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EFFECTS OF FERTILIZER TREATMENT ON THE POLYPHENOL CONTENT IN MAIZE AND VELVETLEAF COMPETITION

Ali F. Alynad[1], Nenad R. Trkulja[2], Sanja B. Đurović[2], Snežana M. Janković[3], Mohamed A. Elahmar[4], Layth Nesseef[5] and Danijela M. Šikuljak[2*]

[1]University of Tripoli, Faculty of Agriculture, Tripoli, Libya
[2]Institute for Plant Protection and Environment, Belgrade, Serbia
[3]Institute for Science Application in Agriculture, Belgrade, Serbia
[4]Biotechnology Research Center, Tripoli, Libya
[5]Faculty for Applied Ecology „Futura“, Belgrade, Serbia

Abstract: The aim of this research was to examine the effect of fertilizer treatment on the content of polyphenols in velvetleaf and maize, grown without use of herbicides under controlled conditions (12h/12h, T 25º/22ºC day/night). Plants were treated with organic - F1 and synthetic fertilizers - F2 and F3. The content of total phenols, individual polyphenolic acids and antioxidant activity of plant extracts were examined, as important indicators of the plant condition and its resistance to oxidative stress. The content of total phenols was determined spectrophotometrically (modified Folin-Ciocalteu method). The content of individual polyphenolic acids was determined by the high-performance liquid chromatography method, while measurement of polyphenols antioxidant activity was determined by the DPPH method. It was established that in maize, the application of fertilizers, neither in monoculture nor in competition with velvetleaf plants, caused statistically significant changes in the content of polyphenolic acids. However, in velvetleaf, the use of fertilizers led to an increase in the content of polyphenolic acids in conditions of competition vs monoculture. The content of chlorogenic, p-coumaric and trans-ferulic acids was statistically, significantly higher in plants of velvetleaf competition vs monoculture, and the content of chlorogenic, p-coumaric and cinnamic acids was lower in plants of maize competition vs monoculture. A statistically significant increase in the content of total polyphenols and their antioxidant activity in velvetleaf plants indicates the difficulty of removing weeds from crops, especially resistant populations. The research conducted and the results obtained confirm that weeds in competitive conditions manifest their natural competitive advantage, which is further enhanced by crop feeding.

Key words: polyphenols, velvetleaf, maize, competition.

*Corresponding author: e-mail: dulekaca@yahoo.com

Introduction

The effects of fertilizer application on cultivated plants have been the subject of numerous studies. There is a significantly smaller number of investigations and studies related to their effects on the environment and the changes they cause in weed plants. The fact is that fertilizers are a source of biogenic elements, primarily nitrogen, for cultivated plants, but also for weed plants. Plants absorb about 50% of the nitrogen from applied fertilizers, and the remaining part ends up in groundwater and the atmosphere (Erisman et al., 2015).

The absorbed nitrogen and other biogenic elements in plants participate in various biochemical processes. Polyphenols represent an important group of metabolites (Marchiosi et al., 2020). They are products of the secondary metabolism of plants and their role in plants is diverse: assimilation of nutrients, photosynthesis, enzyme activity, protein synthesis, photoreceptors, protection from UV radiation, etc. (Wu et al., 2001; Liu et al., 2004; Ikhajiagbe and Ogwu 2021). They are especially necessary for the biosynthesis of aromatic amino acids (Waksmundzka, 1998) and in stressful conditions (competition, herbicide application, drought, etc.). As non-enzymatic antioxidants, polyphenols prevent the oxidation of lipids, carbohydrates, proteins and DNA (Shahidi and Zhong, 2010). Their activity is manifested in several ways: (1) direct binding of free oxygen and nitrogen radicals and hydrogen atom donation, (2) activation of antioxidant enzymes, (3) chelation of metal ions, (4) inhibition of some enzymes, etc. (Lobo et al., 2010). From the aspect of agricultural practice, applied fertilizers through polyphenols can affect the effectiveness of herbicides.

Phosphorus from applied fertilizers is incorporated into a multienzyme complex (Achary et al., 2017) that regulates the "Shikimic acid cycle" (Winkel-Shirley, 1999), i.e., the transformation of L-phenylalanine and L-tyrosine into different groups of polyphenolic compounds (Rodriguez et al., 2015). In addition, fertilizers with lower nitrogen concentrations increase the resistance of the weed, *Setaria viridis* (6x) to a.m. nicosulfuron and *Amaranthus retroflexus* on a.m. nicosulfuron, glyphosate and mesotrione (Cathcart et al., 2009). Heavy metals originating from fertilizers do not only accumulate in the plants, but they are usually deposited in the soil. Therefore, the aim of this research was to examine the effects of fertilizers on the content of polyphenols in velvetleaf (*Abutilon teophrasti*) and maize (*Zea mays*) grown in conditions without the use of herbicides, as well as the accumulation of heavy metals in the environment.

Material and Methods

The test plants *Abutilon teophrasti* (velvetleaf) and *Zea mays* (maize) were grown under controlled conditions (photoperiod 12h/12h, T av. 25º/22ºC day/night). Six seeds of each species were placed in 1-L pots in two systems: monoculture

(velvetleaf or maize) and competition (maize and velvetleaf plants in the same pot). For each group of plants, the control is plants without fertilizer application. The plants were sown in soil certified for growing seedlings – Floragard TKS 1. The plants were treated with different fertilizers 25 days after germination. The effects of organic fertilizer – F1 (mixture of xydroxy acids: chlorogenic, caffeine and chichoric; 15 µl/100 ml of water), synthetic-organic – F2 (2% amino acids [alanine, isoleucine, histidine, etc.], 2% organic carbon, vitamin, 1.7% nitrogen and microelements [ferro, zinc, calcium, molybdenum, etc.]; 1.5 ml/100 ml of water) and synthetic F3 (nitrogen, phosphorus and potassium fertilizer; 1.5 ml/100 ml of water) were examined. The fertilizer was applied with a manual sprayer with a volume of 500 ml. The material samples for all analyses were taken 20 days after fertilizer application. The content of total polyphenols (plant material, soil) and the content of individual polyphenolic acids (plant material), their antioxidant activity (plant material) and the content of heavy metals in the soil were monitored.

The extraction of polyphenols from the plant material and the soil was carried out in 70% methanol in an ultrasonic bath (Asonic Pro 4P, 40 kHz) (2x for 30 minutes with a 15-minute break). For the analysis, 2.5 g of soil and 3 g of plant material crushed by liquid nitrogen in an oven were measured. The extracts were filtered through a 45-µm PTFE filter. The content of total phenols, individual polyphenolic acids and antioxidant activity were determined from the same extracts.

Determination of the total phenol content

The content of total phenols was determined spectrophotometrically according to the modified Folin-Ciocalteu method (Singleton et al., 1999). First, 50 µl of the plant extract was mixed with 250 µl of Folin's reagent (prepared in a 1:1 ratio with distilled water), 750 µl of 20% Na_2CO_3 and 3 ml of water and left at room temperature for 8 minutes. Then, 950 µl of water was added and incubated in the dark for 2 hours. The standard curve was defined by gallic acid in the concentration range 0–500 µg/ml. The content of total phenols was read spectrophotometrically at λ=765 nm (UV/Vis spectrophotometer UV-2100, Shimadzu, Japan), and their content was expressed as milligram equivalent of gallic acid per gram of dry matter (mgekvGK/g d.m.).

Determination of individual polyphenolic acid content

The content of individual polyphenolic acids was determined by high-performance liquid chromatography (HPLC) according to the method of Robbins (2003) with minor modifications. The elution mode was adapted to the length of the column used, Zorbax SB C18 4.6×250 mm, pore diameter of 5 µm, thermostated at 25°C. The Shimadzu Nexera-XR liquid chromatograph was used. The flow of the

mobile phase was set to 1 ml/min, and the amount of injected sample was 10 µl. The injection was performed automatically, using an autosampler. A mixed solution of chlorogenic, p-coumaric, ferulic, and trans-cinnamic acids at a concentration of 1 mg/ml was prepared in 70% methanol and dilutions were made in the mobile phase at a 1:1 ratio. Calibration curves of the polyphenolic acids were constructed in the range of 25–250 µg/ml. Polyphenolic acid content was monitored at two wavelengths, 280 nm (cinnamon) and 325 nm (chlorogenic acid, p-coumaric acid and ferulic acid). The concentration of each polyphenolic acid in the samples was calculated using the LabSolutions software (Shimadzu) and expressed in µg/g s.m.

Monitoring the antioxidant activity of polyphenols

The antioxidant activity of the plant extracts was determined by the DPPH method (Gil et al., 2002). The prepared extracts were diluted 10 times, 200 µl of the diluted extract was pipetted out and 3.8 ml of the 0.1 mM DPPH reagent prepared and was added immediately before the analysis. The samples with the reagent were incubated in the dark for 30 minutes. The antioxidant activity reading was performed on a UV-2100 spectrophotometer (Shimadzu, Japan) at λ=517 nm and expressed as µmol TE/g s.m. A standard curve was generated from a solution of TROLOX (T) in the concentration interval 0–1000 µmol/l. The stock solution of TROLOX with a concentration of 1 mmol/l was prepared in 70% methanol and dilutions of different concentrations (0, 50, 100, 250, 500 and 750 µmol/l) were prepared.

Statistical analysis

All the results obtained were compared by the analysis of variance (LSD test) for the comparison between control and the fertilizer-treated plants and the t-test for independent samples to compare a given parameter between different treatments. The statistical package “Stat 7” was used to analyze the obtained values.

Results and Discussion

Research showed that there were no statistically significant changes in the content of individual polyphenolic acids in maize grown in monoculture and in competition with velvetleaf plants compared to untreated plants. However, in velvetleaf, the use of fertilizers (F1, F2 and F3) resulted in an increase in the content of individual polyphenolic acids in competitive conditions compared to monoculture conditions (Table 1), which indicates the stimulating effect of the fertilizers applied on the weed plants. The measured increase in ferulic acid content in maize plants (Table 1) cannot be clearly attributed to the effect of organic fertilizer (F1) due to the fact that this polyphenolic acid is naturally present in maize tissue (Adom and Liu, 2002). On

the other hand, organic fertilizers are known to have a stimulating effect on plant growth through proteins in whose biosynthesis ferulic acid participates (He and Lin, 2001). All this leads to the conclusion that fertilizers enhance the natural feature of weeds – better competitiveness.

Table 1. Statistical analysis (LSD test) of the content of polyphenolic acids (μg/g fresh mass) in velvetleaf and maize leaves under competitive conditions after fertilizer application.

	velvetleaf competition				maize competition			
	chlorogenic	ferul	kumarn	cinnamon	chlorogenic	ferul	kumarn	cinnamon
K/F1	**	**	**	ns	ns	*	ns	ns
K/F2	**	**	**	ns	ns	ns	ns	ns
K/F3	**	**	**	ns	ns	ns	ns	ns
F1/F2	ns	ns	*	ns	ns	ns	ns	ns
F1/F3	ns	ns	ns	ns	ns	ns	ns	ns
F2/F3	ns	ns	ns	ns	ns	ns	ns	*
SD	6.62	9.75	7.18	12.97	6.626	1.121	1.712	3.350
mean	35.16	34.51	30.39	26.84	46.03	39.33	35.54	27.36

SD – standard error, K – control, ns – differences are not statistically significant, $p<0.05$*, $p<0.01$**, F1– organic, F2 – organic-synthetic and F3 – synthetic fertilizers.

The analysis of the obtained data revealed no difference in the effect between organic and synthetic fertilizers (except for the content of p-coumaric acid in velvetleaf in favor of F1, Table 1). In addition, a difference was found in the content of cinnamic acid in maize (Table 1) compared to synthetic fertilizers, although some studies showed a better effect of organic fertilizers (Stefanelli et al., 2010; Wendy et al., 2012; Ma et al., 2015). Hamouz et al. (2010) agree with this conclusion and explain that higher amounts of mineral fertilizers reduce the synthesis of polyphenols. The effect of fertilizers is mostly related to the amount of nitrogen present. Biesiada et al. (2010) believe that adding nitrogen reduces the content of polyphenolic acids in plants, and Ma et al. (2015) claim that reducing the amount of nitrogen reduces the amount of polyphenols in plant tissue. Similar changes in the content of polyphenols can be caused by fertilizers based on potassium and phosphorus, as well as sulfur. The study on the effect of sulfur fertilizers on the content of polyphenols in weeds (*Raphanus sativus*) showed a positive correlation of synthesis with an increase in the amount of sulfur (Zhou et al., 2013). Generally speaking, the uncontrolled application of fertilizers (especially nitrogen and phosphorus) poses numerous risks: accumulation of anion content in the soil (Kovacevic et al., 2010), salinization and alkalization of agricultural land, groundwater pollution, etc.

The working hypothesis was that fertilizers would affect the content of polyphenols in the cultivated plant due to the fact that maize plants belong to a group

of C4 plants that better absorb available nitrogen from the soil (Bonifas et al., 2005). However, the competitive conditions and fertilizer application stimulated a greater competitive potential of the weeds (velvetleaf) compared to the cultivated plants (maize). Lindquist and Mortensen (1999) additionally indicate that the competitiveness of velvetleaf can be explained by the higher leaf area index of velvetleaf plants. Although both species are equally capable of absorbing the available nitrogen from fertilizers, the larger leaf area of velvetleaf plants (Barker et al., 2006) enables better utilization of available sunlight and the creation of a greater biomass. The addition of nitrogen provides a higher growth rate of velvetleaf plants (from 46 to 82%) than of maize plants (from 29 to 45%) (Bonifas et al., 2005). Similar to our research, the effect of nitrogen fertilizers on other weed species was also observed. Nitrogen stimulates the germination of *Avena fatua* seeds (76.1% vs. control, 21.6%; Agenbag and Villers, 2006) and their competitive advantage over wheat plants (Pourreza and Bahrani, 2015). The comparison of the content of each polyphenolic acid (processed t-test, Table 2) showed that the values were statistically significantly higher in the plants of the velvetleaf competition compared to the monoculture (except cinnamon) and lower in the plants of the maize competition compared to the monoculture (except ferul).

