which Iyayi et al. (2014) reported to be affected by feed crude protein content. Although, Furlan et al. (2004) had earlier concluded that nutritional composition, especially the class of amino acids, the monomeric unit of protein in the feed, was interconnected with the building of structural tissues. Arif et al. (2017) observed that feeding a 20% potash-boiled pigeon pea seed meal diet favoured carcass and breast weight, which is not similar to this study in which breast yield peaked at 12.50% compared to those fed 37.50%. Ojediran et al. (2016a) demonstrated that thigh weight was influenced by the lysine portion of the feed. According to Barbosa Filho et al. (2017), there is a mathematical connection in carcass yield: low breast yield translates to a higher

yield of other parts like shank, back and wings. This is similar to the report of Rance et al. (2002).

The kidney weight observed in this study was higher in birds fed toasted pigeon pea seed meal than those fed the control diet, which may be attributed to the test ingredients used. Holanda et al. (2009) had earlier confirmed that the rate of fat deposition rose by the terminal point of the rearing phase when broiler chickens were butchered at 42 days old. As a crucial organ in the body, the liver has an important role in detoxification. Birds fed the pigeon pea seed meal had varying levels of hepatic degeneration, which increased in intensity as the level of inclusion increased. It ranged from mild sinusoidal congestion and cellular infiltration to necrosis of the cell in the liver. Ojediran et al. (2016b) observed that broiler chicken fed *Jatropha curcas* kernel meal damaged liver due to residual anti-nutrients. The report of Emiola et al. (2007) on broilers fed raw and dehulled kidney bean meals indicated extensive coagulative necrosis, congestion of sinusoids and extensive degeneration of hepatocyte, whereas it was less marked in the liver of birds placed on a toasted-kidney bean-based diet. The findings of Safameher (2008) also showed changes in the liver and kidney of cockerel chicks fed raw full-fat neem seed. However, within this study, the hypertrophy of the liver was not observed, thus the liver was managing the situation which may result in a total breakdown in the long run. This does not agree with the findings of Akande et al. (2012) when broiler chickens were fed *Ricinus communis* because the liver was not enlarged in that study. Despite some level of detoxification attained with toasting, slight changes in hepatic structure are indicative of toxicity of residual anti-nutritional factors.

## Conclusion

This study showed that toasted *C. cajan* seed meal had a satisfactory protein content with reduced anti-nutrients. Up to 37.50% toasted pigeon pea seed meal replacement for soybean meal supported optimum growth, breast and thigh yield, and mild to moderate hepatic disruptions.

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Received: November 17, 2020
Accepted: November 3, 2021

# ODGOVOR PRIRASTA, UJEDNAČENOST JATA, MASA ORGANA, ABDOMINALNA MAST, KVALITET TRUPA I HISTOLOGIJA JETRE BROJLERSKIH PILIĆA HRANJENIH TOSTIRANIM KAJANOM (GOLUBIJI GRAŠAK) U ZAVRŠNOJ FAZI TOVA

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R e z i m e

Tri stotine (300) brojlerskih pilića (Arbor-acre) starih dvadeset i jedan dan raspoređenih u pet (5) hranidbenih grupa sa po šezdeset (60) jedinki, u šest (6) ponavljanja hranjene su različitim nivoima termički tretirane sačme kajana (TSK) kako bi se ocenio učinak, ujednačenost jata, masa organa, kvalitet trupa i histologija jetre u završnoj fazi tova. Smeša na bazi kukuruza i punomasnog sojinog griza poslužila je kao kontrolna smeša (I). TSK je supstituisala punomasni sojin griz u koncentracijama od 12,5%, 25,0%, 37,5% i 50,0% za smeše u II, III, IV odnosno V grupi. Tostiranje je imalo pozitivan efekat na proteine, BEM, sadržaj vlakana, metaboličku energiju i smanjenje antinutritivnih, materija osim oksalata. Završna masa, promena ukupne težine, prosečni prirast, masa, konverzija hrane, masa trupa, masa grudi i masa bataka imale su značajne razlike ($P<0,05$), posebno značajno manje pri 50% supstitucije u eksperimentalnim smešama, za razliku od prosečne konzumacije hrane, mortaliteta i ujednačenosti jata ($P>0,05$). Bilo je značajnog uticaja ($P<0,05$) i na bubrežnu i abdominalnu mast. Postojali su različiti nivoi degeneracije jetre, čiji se intenzitet povećavao kako se nivo uključivanja TSK povećavao. Kretali su se od blage sinusoidne kongestije i ćelijske infiltracije do nerkoze ćelija u jetri. Dodavanje TSK do 37,5% umesto punomasnog sojinog griza, omogućava optimalan porast, masu grudi i bataka, kao i blage do umerene poremećaje u radu jetre.

**Ključne reči:** brojler, *Cajanus cajan*, odgovor prirast, masa organa, masa trupa.

Primljeno: 17. novembra 2020.
Odobreno: 3. novembra 2021.

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**Thesis**
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**Report**
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

**Web site**
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

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All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

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Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim

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Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

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U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

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Primeri navođenja referenci su sledeći:

**Periodičan časopis**
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**Knjiga**
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

**Poglavlje u knjizi**
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

**Zbornik**
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

**Teza**
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

**Izveštaj**
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

**Veb sajt**
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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**JOURNAL of Agricultural Sciences** / editor-in-chief Snežana Oljača. - [Štampano izd.]. - Vol. 44, no. 1 (1999)- . - Belgrade : University of Belgrade, Faculty of Agriculture, 1999- (Belgrade-Zemun : Faculty of Agriculture). - 24 cm

Tromesečno. - Je nastavak: Review of Research work at the Faculty of Agriculture = ISSN 0354-3498. - Drugo izdanje na drugom medijumu: Journal of Agricultural Sciences (Belgrade. Online) = ISSN 2406-0968
ISSN 1450-8109 = Journal of Agricultural Sciences (Belgrade)
COBISS.SR-ID 169380871