Table 2. Content of polyphenolic acids (μg/g fresh mass) in leaves of velvetleaf and maize grown in different systems after application of different fertilizers (t-test).

	velvetleaf				maize			
	chlorogenic	ferul	kumarn	cinnamon	chlorogenic	ferul	kumarn	cinnamon
F1 monoculture vs. competition								
t	54.79	98.865	116.981	-2.615	-4.887	42.374	-12.468	-15.968
p	**	**	**	ns	**	**	**	**
F2 monoculture vs. competition								
t	41.148	10.484	122.24	-2.181	-8.709	86.901	-66.225	-22.639
p	**	**	**	ns	**	**	**	**
F3 monoculture vs. competition								
t	15.481	40.743	63.611	-4.523	-8.452	219.28	-255.04	-14.368
p	**	**	**	*	**	**	**	**

ns - differences are not statistically significant, $p<0.05$*, $p<0.01$**, t-test value, F1 – organic, F2 – synthetic-organic and F3 – synthetic fertilizers.

In general, in addition to the content of polyphenols, the degree of their antioxidant activity is also important for plants as a mechanism for overcoming stress conditions (application of herbicides, drought, moisture, competition, etc.). The antioxidant potential of polyphenols occurs in two ways: donation of H atoms and electrons and neutralization of free radicals (Ksouri et al., 2008) and prevents the oxidation of proteins, lipids and carbohydrates (Shahidi and Zhong, 2010). It is

considered that any disruption of the redox balance in the cells (exposure to free radicals) introduces the plant in a stateof oxidative stress (Vaya and Aviram, 2001). Free radicals trigger a chain reaction that destroys the cells by binding to the nearest stable molecules and taking over their electrons (Kaur and Kapoor, 2001). Therefore, plants/cells increase the synthesis of polyphenols (antioxidants) and antioxidant enzymes (Wu and Cederbaum, 2003). The measurement of the content of total polyphenols and their antioxidant activity was consistent with the changes in the content of individual polyphenolic acids compared to untreated plants in different growing systems (Tables 1, 3 and 4). The conditions of monoculture (conditions without stress) had no statistically significant effect on the change in the content of total polyphenols and their antioxidant activity in both tested species compared to the control, regardless of the application of fertilizers, (Table 3). Based on the results, it can be concluded that fertilizers (F2 and F3) influenced a slight increase in total polyphenols in the maize plants regardless of the level of significance (Table 3), which justifies the use of fertilizers.

Table 3. Statistical analysis and content of total polyphenols and antioxidant activity in leaves of velvetleaf and maize in monoculture after application of different fertilizers.

	velvetleaf				maize			
	UF (µg/g fresh mass)		AOA (µmol TE/g a.i)		UF (µg/g fresh mass)		AOA (µmol TE/g a.i)	
K/F1	1.33 1.20	ns	8.67 7.21	ns	0.51 0.69	ns	1.33 1.09	ns
K/F2	1.33 1.15	ns	8.67 7.76	ns	0.51 0.63	ns	1.33 1.43	ns
K/F3	1.33 1.44	ns	8.67 10.15	ns	0.51 0.82	ns	1.33 0.93	ns
F1/F2	1.20 1.15	ns	7.21 7.76	ns	0.69 0.63	ns	1.09 1.43	ns
F1/F3	1.20 1.44	ns	7.21 10.15	ns	0.69 0.82	ns	1.09 0.93	ns
F2/F3	1.15 1.44	ns	7.76 10.15	ns	0.63 0.82	ns	1.43 0.93	ns
SD	1.344		1.807		0.219		0.417	
mean	7.989		8.456		0.66		1.19	

SD – standard error, K – control, ns – differences not statistically significant, $p<0.05$*, $p<0.01$**, t-test value, UF – total polyphenols, AOA – antioxidant activity, F1 – organic, F2 – synthetic-organic and F3 – synthetic fertilizers.

On the other hand, the effect of the synthetic fertilizer F3 on the velvetleaf weeds points to the risk of using fertilizers and the effectiveness of herbicides in crops where

there are resistant weed populations. However, the growing conditions in the competition clearly show the level of risk of fertilizer application. A statistically significant increase in the content of total polyphenols and their antioxidant activity in the velvetleaf plants indicates the difficulty of removing the weeds from the crops, especially resistant populations (Table 4). This fact is confirmed by the obtained content analysis in the cultivated maize plant (Table 4). The competition between crops and weedsis stressful and risky enough to achieve good yields. However, the expected effect of fertilizers on increasing the competitive advantage of the cultivated plant was significantly reduced based on the obtained results. The application of fertilizers statistically significantly reduced the content of total polyphenols and their antioxidant activity, in contrast to the effects on the weed species (Table 4). Some studies with other weed species partially support the results obtained in the studies conducted. In the case of the weed species *Vicia faba*, a lower content of polyphenols was found after the application of mineral fertilizers and increased after the combined application of mineral and organic fertilizers compared to the control (Cucci et al., 2019). There are also studies showing that *Helianthus tuberosus* plants without nitrogen application contain more chlorogenic acid and its antioxidant activity (Amarowicz et al., 2020).

Table 4. Statistical analysis and content of total polyphenols and antioxidant activity in leaves of velvetleaf and maize in competitive conditions after application of different fertilizers.

	velvetleaf				maize			
	UF (µg/g fresh mass)		AOA (µmol TE/g a.i)		UF (µg/g fresh mass)		AOA (µmol TE/g a.i)	
K/F1	0.59 0.92	0.001 **	2.12 5.63	0.000 **	1.24 0.95	0.008**	8.71 6.23	0.001**
K/F2	0.59 0.73	0.023 *	2.12 5.02	0.000 **	1.24 0.84	0.001**	8.71 4.76	0.000**
K/F3	0.59 1.38	0.000 **	2.12 9.29	0.000 **	1.24 1.20	ns	8.71 7.03	0.011*
F1/F2	0.92 0.73	0.004**	5.63 5.02	0.039*	0.95 0.84	ns	6.23 4.76	0.019*
F1/F3	0.92 1.38	0.000**	5.63 9.29	0.000**	0.95 1.20	0.018*	6.23 7.03	ns
F2/F3	0.73 1.38	0.000**	5.02 9.29	0.000**	0.84 1.20	0.000**	4.76 7.03	0.002**
SD	2.23		2.679		0.197		1.581	
mean	5.442		5.517		1.06		6.68	

SD – standard error, K – control, ns – differences not statistically significant, $p<0.05$*, $p<0.01$**, t-test value, UF – total polyphenols, AOA – antioxidant activity, F1– organic fertilizer, F2 and F3 – synthetic fertilizers.

The effect of the applied fertilizers on the content of total polyphenols in the rhizosphere of the roots of both cultivated species was also observed as part of the study. Under competitive conditions, the plants interact with each other through allelochemicals. Polyphenols as secondary metabolites also affect the level of competition through the soil. Studies on the allelopathic effects of maize chemicals on weed populations have shown that vanillic, ferulic, cinnamic and caffeic acids are responsible for competition (Jabran, 2017). During the research conducted in the rhizosphere of maize and velvetleaf under competitive conditions, only cinnamic polyphenolic acid was detected (out of 4 tested), regardless of the type of applied fertilizers (Figure 1). Based on the conducted research, it can be concluded that the examined species did not exhibit allelopathic action via polyphenols through the soil. However, the research conducted by Balah and Nassar (2011) showed that the water extract of velvetleaf inhibited maize seed germination by 44.4–74.0%, the growth of the root system by 70.51–80.76% and shoot by 53.60–75.94%. The results obtained by Nádasy et al. (2018) agree with these results.

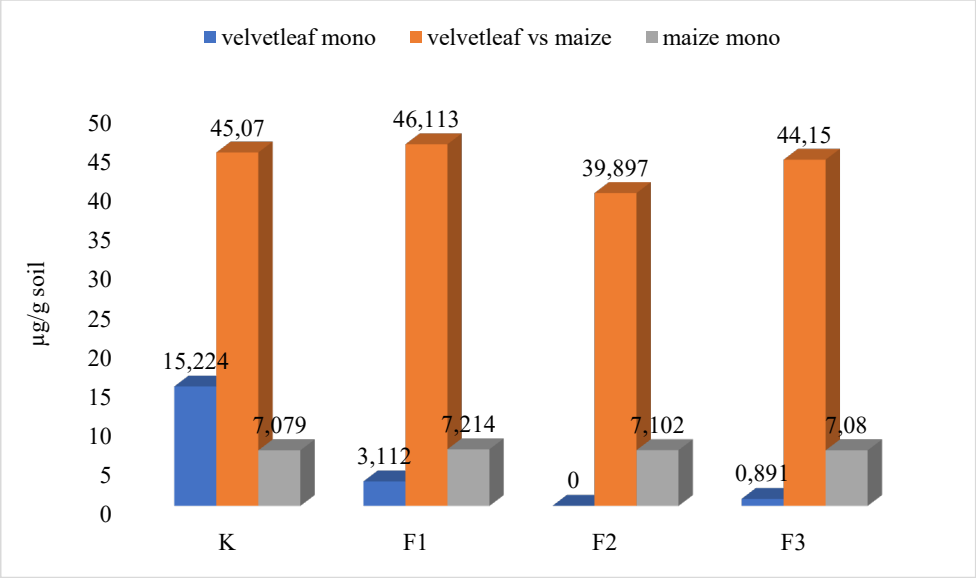

Figure 1. The content of cinnamic acid in the plots where maize and velvetleaf were grown in different cultivation systems.

Conclusion

In maize, the application of organic and synthetic fertilizers, neither in monoculture nor in competition with *Abutilon theophrasti* plants, caused statistically significant changes in the content of polyphenolic acids. However, in velvetleaf, the use of fertilizers resulted in an increase in the content of polyphenolic acids in competitive conditions compared to monoculture. The analysis of the obtained data did not reveal any difference in the effect between organic and synthetic fertilizers, except for the content of p-coumaric acid in velvetleaf and the content of cinnamic acid in maize when synthetic fertilizers were used. The content of individual polyphenolic acids was statistically significantly higher in velvetleaf plants grown with maize compared to plants grown in monoculture (except cinnamon) and lower in maize plants grown with velvetleaf compared with plants grown in monoculture (except ferul). Regardless of the level of significance, fertilizers influenced a slight increase in total polyphenols in maize plants, which justifies the use, but on the other hand, the effect of the synthetic fertilizer F3 on the velvetleaf weeds points to the risk of its use. A statistically significant increase in the content of total polyphenols and their antioxidant activity in velvetleaf plants indicates the difficulty of removing weeds from crops, especially resistant populations.

The research conducted and the results obtained confirm that weeds (velvetleaf) exhibit their natural competitive advantage in competitive conditions, which is further enhanced by crop feeding.

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EFEKAT PRIMENE ĐUBRIVA NA SADRŽAJ POLIFENOLA U KUKURUZU I ABUTILONU U USLOVIMA KOMPETICIJE

Ali F. Alynad[1], Nenad R. Trkulja[2], Sanja B. Đurović[2], Snežana M. Janković[3], Mohamed A. Elahmar[4], Layth Nesseef[5] i Danijela M. Šikuljak[2*]

[1]Univerzitet u Tripoliju, Poljoprivredni fakultet, Tripoli, Libija
[2]Institut za zaštitu bilja i životnu sredinu, Beograd, Srbija
[3]Institut za primenu nauke u poljoprivredi, Beograd, Srbija
[4]Centar za biotehnološka istraživanja, Tripoli, Libija
[5]Fakultet za primenjenu ekologiju „Futura“, Beograd, Srbija

R e z i m e

Cilj istraživanja bio je ispitivanje uticaja tretmana đubrivom na sadržaj polifenola u korovu abutilon i kukuruzu, gajenim bez primene herbicida u kontrolisanim uslovima ($12^h/12^h$, T 25º/22ºC dan/noć). Biljke su tretirane organskim – F1 i sintetičkim đubrivima – F2 i F3. Ispitan je sadržaj ukupnih fenola, pojedinačnih polifenolnih kiselina i antioksidativna aktivnost biljnih ekstrakata, kao važnih pokazatelja stanja biljke i njene otpornosti na oksidativni stres. Sadržaj ukupnih fenola je određivan spektrofotometrijski (modifikovana metoda Folin-Ciocalteu). Sadržaj pojedinačnih polifenolnih kiselina je određivan metodom tečne hromatografije visokih performansi, a antioksidativna aktivnost polifenola DPPH metodom. Ustanovljeno je da kod kukuruza, primena organskih i sintetičkih đubriva, ni u monokulturi ni u kompeticiji sa biljkama abutilona, nije uslovila statistički značajne promene sadržaja polifenolnih kiselina. Međutim kod abutilona, upotreba đubriva uslovila je porast sadržaja polifenolnih kiselina u uslovima kompeticije u poređenju sa monokulturom. Sadržaj hlorogene, p-kumarne i trans-ferulne kiseline bio je statistički značajno veći u biljkama abutilona gajenim sa biljkama kukuruza u poređenju sa biljkama gajenim u monokulturi, a sadržaj hlorogene, p-kumarne i cimetne kiseline manji u biljkama kukuruza gajenim u kompeticiji sa biljkama abutilona u poređenju sa biljkama gajenim u monokulturi. Statistički značajno povećanje sadržaja ukupnih polifenola i njihove antioksidativne aktivnosti u biljkama abutilona ukazuje na poteškoće uklanjanja korova iz useva, posebno rezistentnih populacija. Izvedena istraživanja i dobijeni rezultat potvrđuju da korovi u uslovima kompeticije ispoljavaju svoju prirodnu kompetetivnu prednost, koja se dodatno pojačava prihranom useva.

Ključne reči: polifenoli, abutilon, kukuruz, kompeticija.

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*Corresponding author: e-mail: dulekaca@yahoo.com

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EFFECT OF SAWDUST MULCH ON SOIL PROPERTIES AND PERFORMANCE OF TOMATOES (*LYCOPERSICUM ESCULENTUM*) IN AN ALFISOL IN SOUTHWESTERN NIGERIA

Abiodun Femi Olusola[1*] and Isola John Oluwasina[2]

[1]Forestry Research Institute of Nigeria, Ibadan
[2]Federal College of Forestry, Ibadan

Abstract: Mulching is an effective method of manipulating crop growing environment to increase yield. A field experiment was carried out at the Teaching and Research Farm of the Federal College of Forestry Jericho (Ibadan). The study aimed to examine the effect of sawdust mulching on selected soil properties, growth and yield of two tomato varieties. The study was a 4 x 2 factorial experiment laid out in a randomized completely block design (RCBD) with four replications. Factor one consisted of four treatments, namely: T0 (control), T1 (sawdust mulch [SDM] at 10 t ha^{-1}), T2 (SDM at 20 t ha^{-1}) and T3 (SDM at 30 t ha^{1}). The second factor was the two tomato varieties (UC82B and Ibadan local). Plant height, stem girth, number of leaves and branches were collected at a 2-week interval while the number of fruits, fruit weight (kg/ha) and yield parameters were measured at maturity. The Genstat statistical software package was used for data analysis and an LSD test was performed at the 5% level of significance. The sawdust mulch used had a low nitrogen content (0.60%) and a moderate organic carbon content (38.6%). The use of sawdust mulch had a major impact on tomato yield, while there was not any significant variation among the examined tomato varieties. UC82B (117.92 kg ha^{-1}) at 30 t ha^{-1} SDM plot had the highest yield, followed by Ibadan local (103.93 kg ha^{-1}) at 30 t ha^{-1} SDM plot, while Ibadan local (61.94 kg ha^{-1}) at the control plot had the lowest yield. In this study, the tomato performed best with sawdust mulch at 30 t ha^{-1} and is therefore recommended to farmers in the study area to maximize tomato production.

Key words: tomato, sawdust, mulching, soil.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family *Solanaceae* and is a common and important fruity vegetable. It is rich in vitamins A and B as well as iron. This vegetable is very popular among the various tribes in Nigeria as

*Corresponding author: e-mail: feminikan@gmail.com

an ingredient in salads and sauces/soups (Ploey and Heuvelink, 2005). Tomato is grown all over Nigeria, with an average yield of 15–20 t ha^{-1} recorded in the forest ecological zone of the south and up to 60 t ha^{-1} in the savanna ecozone of the north. Tomato is grown during the period when rainfall is scarce, and the soil moisture (250 mm) is exhausted by evapotranspiration. Water directly affects the fruit production in tomato plants (Santana and Vieira, 2010), which makes the use of irrigation indispensable for cultivation. The success of tomato cultivation largely depends on the adequate application of manures and fertilizers, efficient use of available soil moisture, plant spacing, timing of planting and weed control (Kayum et al*.,* 2008). To achieve a high-quality yield, the tomato requires high soil moisture during the entire growing (vegetation) period and moderate humidity, which is associated with the tomato's high transpiration coefficient. Various factors affect the quality and yield of tomatoes, including inadequate use of available moisture and nutrients, and unfavorable temperature (Brault et al., 2002). Mulching is an effective method of manipulating crop growing environment to increase yield and improve product quality by controlling weed growth, ameliorating soil temperature, conserving soil moisture, reducing soil erosion, improving soil structure and enhancing organic matter content (Hochmuth et al., 2001). Mulching can also protect soils from wind and water, erosion and compaction. Mulching of soil surface can limit evaporation and erosion, improve the available water capacity of the soil, regulate soil temperature, suppress the weed development and improve soil fertility (Clare et al., 2015). Sawdust mulch could increase the soil oxygen diffusion rate, maintain a more uniform soil temperature, reduce the surface crusting and soil bulk density, and increase the aeration porosity and soil moisture (Tran, 2005; Khan et al., 2000). Nigeria produces several million tons of sawdust annually, which is basically waste from the timber industry and pollutes the environment. While most of it is incinerated, an increasing amount is used as mulch by horticulturalists, small fruit growers, and nurseries. It is also increasingly used as litter in barns and feed lots and can become a valuable commodity which can be considered for agricultural utilization in soil conservation. The use of sawdust on a trial and error basis usually leads to good results. Like other highly carbonaceous organic matter, certain woods or barks contain tannins and other extractives that may have more or less toxic effects on plants and soil microorganisms. This study is therefore aimed at determining the effect of sawdust mulch on the soil properties for growth and yield of two tomato varieties.

Material and Methods

The study was carried out at the Crop Section of the Teaching and Research Farm of the Federal College of Forestry, Ibadan, Oyo state. The climatic conditions in the region are tropical with rainfall ranging from 1300 mm to1600 mm. The

average temperature is about 28.9°C, the average relative humidity is 80–85% and the ecological climatic conditions of the region have two distinct seasons: dry season from November to March and rainy season from April to October (FRIN, 2019). The initial soil sample was taken at 0–15 cm, air-dried and sieved with a 2-mm sieve before laboratory analysis was carried out to determine the physical and chemical properties of the soil. The particle size analysis was carried out using the Bouyoucos hydrometer method (Bouyoucos, 1965. The soil bulk density was determined using the core method as described by Blake and Hartage (1986). The soil pH was determined with the pH meter using a glass electrode in a 1:1 soil to water ratio (Udo and Ogunwale, 1986). The organic carbon of the soil was determined using the Walkley Black wet oxidation method (Udo and Ogunwale, 1986). The determination of total nitrogen was done as described by Kjeldahl (1883). The available P was determined with a spectrophotometer using the Mehlich method (Mehlich, 1984). The determination of exchangeable cations (K, Ca, Na and Mg) was done after extraction with ammonium acetate, whereby K and Na were quantified using a flame photometer, Ca and Mg were analyzed with an atomic absorption spectrophotometer. Extractable micronutrients (Fe, Mn, Cu and Zn) were determined after extraction of the soil with 0.1N HCl solution and analyzed with an atomic absorption spectrophotometer. A subsample of one (1) g of sawdust was taken for analysis of total nitrogen, phosphorus content and cations – potassium (K), sodium (Na), magnesium (Mg) and calcium (Ca), using the procedures generally used for plant samples (Tran, 2005). The experimental field was cleared, plowed and leveled manually with a hoe and a cutlass. The entire field was partitioned into 32 experimental plots, each covering a total land area of 36.0 m^2. The study was a 4 x 2 factorial experiment laid out in a randomized completely block design (RCBD) with four replications. Factor one consisted of four treatments, namely: T0 (control), T1 (sawdust mulch at 10 t ha^{-1}), T2 (sawdust mulch at 20 t ha^{-1}) and T3 (sawdust mulch at 30 t ha^{-1}). The second factor included the two tomato varieties (UC82B and Ibadan local) obtained from the National Horticultural Research Institute (NIHORT). The seeds were sown in germination trays containing sieved top soil, while the germinated seedlings were transplanted two weeks after sowing. Watering was done only in the morning, while weeding was carried out regularly to minimize the nutrient uptake by weeds from the soil. The data collected included: plant height, stem girth, number of leaves, branches at a 2-week interval between the 2^{nd} and the 10^{th} week after transplanting, while the number of fruits per plant, fruit weight per plant (kg ha^{-1}) and yield parameters were measured at maturity. The mulched soil was taken to the laboratory to determine the physical and chemical properties of the soil after harvest. The collected data were statistically analyzed using the Genstat statistical software package and significant means were separated using the least significant difference (LSD) at a 5% level of significance.

Results and Discussion

The analysis of the chemical and physical properties of the pre-experimental soil showed that the particle size distribution of the soil was that of a sandy loam. The soil pH (6.07) was slightly acidic, total nitrogen (1.71 g kg^{-1}) and available phosphorus (12.44 mg kg^{-1}) were moderate, which were within the critical range for total nitrogen (1.6–2.0 g kg^{-1}) and available phosphorus (7–20 mg kg^{-1}), respectively (Adeoye and Agboola, 1985). The organic carbon content was 15.51 g kg^{-1} which was above the critical level of 10–14 g kg^{-1} (Adeoye and Agboola, 1985). This implies that the soil was suitable for tomato production. The chemical analysis of the sawdust used in the experiment showed that the nutrient concentration of the sawdust had a low nitrogen content (0.60%) and a moderate organic carbon content (38.6%) (Table 1).

Table 1. Physical and chemical properties of the soil and sawdust used before planting.

Soil parameters	Content in soil	Sawdust (%)
pH (H_2O 1:1)	6.07	6.30
Organic carbon (g kg^{-1})	15.5	38.6
Organic matter (g kg^{-1})	26.72	
Total nitrogen (g kg^{-1})	1.71g	0.60
Available phosphorus (mg kg^{-1})	12.44	0.150
Exchangeable cations (cmol kg^{-1})		
Na	0.40	0.130
K	0.60	0.210
Mg	0.66	0.230
Ca	3.97	1.050
Extractable micronutrients (mg kg^{-1})		
Mn	88.0	158.60
Fe	103.0	174.00
Cu	1.14	22.39
Zn	1.17	11.80
Particle size distribution g kg^{-1}		
Sand	80.0	
Silt	14.0	
Clay	6.0	
Textural class	Sandy loam	

The effect of sawdust mulch on plant height (cm) showed that there was a significant difference in the plant height from the 2^{nd} to the 10^{th} week after transplanting (WAT) as influenced by sawdust across the tomato varieties used while at 10 WAT. UC82B at 30 t ha^{-1} SDM (138.2 cm) had the highest plant height and the lowest value was recorded by Ibadan local variety at the control plot (86.3 cm) (Table 2). This showed that sawdust mulch enhanced the growth of tomato

plants in this study and this is in line with the report of Awodoyin and Ogunyemi (2005) that mulched plants showed growth superiority over unmulched plants due to the change in soil profile and reduced nutrient uptake by weeds. There was no significant difference between the tomato varieties and the interaction of sawdust and tomato varieties at the plant height from 2 to 10 WAT (Table 2).

Table 2. The effect of mulching materials on plant height (cm) of UC82B and Ibadan local plants from the 2nd to the 10th week after transplanting.

Mulching materials	Varieties	2WAT	4WAT	6WAT	8WAT	10WAT
T0	Ibd local	17.87	33.43	59.3	78.9	86.3
	UC82B	20.77	29.53	47.5	81.1	88.9
T1	Ibd local	20.63	39.10	77.3	91.6	110.9
	UC82B	26.17	41.47	83.2	114.9	130.5
T2	Ibd local	23.57	47.00	93.1	102.8	125.3
	UC82B	24.50	44.77	89.0	125.5	131.4
T3	Ibd local	23.8	44.47	88.8	124.8	136.0
	UC82B	25.90	47.10	93.4	123.7	138.2
LSD MM		**	**	**	**	**
LSD VAR		Ns	Ns	Ns	Ns	ns
LSD.MM× VAR		Ns	Ns	Ns	Ns	ns

The effect of mulching materials on the number of leaves of UC82B and Ibadan local from the 2nd to the 10th week after transplanting is presented in Table 3. The result showed that there was no significant difference in the tomato varieties used at the 2nd and the 10th week after transplanting, but there was a significant difference in the effect of sawdust used at 4, 8 and 10 WAT. Likewise, there was a significance difference in the interaction between mulching materials and tomato varieties at 4 WAT (Table 3).

Table 3. The effect of mulching materials on the number of leaves of UC82B and Ibadan local plants from the 2nd to the 10th week after transplanting.

Mulching materials	Varieties	2WAT	4WAT	6WAT	8WAT	10WAT
T0	Ibd local	25.33	41.00	58.00	69.00	81.00
	UC82B	23	39	56	69.33	85.3
T1	Ibd local	25	38.67	58	75.33	110.7
	UC82B	25	44.67	56	75.33	112.7
T2	Ibd local	25	41.67	59	74.33	110.2
	UC82B	26	42.67	56.67	80.00	116.7
T3	Ibd local	25	43.67	58.33	86.33	123.7
	UC82B	25	44	60	89.33	130.3
LSD MM		Ns	*	Ns	**	**
LSD VAR		Ns	Ns	Ns	Ns	ns
LSD.MM× VAR		Ns	*	Ns	Ns	ns

At 10 WAT, UC82B also recorded the highest number of leaves produced at 30 t ha^{-1} SDM (130.3), while the lowest number was produced by Ibadan local (81.0) at the control plot. This also agrees with the primary objective of mulching which includes weed control, soil moisture conservation and temperature modifications (Hochmuth et al., 2001) to promote better crop growth.

The effect of the mulching materials on the number of branches per plant is shown in Table 4. The result shows that there was no significant difference in the tomato varieties used and the interaction between mulching materials and varieties used from 2 to 10 WAT, but there was a significant difference in the effect of mulching materials used at 2, 4, 8 and 10 WAT. At 10 WAT, UC82B (15.67) had the highest number of branches in a plot with 30 t ha^{-1} SDM, followed by Ibadan local (15.67) in a plot with 30 t ha^{-1} SDM and the lowest number of branches was recorded by UC82B (12.33) at the control plot (Table 4).

Table 4. The effect of mulching materials on the number of branches of UC82B and Ibadan local plants from the 2nd to the 10th week after transplanting.

Mulching materials	Varieties	2WAT	4WAT	6WAT	8WAT	10WAT
T0	Ibd local	7.67	8.33	11.33	12	12.33
	UC82B	7	8.67	10.67	11.33	13.33
T1	Ibd local	8.00	10.67	11.67	12.67	14.67
	UC82B	7.33	10	11	13	14.67
T2	Ibd local	8.67	10.33	12	13.33	15.33
	UC82B	8.33	10	11.33	12.67	15.33
T3	Ibd local	8.33	9.67	11.33	13.33	15.64
	UC82B	8.00	10	11.67	12.33	15.67
LSD MM		*	*	Ns	*	**
LSD VAR		Ns	Ns	Ns	Ns	ns
LSD.MM× VAR		Ns	Ns	Ns	Ns	ns

Mulch provides numerous benefits to crop production by improving the physical, chemical and biological properties of the soil. Organic mulches reduce soil temperature while mulches generally reduce soil moisture depletion and weed infestation, which enhances the tomato growth and fruit yield (Hochmuth et al., 2001*).*

The results showed that there was no significant difference in the interaction between the mulching materials and the varieties used at 6 WAT on plant stem diameter, but a significant difference was observed at 2, 4, 8 and 10 WAT. There was no significant difference in the tomato varieties used from 2 to 8 WAT, but there was a significant difference at 10 WAT where UC82B (2.00 cm) had the highest stem girth at 10 t ha^{-1} SDM plot, followed by Ibadan local (1.98 cm) at 10 t ha^{-1} SDM plot and the lowest was obtained by Ibadan local (1.73 cm) at the control plot (Table 5). The lower moisture depletion under the mulches was due to the fact that the soil was not in contact with dry air, which reduced water loss and prevented soil compaction.

Table 5. The effect of mulching materials on stem girth (cm) of UC82B and Ibadan local plants from the 2nd to the 10th week after transplanting.

Mulching materials	Varieties	2WAT	4WAT	6WAT	8WAT	10WAT
T0	1	0.57	1.17	1.53	1.63	1.73
	2	0.60	1.20	1.50	1.63	1.80
T1	1	0.63	1.30	1.57	1.83	1.98
	2	0.67	1.33	1.50	1.84	2.00
T2	1	0.73	1.37	1.53	1.77	1.87
	2	0.63	1.30	1.57	1.80	1.90
T3	1	0.67	1.30	1.50	1.70	1.83
	2	0.73	1.33	1.53	1.77	1.90
LSD MM		*	*	Ns	*	**
LSD VAR		Ns	Ns	Ns	Ns	ns
LSD MM × VAR		Ns	Ns	Ns	Ns	ns

The tomato yield determined in this study revealed that the use of sawdust mulch significantly influenced the number of fruits and the weight of the freshly harvested tomatoes. There was no significant difference in the tomato varieties used in terms of the number of fruits, but there was a significant difference in the weight of freshly harvested tomatoes. In the interaction between varieties and mulching materials, there was a significant difference in the number of fruits but no significant difference in the weight of freshly harvested fruits. UC82B (117.92 kg ha^{-1}) at 30 kg ha^{-1} SDM plot had the highest yield followed by Ibadan local (103.93 kg ha^{-1}) at 30 kg ha^{-1} SDM plot and the lowest yield was recorded by Ibadan local (61.94 kg ha^{-1}) at the control plot (Table 6).

Table 6. Effects of mulching materials on the yield of UC82B and Ibadan local plants.

Mulching materials	Varieties	Number of fruits	Fruit weight (kg ha^{-1})
T0	1	13.6	61.94
	2	15.7	80.22
T1	1	16.4	80.44
	2	17.2	94.31
T2	1	17.8	100.81
	2	21.4	100.46
T3	1	227	103.93
	2	21.6	117.92
LSD MM		**	**
LSD VAR		Ns	**
LSD MM × VAR		**	Ns

After harvest, the laboratory results of the soil mulched with sawdust mulch (SDM) showed that the organic carbon (g kg^{-1}) was moderate at 11.4, total nitrogen

(g kg^{-1}) was low at 1.3, available phosphorus (mg Kg^{-1}) was moderate at 14.36, while the exchangeable cations (cmol kg^{-1}) were moderate and extractable micronutrients (mg kg^{-1}) were also moderate (Adeoye and Agboola, 1985).

Table 7. Soils mulched with sawdust after harvesting.

Soil parameters	SDM
pH (H_2O 1:1)	5.60
Organic carbon (g kg^{-1})	11.4
Total nitrogen (g kg^{-1})	1.3
Available phosphorus (mg kg^{-1})	14.36
Exchangeable cations (cmol kg^{-1})	
Na	0.28
K	0.37
Mg	0.91
Ca	2.34
Extractable micronutrients (mg kg^{-1})	
Mn	95.00
Fe	108.00
Cu	1.28
Zn	1.50

Conclusion

The incorporation of mulch materials improved the soil properties, growth and yield of both tomato varieties in the study area. Sawdust when applied as mulch at a rate of 30 t ha^{-1} had an excellent effect on the growth and yield of both tomato varieties. The study therefore recommends that farmers in the study region could use this material to maximize fruit yield in tomato production. The farmers should also be educated on the use of sawdust mulch to improve soil health, which will in turn give rise to a better performance of the crops grown.

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UTICAJ MALČA OD PILJEVINE NA SVOJSTVA ZEMLJIŠTA I PRODUKTIVNOST PARADAJZA (*LYCOPERSICUM ESCULENTUM*) NA ALFISOLU U JUGOZAPADNOJ NIGERIJI

Abiodun Femi Olusola[1*] i Isola John Oluwasina[2]

[1]Forestry Research Institute of Nigeria, Ibadan
[2]Federal College of Forestry, Ibadan

R e z i m e

Malčiranje je efikasan metod poboljšanja uslova gajanja useva, kako bi se povećao prinos. Poljski eksperiment izveden je na Nastavno-istraživačkom poljoprivrednom imanju Federalnog koledža šumarstva Jeriho (Ibadan). Istraživanje je imalo za cilj da ispita uticaj malčiranja piljevinom na odabrana svojstva zemljišta, rast i prinos dveju sorti paradajza. Istraživanje je predstavljalo četvorofaktorijalni (4 x 2) eksperiment postavljen po potpuno slučajnom blok dizajnu (engl. *randomized completely block design – RCBD*) sa četiri ponavljanja. Faktor jedan se sastojao od četiri tretmana, i to: T0 (kontrola), T1 (primena 10 t ha^{1} malča od piljevine), T2 (primena 20 t ha^{-1} malča od piljevine) i T3 (primena 30 t ha^{-1} malča od piljevine). Drugi faktor su bile dve sorte paradajza (UC82B i lokalna sorta iz Ibadana). Visina biljke, obim stabljike, broj listova i grana prikupljeni su u intervalu od 2 nedelje, dok su broj plodova, masa ploda (kg ha^{-1}) i parametri prinosa mereni u zrelosti. Za analizu podataka korišćen je statistički softverski paket Genstat i urađen je LSD test na nivou značajnosti od 5%. Korišćeni malč od piljevine imao je nizak sadržaj azota (0,60%) i umeren sadržaj organskog ugljenika (38,6%). Upotreba malča od piljevine imala je veliki uticaj na prinos paradajza, dok među ispitivanim sortama paradajza nije bilo značajnijih varijacija. Najveći prinos imao je UC82B (117,92 kg ha^{-1}) na parceli na kojoj je primenjeno 30 t ha^{-1} malča od piljevine, zatim lokalna sorta iz Ibadana (103,93 kg ha^{-1}) na parceli na kojoj je primenjeno 30 t ha^{-1} malča od piljevine, dok je lokalna sorta iz Ibadana (61,94 kg ha^{-1}) na kontrolnoj parceli imala najmanji prinos. U ovom istraživanju, paradajz je pokazao najbolji učinak prilikom primene 30 t ha^{-1} malča od piljevine i stoga se preporučuje poljoprivrednicima na istraživanom području kako bi poboljšali proizvodnju paradajza.

Ključne reči: paradajz, piljevina, malčiranje, zemljište.

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[*]Autor za kontakt: e-mail: feminikan@gmail.com

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YIELD, WATER PRODUCTIVITY AND ECONOMIC RETURN OF DEFICIT-DRIP-IRRIGATED TOMATOES IN KADUNA (NIGERIA)

Donatus Obiajulu Onwuegbunam[1*], Muyideen Abubakar Oyebode[2], Henry Evonameh Igbadun[2] and Habibu Ismail[2]

[1]Department of Agricultural and Bio-environmental Engineering, Federal College of Forestry Mechanization, Afaka, Kaduna; Forestry Research Institute of Nigeria
[2]Department of Agricultural and Bio-resources Engineering, Ahmadu Bello University, Zaria, Nigeria

Abstract: Compared to gravity flow systems, pressurized drip irrigation provides more efficient control of the amount of water applied, better irrigation uniformity, and a higher initial capital and operation costs. Hence, an economic analysis is required to determine its profitability over a projected time period. Field experiments were conducted in Afaka (Kaduna, Nigeria), during two dry seasons of 2018 and 2019 to determine the effect of regulated deficit irrigation on the yield, crop water productivity, and projected economic returns of UC 82B tomatoes under pressurized drip irrigation. The economic returns were evaluated using the benefit-cost ratio, net present values, and payback period analyses. The highest fresh fruit yield (19.0 t/ha) was obtained in the full irrigation treatment (T_1), while the highest crop water productivity (4.94 kg/m^3) was obtained in the deficit treatment with full irrigation at the vegetative and flowering stages followed by 60% of reference evapotranspiration at maturity (T_7). The project was found to be profitable over the projected years, with benefit-cost ratios of 1.90 and 1.69; payback periods of 2.7 and 3.2 years for T_1 and T_7, respectively. The full irrigation of tomatoes was therefore found to be more economical than deficit irrigation in the area, with water not being considered as a limiting factor in terms of costs. Gravity drip irrigation was recommended to reduce the pumping cost of irrigation and thereby increase the profit margin.

Key words: drip irrigation, tomato, yield, water productivity, economic return, Nigeria.

Introduction

Water stands out as the most dominant among the limiting factors for crop production and diversification. Water has been described as a limited resource and

*Corresponding author: e-mail: donancy2001@yahoo.com

with the increasing world population and diverse use of water for domestic, agricultural, urban, and industrial purposes, there is a competing demand for its use among these variables (Kuşçu et al., 2009; FAO, 2019). Globally, water consumption is estimated to have increased more than twice as fast as the population in the last century, and the number of regions reaching the limits of sustainable water supply is increasing (UN-Water, 2021). Almost 70 percent of all water withdrawals are used in farming; with up to 95 percent in some developing countries. Hence, we need to use our water resources more wisely over time (FAO, 2019) and water has to be treated as a scarce or limited resource, with a much stronger focus on how its demand should be managed (UN-Water, 2021).

Essentially, for better water resource utilization at the farm level, an irrigation scheduling criterion should be applied so that the crop is irrigated at the right time with the right volume. However, under the condition of limited water supply, a so-called 'regulated water deficit' can be applied with the aim of supplying lower irrigation volumes compared to the crop water requirements during the whole crop cycle, but coinciding with some particular phenological stages that are the most sensitive to water stress (English et al., 1990; Kirda, 2002). In this way, smaller water amounts maximize the productive result (Mannini, 2004).

Kaduna State, including the study area (Afaka, Kaduna, Nigeria), is known for the cultivation of horticultural crops in the dry season through irrigation. Crops usually grown through irrigation in the area include cucumber, cabbage, carrots, tomatoes, maize, pepper, and onions (Plaisier et al., 2019) and the irrigation schemes are mostly small and medium sized.

Drip irrigation technology is relatively new in Nigeria and the Federal Government in collaboration with the Food and Agriculture Organization (FAO) of the United Nations, has considered its adoption to increase food production. In this vein, the FAO has expressed support for the promotion of a 20–25 ha pilot drip irrigation system in Nigeria through its Technical Cooperation Programme (TCP), starting with the identification of suitable sites for the project (Ewepu, 2022). The promotion also aims to provide an enabling and attractive environment to encourage more youth, smallholder farmers, and other vulnerable groups to produce high-value crops through drip irrigation. The drip irrigation project was supported by the FAO through a $350,000 grant for its promotion in Niger State, Nigeria. The funding is part of the Technical Cooperation Programme for FAO member nations (Staff Reporter, 2021).

Drip irrigation is a fixed system requiring high investment in labor and the acquisition of equipment for water collection, conveyance, control, and distribution. Hence, energy and labor costs are important factors to consider for the effective operation and management of the system. These represent significant additional costs of production. In comparison with a gravity (low pressure) drip irrigation system, the pressurized (high pressure) system provides better irrigation

uniformity as well as better control of the amount of water applied through its pressure compensating emitters; water and nutrients (through fertigation) are most evenly distributed across the field irrespective of the field size, shape, or slope, thus giving every plant the first-class treatment (NETAFIM, 2021). A non-pressure-compensating drip emitter has varying output flows at varying inlet pressures, so the flow varies along uneven terrain, with each dripper emitting a different amount of water depending on its location on the supply line. In such instances, the pressure on a drip emitter varies due to the slope of the land and the length of the supply tube (Drip Depot, 2018). Although the high-pressure drip system is necessary for the development of precision agriculture, it requires a higher initial capital cost than gravity drip systems.

The choice of pump power to run a high-pressure drip irrigation system depends on how available and accessible the energy resources are in an area. Electricity is mostly preferred because it has reduced labor requirements and higher efficiency, and results in lower energy costs (NETAFIM, 2015). However, when electricity is unavailable or irregular (as is the case in the study area), alternative power sources such as gasoline, diesel, or solar may be used. Generally, the challenge with electricity supply in Nigeria is so serious that fossil fuel generators power about thirty percent of micro, small, and medium enterprises (Omorogbe, 2021). The smallholder irrigation farmers in the study area use gasoline engine pumps for irrigation, but this is not adequate for large-scale farms requiring a larger pump capacity (greater than 10 hp); diesel engine pumps have to be employed to meet the higher power requirements (NETAFIM, 2015). Hence, the cost estimation for a hectare of field in the study location is based on diesel as the pump power source.

An economic analysis is required to determine whether the improved performance of the pressurized system justifies its use in terms of returns on investment. That is, an economic evaluation estimating all the expected annual or seasonal expenditures and returns in the irrigation project is required as an indicator of whether the implementation of the irrigation system is worthwhile (Letey et al., 1990; Silva et al., 2003). Generally, there is no information on the economic viability of drip irrigated tomatoes for the study area. The objectives of this study are to evaluate the yield, water productivity and economic returns of field-grown drip-irrigated tomatoes in response to full and deficit irrigation regimes.

Material and Methods

Study area

The study was carried out at the experimental farm of the Federal College of Forestry Mechanization, Afaka, Kaduna, located at latitude $10^0$36‘N and longitude

$07^0$25‘N. The climate of Kaduna is characterized by a clear distinction between the dry and rainy seasons. The rainy season lasts from mid-April to early October. Kaduna has an annual mean rainfall of 1200 mm (Onwuegbunam et al., 2018). The temperature range is 28–36^0C for the maximum scale and 15–23^0C for the minimum scale. The harmattan is at its peak between December and February and the relative humidity is very low. Thereafter, the weather is hot in March and April, with March recording the highest mean temperature of 35^0C (Onwuegbunam et al., 2018). The humidity ranges from 24% to 83%, with the lowest occurring in February and the highest in August (NIMET, 2015; KSWB, 2015).

Experimental procedures

The research was carried out as growth stage-based deficit irrigation trials in the 2017/2018 and 2018/2019 irrigation seasons. The trial spanned from December 12 to March 11 in both seasons. The experiment was laid in a randomized complete block design and replicated three times. The experimental factor is the level of deficit irrigation applied at three crop growth stages as described in Table 1.

The inter-row spacing was 0.55 m while the intra-row spacing was 0.457 m. The intra-row spacing (between the plants along the row) fitted into the spacing between emitters on the lateral. The field layout comprised ten plots (each representing a treatment), which were replicated three times. Each plot was of dimensions 5 m by 1.1 m, and hence, 5.5 m^2 per block (replication). This spacing resulted in an approximate plant density of 40,000 plants/ha, as recommended by FAO (2013).

Irrigation system

Irrigation was carried out by means of a pressurized drip irrigation system with an average discharge of 2.44 l/hr, an emission uniformity of 94% and an optimum operating pressure of 240 kPa. The water source for the irrigation system was fresh water from a borehole within the site. A gasoline-powered centrifugal water pump was used in powering the irrigation system.

The drip irrigation running time for administering water according to the treatments was expressed by Kumari et al. (2014) in the form:

$$T_{drip} = \frac{N_p V}{N_e Q \times EU} \quad (1)$$

where T_{drip} = drip irrigation time (hours), Np = number of plants served by one lateral, V = volume of water applied per plant in drip irrigation system (l), Ne = number of emitters in one lateral, Q = average emitter discharge (l/hr), EU = emission uniformity (fraction).

Table 1. Treatment descriptions.

Treatment number	Treatment tag	Treatment descriptions
T_1	$V_{100}F_{100}M_{100}$	Full irrigation (100% ET_o) at all crop growth stages (control)
T_2	$V_{80}F_{100}M_{100}$	Irrigating with 80% ET_o at vegetative stage, full irrigation at flowering and maturity stages
T_3	$V_{100}F_{80}M_{100}$	Irrigating with 80% ET_o at flowering stage, full irrigation at vegetative and maturity stages
T_4	$V_{100}F_{100}M_{80}$	Irrigating with 80% ET_o at maturity stage, full irrigation at vegetative and flowering stages
T_5	$V_{60}F_{100}M_{100}$	Irrigating with 60% ET_o at vegetative stage, full irrigation at flowering and maturity stages
T_6	$V_{100}F_{60}M_{100}$	Irrigating with 60% ET_o at flowering stage, full irrigation at vegetative and maturity stages
T_7	$V_{100}F_{100}M_{60}$	Irrigating with 60% ET_o at maturity stage, full irrigation at vegetative and flowering stages
T_8	$V_{40}F_{100}M_{100}$	Irrigating with 40% ET_o at vegetative stage, full irrigation at flowering and maturity stages
T_9	$V_{100}F_{40}M_{100}$	Irrigating with 40% ET_o at flowering stage, full irrigation at vegetative and maturity stages
T_{10}	$V_{100}F_{100}M_{40}$	Irrigating with 40% ET_o at maturity stage, full irrigation at vegetative and flowering stages

Field experimental design (2017, 2018)

Water productivity

The water productivity (WP) was expressed as the crop output (yield) per unit of water consumptively used (Ragab, 2017; Igbadun et al., 2012; Talukder and Ali, 2008):

$$WP = \frac{Y}{SCWU} \tag{2}$$

where WP = crop water productivity (kg/m^3), SCWU = seasonal crop water use (mm), Y = fruit yield (t/ha).

Economic considerations

The economic returns of the drip-irrigated tomatoes were evaluated in terms of the benefit-cost ratio (BCR), net present value (NPV), and payback period (PBP). The analyses were based on the comparison between the full irrigation treatment (control) and the deficit irrigation treatment, which had the highest value of water productivity.

The production cost comprised the initial investment in procuring the irrigation equipment and the costs for the system operation and maintenance. The various costs considered relate to land rent, land preparation, repairs and

maintenance, scheduled irrigation durations, seeds (seedlings), transplanting, fertilizer application, weeding, pest control and harvesting. Water charges were estimated as the cost of installing the borehole for water supply and subsequently, the pumping energy requirement. The repair and maintenance cost was estimated as a percentage (4%) of the total capital cost. Kuşçu et al. (2009) and Cetin et al. (2004) used values of 3% and 6% of total system cost for repair and maintenance, respectively.

The pumping energy determined the amount of fuel consumed in powering the irrigation system. For the pressurized system, the pumping energy cost was determined as a function of the pump brake horsepower needed to discharge the design volume for the irrigated area. Harrison (2012) and Scherer (2017) expressed the pump brake horsepower as follows:

$$BHP = \frac{Q \times TDH}{3960 \times E_p} \tag{3}$$

where BPH is the brake horsepower, Q is the total discharge, TDH is the total dynamic head, and E_p is the pump efficiency (= 0.75).

The total dynamic head of the pump is the sum of the total static head, the pressure head, and the velocity head. For the source borehole, the total static head is the distance from the pumping water level in the well to the ground surface, plus the vertical distance of lift of the water to the discharge point (irrigated area). The pressure head is the optimum operating pressure of the pressurized drip irrigation system, converted into a head (m). The velocity head is the energy of the water based on its velocity. Its value is very small and negligible when computing the losses in an irrigation system (Scherer, 2017).

For each treatment, the seasonal fuel consumption is calculated based on the total irrigation time, which was determined by the seasonal water applied. The fuel consumed per unit time of design pump use as expressed by Martin et al. (2011) and Harrison (2012) is as follows:

$$C_f = \frac{EPS}{BHP} \tag{4}$$

where C_f is the fuel consumed (gal/hr), EPS is the fuel energy performance efficiency (hp-hr/gal), and BPH is brake horsepower (hp).

EPS values are given as 12.5 hp-hr/gal and 8.66 hp-hr/gal for diesel and gasoline, respectively (Martin et al., 2011; Harrison, 2012). Hence, the seasonal fuel consumption is as follows:

$$C_f \times IT_{seasonal} \tag{5}$$

where $IT_{seasonal}$ is the seasonal irrigation time per treatment (hr).

Benefit-cost ratio

The benefit cost ratio (BCR) is obtained by dividing the present value of the benefits by the present value of the costs (Savva et al., 2002; Michael, 2009):

$$BCR = \sum_{t=1}^{n} \left[\frac{\frac{B}{(1+r)^i}}{\frac{C}{(1+r)^i}} \right] \quad (6)$$

where BCR = benefit-cost ratio, n = number of years of anticipated project life, i = discount rate selected on the basis of cost of capital, r = interest rate, B = benefits accrued, C = costs incurred.

The discount rate i was determined as follows:

$$i = \frac{1}{(1+r)^n} \quad (7)$$

where the terms are defined as above.

Returns on the produce were based on a prevailing market price of $0.51 per kg on a fresh fruit basis in 2018 and 2019. The interest rate used in the study was 11% and was based on an 11-year average interest rate of 10.81% between 2007 and 2018 in Nigeria (CBN, 2018). The capital and operating costs for the project were stated on a per hectare basis.

It has been recommended that using the BCR alone may not be sufficient to determine how profitable a project is and therefore other forms of financial analysis must also be used to make better financial decisions (FundsNet, 2022).

Net present value

The worth of the project is estimated by subtracting the costs from the returns on a year-to-year basis to obtain the net return stream (cash flow). This is then discounted to the present values, which are added over the project life period to obtain the net present value or worth. The net present value is estimated as follows (Michael, 2009):

$$NPV = \sum_{t=i}^{n} \frac{B_t - C_t}{(1+i)^t} \quad (8)$$

where B_t = benefit estimated for each year of the project, $; C_t = cost estimated for each year of the project, $; t = time period of project life, that is, 1, 2, 3 … n; n = number of years of the anticipated project life; i = discount rate based on the cost of capital.

Payback period of the investment

The payback period is the time it takes the cash flow of a project to pay back the initial investment. A discounted payback period is used to take into account the

depreciation of the project assets. The discounted payback period shows the period in which an investment reaches its break-even point, considering the time value of money. The discounted payback period (Discounted payback period: method and example, 2017) is computed as follows:

$$DPP = LPNC + \frac{AVC}{DCD} \quad (9)$$

where DPP = discounted payback period (year); LPNC = last period with a negative total discounted cash flow (year); AVC = absolute value of the total discounted cash flow at the end of LPNC ($); DCD = discounted cash flow during the period after LPNC ($).

Results and Discussion

The yield and water productivity (WP) for all the treatments are shown in Table 2. The pooled WP in terms of crop water use varied between 3.61 kg/m^3 and 4.94 kg/m^3. The WP showed significant differences among the treatments, with the highest occurring in T_7, which is, the application of 60% ET_o irrigation amount at the maturity stage, and 100% ET_o at both vegetative and flowering stages. The WP values obtained in several studies on tomatoes showed a wide range of results, depending mainly on the cultivar type, yield values, amount of water applied, amount of water consumptively used, and the irrigation practice adopted. The WP values are within the range (4.2–13.4 kg/m^3) obtained by Singh et al. (2009) for the Rupali cultivar with a yield of 13.70 to 29.90 t/ha under SWA of 337 to 700 mm in Abohar, Punjab, India. Tya and Othman (2014) obtained a WP range of 0.32 to 0.85 kg/m^3 for the pevabo tomato cultivar (determinate type) with a fresh fruit yield range of 9.3 to 14.2 t/ha and seasonal irrigation application depths of 1360 to 3080 mm under a basin irrigation system at Yola, the ecological savanna zone of Nigeria.

Table 2. The yield, crop water use and water productivity of drip-irrigated tomatoes.

Treatment	Y (t/ha)	CWU (mm)	WP (kg/m^3)
T_1 ($V_{100}F_{100}M_{100}$)	19.0a	393.5a	4.82c
T_2 ($V_{80}F_{100}M_{100}$)	18.4b	388.0b	4.74d
T_3 ($V_{100}F_{80}M_{100}$)	18.1c	377.0c	4.80cd
T_4 ($V_{100}F_{100}M_{80}$)	17.6d	360.5e	4.88b
T_5 ($V_{60}F_{100}M_{100}$)	17.9cd	368.0d	4.86bc
T_6 ($V_{100}F_{60}M_{100}$)	17.6d	358.0e	4.92ab
T_7 ($V_{100}F_{100}M_{60}$)	16.8e	339.5f	4.94a
T_8 ($V_{40}F_{100}M_{100}$)	16.3f	335.5g	4.87b
T_9 ($V_{100}F_{40}M_{100}$)	15.6g	325.5h	4.79cd
T_{10} ($V_{100}F_{100}M_{40}$)	11.0h	305.0i	3.61e
SE±	0.7219	8.9779	0.1252

Y is the fresh fruit yield (t/ha); CWU is the crop water use (mm); WP is the water productivity (kg/m^3).

The economic analyses of the drip-irrigated tomatoes were based on a comparison between the deficit treatment with the highest water productivity, that is T_7, and the fully irrigated treatment, T_1. The pooled mean annual benefit-cost ratios (BCRs) for the two irrigation seasons are presented in Table 3 for T_1, T_7, and T_{7+}; T_{7+} being a re-designation of T_7 to depict the extra yield and the additional irrigated land with the water saved. The BCR values for T_1, T_7, and T_{7+} were 1.90, 1.69, and 1.74, respectively, showing that the investment in pressurized drip irrigation of UC 82B tomatoes in Afaka (Kaduna, Nigeria) was profitable following each of the irrigation strategies; the BCR was higher than 1. This means that for every \$1 invested in the drip irrigation project, there was a discounted profit value of \$1.90, \$1.69 and \$1.74 for T_1, T_7 and T_{7+}, respectively. T_1 gave the highest BCR compared to T_7 and T_{7+}, regardless of the additional yield from the water saved in T_7. The reason for this was the additional capital cost of procuring drip irrigation units to cater for the extra land cultivated with the water saved. The cost of procuring the additional drip irrigation unit (\$3,765) was higher than the cash inflow from the yields of the additional cultivated land (\$1,387) in T_{7+} with the water saved. The gain from the additional land cultivated with the water saved (1,146 m^3) was 4.2 tons, an economic gain that was not significant for the cash outflow. It was, therefore, inferred that economic returns on pressurized drip irrigation were higher under full irrigation than deficit irrigation in the study area. Tewelde (2019) obtained a similar outcome when evaluating the economic water productivity of sesame crops under full and deficit irrigation in Woreda Kafta-Humera, Tigrai-Ethiopia, with full irrigation having the highest economic returns in comparison to deficit treatments.

Table 3. Benefit-cost ratio analysis of drip-irrigated tomatoes for a 10-year project period.

Cost parameters	Treatment		
	T_1	T_7	T_{7+}
Capital cost (\$)	19261	19261	23026
Operation and maintenance cost (\$)	26444	25871	31962
Cash outflow (\$)	45705	45131	54988
Cash inflow (\$)	103749	91500	114415
Interest rate (%)	11	11	11
Discounted cash outflow (\$)	32291	31967	38800
Discounted cash inflow (\$)	61374	54128	67684
Benefit-cost ratio	1.90	1.69	1.74

However, the experiment involved non-conventional irrigation methods and made use of jars, bottles and large collectors. Adeboye et al. (2015) evaluated the economics of drip-irrigated soybeans in Ile-Ife, Nigeria and obtained the highest economic water productivity under full irrigation as a reference treatment. This

suggests that while deficit irrigation has proved to be the most viable option in water-scarce regions, its use is not economically justifiable in areas where water is not a limiting factor and the water price is relatively low.

The results generally showed that pressurized drip irrigation of tomatoes was profitable in the study area. As stated by Adeboye et al. (2015), a low-income farmer can benefit from the use of high cost (imported) drip lines, but if the drip irrigation system is properly maintained, it will be continuously used for crop production after several years. Local production of drip lines is necessary to avoid the high costs of importation. Also, the gravity drip irrigation system eliminates the cost of operating and maintaining the pumps, except for the lifting of water to the supply reservoir. The system is recommended for crops of higher economic value because of the high initial costs. Cetin et al. (2004) recommended a drip irrigation system for crops with higher economic value such as apples, as all growers of drip irrigated apples in the Inegöl district of Bursa province, Turkey reported positive returns despite relatively high initial investments.

Net present value analysis and payback period

The net present values (NPVs) for the drip irrigation project on a per hectare basis were computed over the system's service life. The NPV and payback period (PBP) for the fully irrigated and deficit irrigated treatments are presented in Tables 4, 5 and 6. T_1 gave a payback period of 2.7 years with a corresponding NPV of \$1,966 and a cumulative NPV of \$29,083 at the end of the projected service life of the irrigation system. A regression analysis of the discounted cash flows over the system's service life, given by Equation 10, showed that the cash flow would cease in the 14th year, that is, four years after the useful life of the system.

$$Discounted\ cash\ flow = -214359 * year + 3 * 10^6 \quad (10)$$

On the other hand, the deficit treatment (T_7) gave a payback period of 3.2 years with a corresponding NPV of \$4,088 and a cumulative NPV of \$22,158 at the end of the projected system's service life. Similar to equation (10), the cash flow would cease in the 11th year, that is, one year after the useful life of the system.

$$Discounted\ cash\ flow = -186231 * year + 2 * 10^6 \quad (11)$$

The full irrigation treatment produced better economic returns in terms of the net present values and payback periods than the deficit treatments. It can be concluded that the full irrigation of tomatoes was preferred in the study area, as the water saved in deficit irrigation, which was used for extra cultivation, did not produce yields that could outweigh the cash outflow for the additional cultivation.

Table 4. Pooled net present value analysis for the fully irrigated treatment (T_1).

Yr.	Capital ($)	O & M ($)	CO ($)	CI ($)	CF ($)	i, 11%	DCF ($)	Σ DCF ($)	PBP (Yr)
1	19,261	2,102	21,363	9,651	-11,712	0.9009	-10,552	-10,552	
2		2,208	2,208	10,134	7,926	0.8116	6,433	-4,119	
3		2,318	2,318	10,640	8,322	0.7312	6,085	1,966	2.68
4		2,434	2,434	11,172	8,738	0.6587	5,756	7,722	
5		2,556	2,556	11,731	9,175	0.5935	5,446	13,168	
6		2,683	2,683	11,144	8,461	0.5346	4,523	17,691	
7		2,817	2,817	10,587	7,770	0.4817	3,743	21,434	
8		2,958	2,958	10,058	7,099	0.4339	3,080	24,514	
9		3,106	3,106	9,555	6,449	0.3909	2,521	27,035	
10		3,262	3,262	9,077	5,815	0.3522	2,048	29,083	

O & M is operation and maintenance cost; CO is cash outflow; CI is cash inflow; CF is cash flow; DCF is discounted cash flow; PBP is payback period.

Table 5. Pooled net present value analysis for 1 ha of the deficit irrigated treatment (T_7).

Yr.	Capital ($)	O & M ($)	CO ($)	CI ($)	CF ($)	i, 11%	DCF ($)	Σ DCF ($)	PBP (Yr)
1	19,261	2,057	21,318	8,512	-12,806	0.9009	-11,537	-11,537	
2		2,160	2,160	8,937	6,777	0.8116	5,501	-6,036	
3		2,268	2,268	9,384	7,116	0.7311	5,203	-834	
4		2,381	2,381	9,853	7,472	0.6587	4,922	4,088	3.17
5		2,500	2,500	10,346	7,846	0.5934	4,656	8,744	
6		2,625	2,625	9,829	7,203	0.5346	3,851	12,595	
7		2,756	2,756	9,337	6,581	0.4816	3,169	15,764	
8		2,894	2,894	8,870	5,976	0.4339	2,593	18,357	
9		3,039	3,039	8,427	5,388	0.3909	2,106	20,463	
10		3,191	3,191	8,005	4,815	0.3521	1,695	22,158	

Table 6. Pooled net present value analysis for 1 ha of the deficit irrigated treatment (T_{7+}).

Yr.	Capital ($)	O & M ($)	CO ($)	CI ($)	CF ($)	i, 11%	DCF ($)	Σ DCF ($)	PBP (Yr)
1	23,026	2,640	25,666	10,643	15,023	0.9009	13,534	-13,534	
2		2,772	2,772	11,175	8,403	0.8116	6,820	-6,714	
3		2,910	2,910	11,734	8,824	0.7311	6,451	-263	
4		3,056	3,056	12,321	9,265	0.6587	6,103	5,840	3.04
5		3,209	3,209	12,937	9,728	0.5934	5,773	11,613	
6		3,369	3,369	12,290	8,921	0.5346	4,769	16,382	
7		3,538	3,538	11,676	8,138	0.4816	3,919	20,301	
8		3,715	3,715	11,092	7,377	0.4339	3,201	23,502	
9		3,900	3,900	10,537	6,637	0.3909	2,594	26,096	
10		4,095	4,095	10,010	5,915	0.3521	2,083	28,179	

Beyond the useful life, an asset is deemed to be cost-ineffective or not fit for operation or usage but it has been proved that the useful life of a system can be extended following a regular maintenance schedule as recommended by the original equipment manufacturer (ToolSense, 2022).

Conclusion

The highest water productivity of tomatoes in terms of yield per water consumptively used was obtained under deficit irrigation when the crop was irrigated with 100% ETo at the vegetative and flowering stages, then with 60% ETo at the maturity stage. However, this did not translate to higher economic returns as full irrigation treatment at all the growth stages produced the highest benefit-cost ratio and net present value as well as the lowest payback period in comparison to the deficit treatments. Full irrigation at all crop growth stages is, therefore, recommended for the study area. The economic gains from the water saved under deficit irrigation were not significant, as the cash outflow from the cultivation of extra land outweighed the cash inflow.

For higher economic returns, the pressurized drip irrigation system can be replaced by the gravity type since the energy requirement is restricted to lifting the water to the irrigation overhead storage tank, thus reducing, or eliminating the pumping costs of water application.

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PRINOS, PRODUKTIVNOST VODE I POVRAĆAJ ULAGANJA U PROIZVODNJI PARADAJZA PRI PRIMENI DEFICITARNOG NAVODNJAVANJA KAPANJEM U KADUNI (NIGERIJA)

Donatus Obiajulu Onwuegbunam[1*], Muyideen Abubakar Oyebode[2], Henry Evonameh Igbadun[2] i Habibu Ismail[2]

[1]Department of Agricultural and Bio-environmental Engineering, Federal College of Forestry Mechanization, Afaka, Kaduna; Forestry Research Institute of Nigeria
[2]Department of Agricultural and Bio-resources Engineering, Ahmadu Bello University, Zaria, Nigeria

R e z i m e

Za razliku od gravitacionih sistemima za navodnjavanje, sistem navodnjavanja kapanjem pod pritiskom omogućava efikasniju kontrolu količine vode koja se koristi u te svrhe, veći stepen ujednačenosti prilikom navodnjavanja i veće početne kapitalne i operativne troškove. Stoga je potrebno sprovesti ekonomsku analizu kojom bi se utvrdila profitabilnost ovih sistema tokom projektovanog vremenskog perioda njihovog korišćenja. Eksperimenti na terenu sprovedeni su na lokalitetu Afaka (Kaduna, Nigerija), tokom dve sušne sezone, 2018. i 2019. godine, kako bi se utvrdio uticaj regulisanog, deficitarnog navodnjavanja na prinos, produktivnost vode u usevu i projektovani povraćaj ulaganja u proizvodnju paradajza UC 82B, u uslovima navodnjavanja kapanjem pod pritiskom. Povraćaji ulaganja procenjeni su korišćenjem cost-benefit analize, metode neto sadašnje vrednosti i analize perioda otplate. Najveći prinos svežeg ploda (19,0 t/ha) dobijen je u tretmanu potpunog navodnjavanja (T_1), dok je najveća produktivnost vode u usevu (4,94 kg/m3) postignuta u deficitarnom tretmanu sa potpunim navodnjavanjem u fazi vegetacije i cvetanja, što je praćeno sa 60% referentne evapotranspiracije pri zrelosti (T_7). Utvrđeno je da je projekat profitabilan tokom projektovanih godina, sa odnosom koristi i troškova od 1,90 odnosno 1,69; sa periodom otplate od 2,7 odnosno 3,2 godine za T_1 odnosno T_7. Stoga je utvrđeno da je potpuno navodnjavanje paradajza ekonomičnije od deficitarnog navodnjavanja u ovoj oblasti, pri čemu se voda ne smatra ograničavajućim faktorom u pogledu troškova. Gravitaciono navodnjavanje kapanjem preporučeno je da bi se smanjili troškovi rada pumpe i time povećao profit.

Ključne reči: navodnjavanje kapanjem, paradajz, prinos, produktivnost vode, povraćaj ulaganja, Nigerija.

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[*]Autor za kontakt: e-mail: donancy2001@yahoo.com

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EFFECTS OF DIFFERENT SPATIAL AND PRECIPITATION INPUT DATA ON SWAT-DERIVED CATCHMENT FEATURES

Senka D. Ždero*, Zorica B. Srđević and Pavel P. Benka

University of Novi Sad. Faculty of Agriculture, Department of Water Management, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

Abstract: Computer-based mathematical models are used in water management research to represent ecological problems, simulate different processes, deal with such problems and support their solution. Such studies require an interdisciplinary approach that is both user-friendly and comprehensive to integrate all of the processes that occur in nature. The use of available hydrological models requires a model setup with a reasonable level of data quality and quantity to represent catchment features to emphasize the distinctive local character of ecosystems. One of the models that comply with such requirements is the Soil and Water Assessment Tool (SWAT). This paper aims to address the possible impact of different spatial and precipitation input data on the catchment features by using SWAT for the case study of the floodplain located within the Danube River Basin in Serbia. The objective was to evaluate the impact of digital elevation models, land use, and soil types with different resolutions (1) SRTM and ASTER (30 m), TanDEM-X (12.5 m), (2) CORINE and GlobCover land use databases, and (3) FAO/UNESCO world soil map and digitized soil map of Vojvodina Province on catchment delineation. The research was conducted alongside the analysis of precipitation, using data from the CFSR, CarpatClim, and the national yearbooks. Regarding the spatial data, the results indicate that the high-resolution data need to be adjusted for this area, while the ASTER layer is suitable at an acceptable level for further modeling in SWAT. Interpolated precipitation data are better to use due to their higher resolution (10km) and the heterogeneous distribution of rain gauge stations.

Key words: spatial data, precipitation data, SWAT, hydrological modeling.

Introduction

Water stands out as the most dominant among the limiting factors for crop production. Urbanization, the rapidly growing human population, climate change, and land use change have transformed the environment and altered the quality of

*Corresponding author: e-mail: senka.zdero@polj.uns.ac.rs

the main environmental elements (water, air, soil). Any changes in the environment are at the same time pressures that must be analyzed and processed to establish the optimal condition for the survival of ecosystems. Nowadays, there are scientifically confirmed statements that indicate that one of the factors on which the quality of the (local) water and the quality of the environment depend is the landscape of the surroundings (Galan et al., 2023). Changes in land use through deforestation, conversion of agricultural land, and modern urbanization, are changing the hydrological cycle and its components, such as volume and flow rate, infiltration, evapotranspiration, etc. Assessing the effects of anthropogenic activities, principally at the local level, is primarily important for decision-makers to whom environmental quality information is relevant when creating new management policies (Huang et al., 2020).

Hao et al. (2022) point to the lack of an integrated approach to the assessment that leads to inconsistent strategies and inefficient use of water resources, but they also point out the availability of adaptive, mostly mathematical and computer tools and models that could help in solving such problems. Nowadays, computer-based mathematical models are primarily used in scientific research to represent and simulate real-world system problems. The development of mathematical simulation models has contributed to a more efficient prediction of complex bio-geo-hydro-chemical mechanisms that occur in nature (Latinopoulos et al., 2020), which mainly require an understanding of the process, the mathematical basis, and the permissible conditions of uncertainty. The models have been developed over the years so that they can be used to monitor the quality and quantity of water resources under the pressure of current and predicted climatic and hydrological conditions around the world. One of the commonly used models is an open-source code SWAT (Soil and Water Assessment Tool), which is used to address various eco-hydrological problems worldwide (Tan et al., 2020). The model considers the effects of multiple factors, such as time distribution, changes in water resources, land quality, land use, topography, vegetation, and the impact of human activities in the study area. By using SWAT, it is possible to integrate different modules that evaluate the effects of numerous hydrological and chemical processes in the environment, as well as the complex relationships between climate, soil, water, environmental stressors, scattered sources of pollution, and climate changes.

The application of the SWAT model refers to a wide range of hydrological, environmental, and agricultural problems and can contribute to their better understanding (Gassman et al., 2014). It increases the understanding of natural processes, predicts the state of the system in different time steps, and allows users to perform simulations with different input data by constructing different management strategies for a selected catchment area. Catchment delineation can be performed automatically by loading the required input data for the SWAT model. The data can be roughly divided into two groups: the first group consists of spatial

data, and the second group includes meteorological data. The main objectives of the catchment process modeling in SWAT are (1) to assess hydrological and polluting impacts as a result of current water use and land management, (2) to quantify current and projected impacts and climate change scenarios, (3) to assess soil erosion, 4) to predict future conditions, and (5) to address the main drivers of changes in land use and water cycle dynamics. Therefore, it is advisable to accurately simulate all the hydrologic processes in the catchment before performing parameter sensitivity analysis, calibration, and validation.

Global datasets are approved for modeling large-scale catchments (regional, international, transboundary), but for small-scale or ungauged catchments, the precision and resolution of these types of datasets could be unfavorable for further examination. The studies on evaluating the impact of different input databases on SWAT simulations, especially in data-poor catchments, have been recently gaining attention (Mararakanye et al., 2020; Akhtar et al., 2021; Kmoch et al., 2022). In recent research by Weber et al. (2020), it was found that global data could be a valuable source to substitute single missing meteorological variables or topographic information, but this fact can lead to a gap in important information about the local character of the river basin. Uncertainties in the SWAT modeling output can be caused by two different sources of error: (1) inappropriate input parameters or representation (input error), or (2) error in the model structure and algorithms (structural error). Uncertainties in the simulation output data can be reflected in (1) the delimitation of the basin that could be influenced by different resolutions of digital elevation models (Ortíz-Rodríguez et al., 2022), (2) land cover and soil datasets which can contribute to the creation of a different number of hydrological units for the same watershed (Busico et al., 2020), and (3) model execution that is sensitive to meteorological input datasets (Koo et al., 2020). Therefore, it is necessary to provide real-time datasets to obtain a model with high calibration and validation coefficients.

The purpose of this research is to analyze the potential effects of different input data that affect the catchment delineation of the selected case study – the Special Nature Reserve "Koviljsko-petrovaradinski rit" (KPR), Serbia. As Serbia is a developing country, almost no field data required for this type of environmental analysis could be collected. Finally, the results of this analysis (1) highlighted the critical importance of dataset resolution selection for SWAT applications and (2) improved the accuracy and understanding of the impact of different input data resolutions on the derived watershed information for similar case studies.

Material and Methods

The geographic information system (GIS) is one of the most promising information technologies today, given the wide range of application areas.

Hydrological models simulate hydrological processes using spatial parameters derived from geospatial data within a GIS framework, and geospatial data are used to represent the spatial variation of catchment properties that cause hydrologic processes. The SWAT model is a semi-distributed and physically based watershed-scale hydrological model. It was developed by Arnold et al. (1998) and a detailed description of the model operation and its components can be found in the suggested reference. The SWAT model requires various input parameters related to water quantity and quality, as well as input data related to climate parameters, topography, soil, and land use. After loading all the required input data, the model is ready to run (Figure 1), and the first two steps of the SWAT framework were performed in this paper.

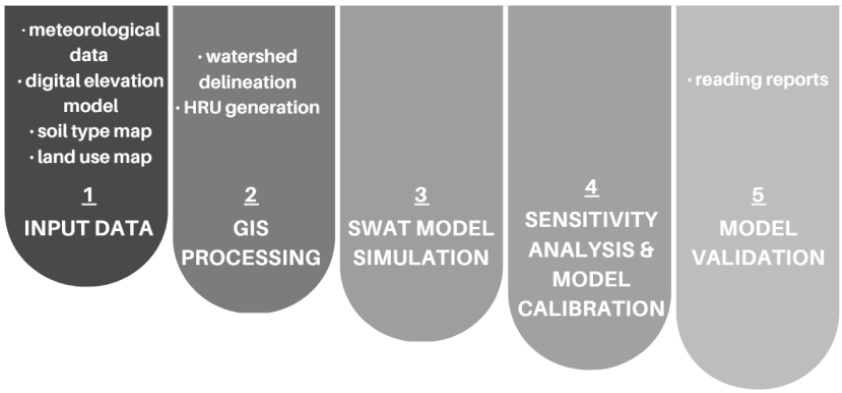

Figure 1. The SWAT model framework.

The main steps in creating the observed catchment are (1) the delimitation of the basin and the division of the basin into sub-basins interconnected by flow networks, and (2) the division of sub-basins into individual, smallest spatial units of Hydrological Response Units (HRUs), which the model automatically executes after loading the input data. The first step requires the input of a digital elevation model (DEM), from which the topographic properties of the catchment are calculated. In a subsequent step, the analysis of each formed HRU is performed based on loaded land use layers and a soil map. The last step in defining the observed catchment area, before the model run, is adding the locations of the weather stations and loading meteorological data. In the Results and Discussion section, the comparative results of the developed hydrographic networks created by different resolution DEMs and identified soil types based on different input data from soil type and land use databases are presented. Furthermore, the statistical analysis of mean annual precipitation values from different databases for the selected case study is shown.

Case study: The Special Nature Reserve "Koviljsko-petrovaradinski rit"

The selected case study is located in the southern part of Vojvodina Province (Serbia) (Figure 2) and has been declared a special nature reserve "Koviljsko-petrovaradinski rit" (KPR), which is a home to numerous endangered animal and plant species. The KPR reserve covers 5,895 ha along the Danube River and represents a very important floodplain in the Danube catchment area. The floodplain complex is a combination of forest, meadow, swamp, pond, and wetland ecosystems recognized on an international level. A fertile plain surrounds the reserve on one side, while the foothills of the National Park "Fruška Gora" are located on the other. It covers the territory of four municipalities where approximately 56,000 inhabitants are directly or indirectly connected to it (KPR Management Plan, 2012). For modeling purposes, the KPR area is expanded with a 10 km buffer zone (KPRbuff) and occupies 87,640 ha. The reason for this is that the protected area is heavily dependent on the water regime of the Danube River. It is not isolated from external pressures, and inadequate water and forest management can cause degradation of the quality of the environment and human well-being in the protected area.

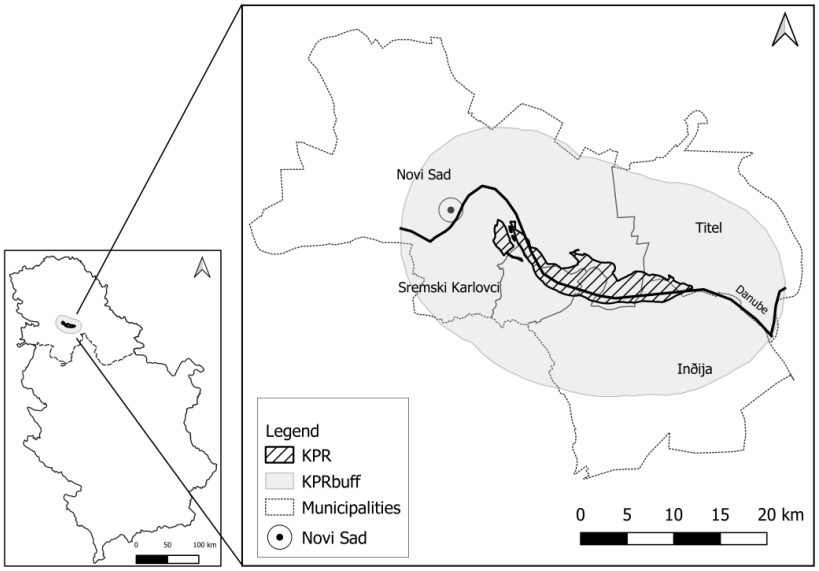

Figure 2. The location of the case study in Serbia.

To continue the research on the impact of land use change and anthropogenic activities on the quality of the environment in KPR, it was necessary to examine the impact of the quality of available data on the definition of catchment features in the pre-validation stage of modeling. The KPRbuff catchment delineation was built

as a SWAT model for the case study using an extension of the QGIS software (version 3.18.1). The automatic catchment delineation was used to calculate and define features such as boundaries, river and drainage networks, and sub-basins, as well as to derive terrain slope parameters as well. All of the spatial input data were created in the UTM zone 34 projection system, which covers the Balkan region (WGS84/UTM zone 34N; EPSG: 32634). The computation of the catchment delineation parameters was conducted on an i5-4590 CPU Core Intel 3,30GHz, 16GB running on a 64-bit operating system. Overall, the real computational time for processing all the input data amounted to approximately 4 hours.

Model set-up

The input data for the KPRbuff model included data related to morphology, land use, soil properties, and climate data. As in other studies from Serbia where SWAT was applied (for example, Šabović et al., 2019; Gregorić et al., 2020; Potić et al., 2022), the lack of continuous, accurate, and quality-checked data were also a challenge when creating a hydrological model of SWAT for KPRbuff.

Table 1. Available online open-source databases for the case study.

SPATIAL DATA			
Data	Database	Resolution	Source
DEM	SRTM	1 arc-second 30 m	https://earthexplorer.usgs.gov/
	TanDEM-X	0.4 arc-second 12.5 m	https://tandemx-science.dlr.de/
	ASTER	1 arc-second 30 m	https://search.earthdata.nasa.gov/search
Land use map	CORINE	1:100,000	https://land.copernicus.eu/pan-european/corine-land-cover/
	The GlobCover 2009	300 m	http://due.esrin.esa.int/page_globcover.php
Soil type map	FAO/UNESCO soil map of the world	1: 5 000 000	http://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/faounesco-soil-map-of-the-world/en/
	Digitized soil map of Vojvodina Province	1: 50,000	Internal GIS database
METEOROLOGICAL DATA			
Precipitation; Temperature; Relative humidity; Solar radiation; Wind speed	CFSR	38 km	https://swat.tamu.edu/data/cfsr
	CarpatClim	10 km	http://www.carpatclim-eu.org/pages/download/default.aspx
	Meteorological Yearbooks	/	http://www.hidmet.gov.rs/latin/meteorologija/klimatologija_godisnjaci.php

Some datasets were available at the local level (internal GIS database for soil type map or meteorological data from yearbooks), while others were downloaded from the global open-source datasets (Table 1).

Spatial data related to the catchment delineation

The DEMs loaded for the case study in this paper were chosen in the UTM projection system. The Shuttle Radar Topography Mission (SRTM) and the Advanced Spaceborne Thermal Emission and Reflection Radiometer, Version 3 (ASTER) DEM at a resolution of approximately 30 m and TanDEM-X at a spatial resolution of approximately 12.5 m served as data input. Accurate information on the topographic model of the Earth's terrain could be particularly critically important in hydrological models when the DEM represents the input layer (Roostaee and Deng, 2023). Raster data are represented as a matrix of cells where each cell contains information and is independent of the other cells. The SWAT model delineates the investigated basin and creates the catchment based on topographic attributes (slope, field slope length, channel lengths, channel width, etc.), estimating this information from the loaded DEM. As a result of using different spatial resolution DEMs in lowland catchment, significant geomorphologic changes, such as an improved representation of the surface and watershed characteristics (mainly to capture water bodies), are expected (Rocha et al., 2020). To illustrate the impact of the different resolution DEM layers on the catchment delineation, the percentage of matching of the created hydrographic network by the SWAT model with the real hydrographic network, extended by a buffer zone of 100 m, was calculated.

Land use

Land use maps are a valuable source of information for understanding the different features of the environment: the geographical distribution and state of natural and protected areas, the geographical distribution and abundance of wild fauna and flora, an abundance of natural resources (water bodies, arable land, forests, wetlands, etc.) as well as urban landscape characteristics. In addition to the numerous available databases providing land use data around the world, the most commonly used for the SWAT model is a product of the European land mapping project CORINE (CLC) with five main categories (artificial surfaces, agricultural areas, forests, and semi-natural areas, wetlands and water bodies) and 44 types of land use. To date, there are five created CLC databases for referenced years: 1990, 2000, 2006, 2012, and 2018. To compare the CLC datasets from 2006 and 2012, the GlobCover 2009 database were used in this research. The GlobCover 2009 land cover product is a 300-m land cover map and the information is in raster format. It is generated from an automated classification of MERIS Full Resolution time series and covers 22 globally characterized land classes based on the UN Land Cover Classification System from an independent reference dataset. The land use

information is classified into three main categories: closed-to-open shrubland, rainfed land, and closed-to-open forest. In this research, the proportion of the area of polygons representing different land use classes was calculated for 2006, 2009, and 2012.

Soil type

The most common compatible soil databases that can be loaded in the SWAT model are the open-source State Soil Geographic Database (STATSGO) and the Soil Survey Geographic Database (SSURGO). These databases are digital soil databases that include all the necessary data on soil parameters in vector format. Both are the most widely used soil databases for hydrological simulation models but cover only a limited part of the world. In addition, it is possible (or even necessary) to load other databases on soil types in the model, i.e., databases that are created or reprocessed by the user (Hao and Wu, 2023). For this research, the digitized soil map of Vojvodina and the FAO/UNESCO layer were used. The soil map of Vojvodina contains 231 soil types based on the general principles of the World Reference Base Classification System (Mrvić et al., 2016). At the European level, the FAO/UNESCO map contains 64 soil types that differ in their physical and chemical properties. For the study area, no comprehensive data on soil properties for the soil map of Vojvodina were available, so impact analysis on the catchment delineation could not be performed and discussed. Soil plays a key role in the hydrological cycle, so the use of high-resolution and high-quality soil-type data can be reflected in more efficient model performance and more accurate model results (Bhandari et al., 2018). Such information can be obtained from field observations and research, but the effort required to collect and statistically process newly created databases often requires time. Nevertheless, it is important to examine whether the available global databases meet the conditions of the research area, which was achieved by comparing the percentages of polygon areas of different soil types using two soil maps.

Precipitation data

The climatic conditions of the studied catchment area provide the parameters needed to manage the water balance by running the model. It is also possible to determine the relative impact of the individual components of the hydrological cycle. The meteorological inputs are required to be in text format and include daily precipitation, maximum and minimum daily temperature, solar radiation, wind speed, and relative humidity. It is important to point out that the required data are often missing (due to their length or lack of spatial coverage), so a time generator module in the SWAT could be used when necessary (Alodah and Seidou, 2019). The SWAT model also allows preparing and processing of meteorological data manually by the users. To do so, some of the statistical results need to be entered, such as average values, standard deviations, skewness values of temperatures, wind

speed, solar radiation, and sequences of wet or dry days derived from the precipitation data.

The most commonly used meteorological database for SWAT modeling is the Climate Forecast System Reanalysis (CFSR), whose precipitation data were used for this research. The CFSR database contain data interpolated at a spatial resolution of 38 km and it is available in SWAT file format and does not require additional time for processing. The second online available open-source database is from CarpatClim with 10 km grid resolution interpolated data. The disadvantage of this database is that it is limited to a certain region, in this case, the Carpathian Mountains. The third database included in the analysis is precipitation data from the Serbian Meteorological Yearbooks. The aim was to detect statistical differences in datasets based on true measurements and the interpolation of different gridded data. This was done by statistically analyzing the annual precipitation amount for the period 1979–2009.

Results and Discussion

Impact of the use of different DEMs

The hydrographic network affects the simulated hydrologic response of a catchment, which implies that accurate water streams are important for accurate model performance. Due to the availability of different databases of DEMs, the extraction of the hydrographic networks and the definition of the topography were performed automatically. The impact of DEM layer resolution on minimum elevation, maximum elevation, number of cells, cell size, cell area, number of sub-basins, and computational time was analyzed using three DEM layers: SRTM, Tandem-X, and ASTER (Table 2). The SWAT model divided the watershed of the selected KPRbuff into 19 sub-basins, and the input data led to the production of 214 HRUs by using the SRTM DEM. Watershed delineation when loading ASTER and TanDEM-X resulted in 16 and 19 sub-basins with 92 and 368 HRUs, respectively.

Table 2. Impacts of DEM resolution on watershed properties.

	SRTM (a)	TanDEM-X (b)	ASTER (c)
Minimum elevation [m]	12	12	9
Maximum elevation [m]	512	568	535
Number of cells	24,456	128,135	20,470
Cell size [m]	22.09 x 30.59	8.83 x 12.23	22.09 x 30.59
Cell area [ha]	0.0488	0.0093	0.0583
Number of sub-basins	19	22	19
Computational time [min]	10	180	5

The highest point of the National Park "Fruška Gora" is 539 m above sea level, hence using the aforementioned DEMs with recognized heights of 512 m, 568 m, and 535 m could result in misconceptions in the subsequent modeling processes. The number and size of cells that were automatically generated by loading one of the observed DEM layers indicate the degree of resolution of the model itself. Accordingly, TanDEM-X had the highest number of cells, which consequently increased the time to process such data. After loading TanDEM-X as an input layer, significant errors in terrain heights were noted, which affected the creation of the Danube River stream inappropriately (Figure 3b).

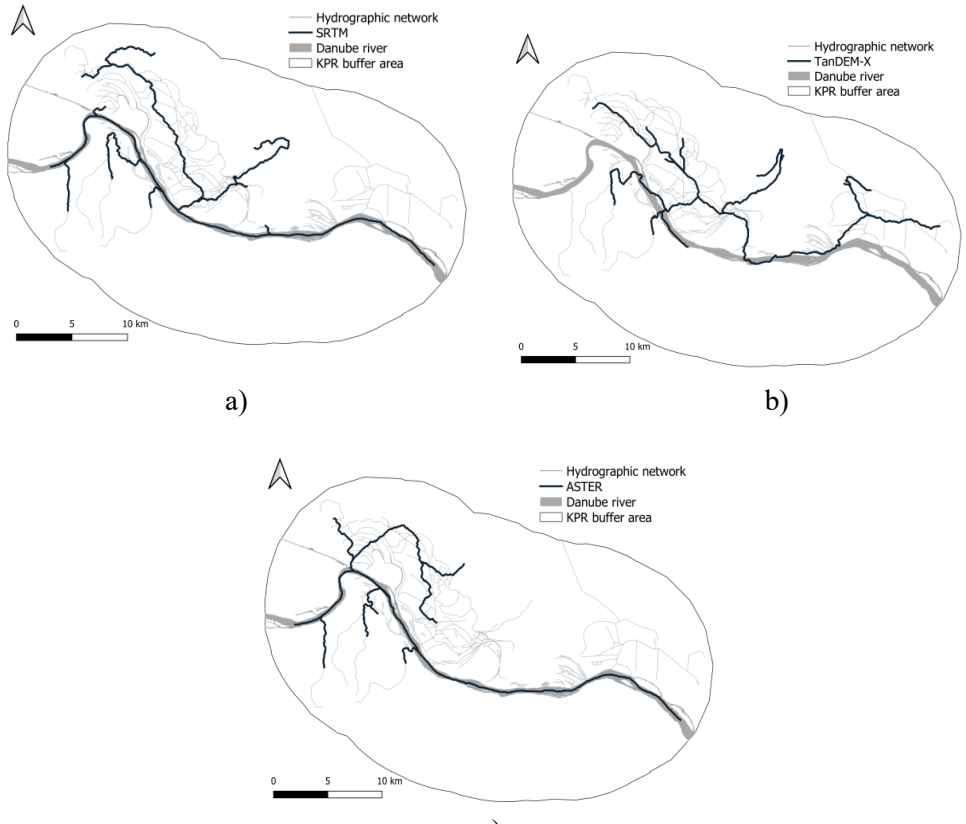

Figure 3. Different digital elevation models for KPRbuff derived by (a) SRTM, (b) TanDEM-X, and (c) Aster DEM.

Due to the lack of data in terms of no values (blank data) for the pixels of the water surfaces, TanDEM-X recognized surface water height as a special, unreal

height (-32,767 m). This caused difficulties in catchment delineation where the produced hydrographic network in the SWAT model only matched the real network up to 3%. Because the produced hydrographic network did not match the real network sufficiently, further analysis should not be performed using the above DEM layer. Additionally, the highest resolution TanDEM-X for this case needs to be further corrected before it can be used. The delineation of the catchment when loading two other DEM layers lasted much shorter and more than 70% of the hydrographic network that was created matched real data of the watercourse of the Danube River and drainage networks (Figure 3a, c).

Respectively, 72% of the hydrographic network matched the real network when SRTM was used as DEM, and 77% when ASTER was used. The lack of proper delineation of the drainage network, when using ASTER DEM and SRTM DEM, was also noted in Rana and Suryanarayana (2019), which is certainly a shortcoming of these DEM layers. The high percentage of correspondence between the created hydrographic network and the same number of created sub-basins implies that these two layers are convenient for further use. However, ASTER DEM should be preferred due to the shorter computation time required for catchment delineation.

Impact of the use of different land use datasets

A layer of land use is another dataset required for watershed delineation. The study results revealed that there were no noticeable changes in the percentages of recognized classes of CLC in the KPR land use between the years 2006 and 2012. There were no significant changes in urban areas, forest areas and water bodies as a result of the designation of the case study as a protected area. When the CLC land use maps were produced, urban areas made up 12% of the total KPRbuff, water surfaces accounted for 15%, and grasslands occupied 33%. By loading the GlobCover 2009 map, 37% of the total study area was found to be covered with mosaic crops, 36% with rainfed crops, and 10% of the total KPRbuff area was identified to be covered by mosaic vegetation. It has been shown how different land use classifications can be identified for the same area, and the study by Cüceloğlu et al. (2021) has shown that different land use datasets can be used successfully for SWAT modeling without having a major effect on the model outcome. For example, when using GlobCover 2009, one class of water bodies was identified, but when using the CLC layer, it can be assumed that several classes were identified as water surfaces. These results were influenced by the methodological classification, where GlobCover contained 22 land use classes and CLC contained 44 land use classes.

In addition to the vegetation cover, the CLC land use map also identified urban areas and water bodies. It can be concluded that the use of this layer contributed to a better understanding of the dynamics of anthropogenic activities in

that area. Since GlobCover 2009 was released in 2010 and there are no later versions, this type of data should not be used in hydrological models. Furthermore, up-to-date information on land cover is of major importance due to the accelerated ongoing processes that affect land cover and land use dynamics. Kmoch et al. (2022) illustrated that local high-resolution datasets can improve SWAT performance over the CLC data, but it has been noted by Szarek-Iwaniuk (2021) that a larger number of classes do not necessarily mean a larger amount of information.

Impact of using different soil type datasets

The last set of required data in the process of catchment delineation was the soil type map. Before loading the soil type database, an analysis of the soil types contained in the different databases was performed. The identification of the present soil types in the KPRbuff case study was determined by calculating the percentage of soil type polygons. By loading a digitized soil map of Vojvodina Province, it was found that ten soil types were recognized, and five soil types were identified by using the FAO/UNESCO soil map of the world (Table 3). Chernozems and Fluvisols, the dominant soil types in Vojvodina, were identified by loading both soil databases, and Vertisols, also a common soil type in Vojvodina, were identified only by loading in the digitized soil map. The fact is that the soil map of Vojvodina Province was created at a scale of 1: 50 000, which led to the production of a larger number of soil types with a lower percentage of coverage.

Table 3. The soil types on KPRbuff determined by calculating the percentage of soil polygons.

Digitized soil map of Vojvodina Province		FAO/UNESCO soil map of the world
Chernozems	54.3%	31%
Fluvisols	23%	56%
Cambisols	6.3%	13%
Vertisols	5.1%	
Plansols (0.1%), Solonchaks (0.1%), Solonetz (0.5%)		
Regosols (0.7%), Gleysols (2.9%), Leptosols (7.1%)		

The framework of SWAT modeling required additional soil characteristics that none of the databases used in this study contained. This step can be performed by the supplemental efforts of users to aggregate all the necessary soil type information. The KPR catchment delineation was completed by loading the available SWAT soil-type databases, STATSGO and SSURGO, to investigate the influence of different DEM layers on the identification of soil types (Table 4). The presented soil type percentage may indicate that different terrain models may affect

the production of the different percentages of soil types, even of different soil type categories. This can be induced by using available databases loaded from the SWAT (STATSGO and SSURGO) with adjusted soil properties that recognize soil types with similar properties. Cambisols are especially common in alluvials. KPR is located in a part of Serbia known as the Pannonian alluvial plain.

Table 4. Different soil types identified in the SWAT report on KPRbuff.

SRTM DEM		ASTER DEM	
Fluvisols	75%	Fluvisols	74%
Chernozems	24%	Chernozems	25%
Gleysols	1%	Cambisols	1%

The provided soil results demonstrate the potential for using various databases, i.e., information differing in precision about the type of land that can cause some of the problems. For example, it is possible that if the soil types differ significantly in their characteristics, the model can show inaccurate results of stream flow, sediment, and nutrient predictions, thus losing the purpose of modeling. Busico et al. (2020) highlighted that different spatial discretization schemes of soil types could contribute to the complexity of the model, influencing the production of a different number of HRU units. Similarly, users must generate and extract a large number of physical and chemical characteristics to use their self-created databases. This requires the time to collect all the data for the model, making the creation of the SWAT input database a tedious task.

Impact of the use of different meteorological datasets

The next section compares open-source databases with the precipitation time series (1979–2009) currently available for the Rimski Šančevi rainfall gauge station. The precipitation data from the Rimski Šančevi rainfall gauge station (45°07′01"N, 19°41′16"E), the highest priority station in Serbia, were used. On the territory of Vojvodina Province, where the case study is located, the amount of precipitation is unevenly distributed throughout the year – extremely rainy June and almost no precipitation in March and November.

Table 5. Statistical characteristics of mean annual precipitation for the Rimski Šančevi rainfall gauge (1979–2009).

	CarpatClim	Serbian meteorological yearbooks	CFSR
Average	632 mm	640 mm	724 mm
Median	612 mm	596 mm	729 mm
Min	290 mm	289 mm	364 mm
Max	954 mm	999 mm	940 mm

The average annual precipitation amount ranges from 550 to 600 mm (Malinović-Milićević et al., 2018) and the results in Table 5 show that the average precipitation from the CFSR database was 724 mm, which also affected the value of the median. A comparison of meteorological data from three databases showed similar dynamics of annual average precipitation for data formed locally and regionally, while data from the most commonly used global CFSR database showed small to significant (up to 30% of the overall average) deviations (Figure 4). The biggest difference in average annual precipitation was between the Serbian Yearbook data (519 mm) and the open-source CFSR (940 mm) in 1985. The aforementioned differences between the data can cause imprecise and incorrect results and lead scientists to wrong conclusions since precipitation is the most important flux in hydrological processes. There are numerous examples of high SWAT model performance using the CFSR database (Tomy and Sumam, 2016; Jaberzadeh et al., 2022), but there are also negative experiences confirmed, for example, by Zhu et al. (2016) and Duan et al. (2019).

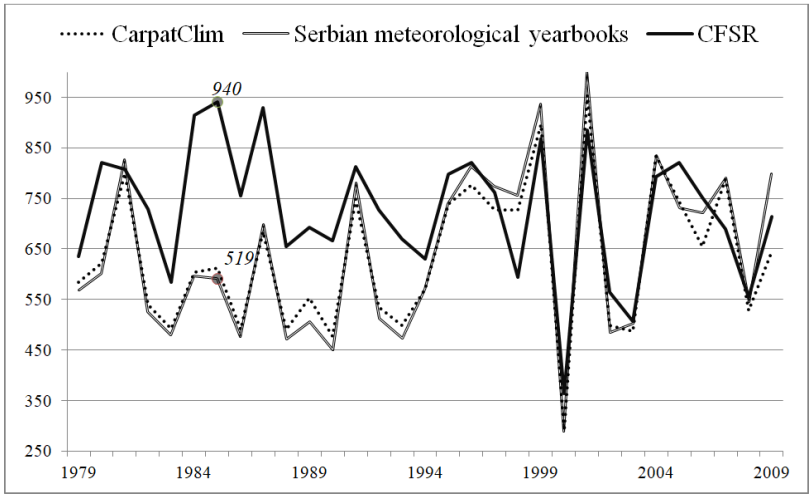

Figure 4. Average annual precipitation collected from different databases for 30 years.

Since the SWAT model takes into account both dry and wet days, the precipitation data need to be appropriate to represent the climate conditions to enable accurate predictions and impacts of climate change. Furthermore, based on the input precipitation data, the reliability of the simulation and the accuracy of the results depend on the input precipitation data, as concluded by Tobin and Bennett (2009). Although the resolutions (38 km, 10 km, and observed grid resolution) of the input data in the available databases vary widely, the authors suggest the use of

the higher resolution data in hydrological modeling of smaller areas that require additional data validation. In this case, the average precipitation values using CarpatClim highest-resolution interpolated data were similar to the values recorded at the gauge stations. However, this database cannot be used for further, subsequent analysis because the database has not been updated since 2010. Other databases containing the same type of climatic data include, i.e., WorldClim and Copernicus sources.

Conclusion

In river catchments, where various hydrological and climatic factors must be examined simultaneously, computer simulations are commonly used. This study assists researchers to select reasonable and feasible data for environmental models and comprehend how sensitive SWAT is to spatial resolution. For the Balkan region, which is covered in UTM zone 34N, additional data processing time and a coordinate transformation from a curved surface to a plane are required because the longitude and latitude system does not work in a rectangular coordinate system, but the SWAT model does. Although the choice of the hydrological model remains a preference of the researchers and the resolution of the model depends on the availability of data, the results of this study can provide guidelines for identifying an appropriate approach for future SWAT applications in similar floodplain regions. The availability of regional, national, and international climate and other datasets has allowed modelers to scale up hydrologic models from the global to the local scales. Undoubtedly, the local character of natural ecosystems must be taken into account, so that, if possible, the highest possible resolution of data should be used, since (1) data at local measurement stations can be missing or are not verified and are therefore uncertain; (2) the number and distribution of stations are not homogeneous; (3) data interpolations are accomplished by neglecting measurement uncertainties.

The aim of this research was to evaluate the effects of input data at different resolutions in the initial steps of SWAT modeling. The ASTER layer with a resolution of 30 m should be preferred (with appropriate adjustment if needed), as it showed a better percentage of matching in the building of the hydrographic network and the same soil types were recognized with the digitized soil map of Vojvodina. Additionally, the computation time for catchment delineation was shorter when loading the ASTER layer. Regarding precipitation data, interpolated data were better to use due to their higher resolution (10 km) and heterogeneous distributions of rain gauge stations in Vojvodina.

Unfortunately, high-resolution data are often not available for many regions around the world, especially in developing countries. Combining remote sensing techniques that can be used to obtain both climate and land cover data results in a

potent tool for the spatial analysis of hydrological changes. Thus, it is plausible to acquire the essential data required to make real and equitable decisions about the conservation and management of water resources by using high-resolution watershed models.

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UTICAJ ULAZNIH PROSTORNIH PODATAKA I PODATAKA O PADAVINAMA RAZLIČITE REZOLUCIJE NA FORMIRANJE SLIVNOG PODRUČJA PRIMENOM MODELA *SWAT*

Senka D. Ždero*, Zorica B. Srđević i Pavel P. Benka

Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za uređenje voda, Trg Dositeja Obradovića 8, 21000 Novi Sad, Srbija

R e z i m e

Kompjuterski zasnovani matematički modeli nalaze široku primenu u oblasti upravljanja vodama za predstavljanje, simulaciju i rešavanje ekoloških problema. Takva istraživanja zahtevaju interdisciplinarni, korisnički orijentisan, sveobuhvatni pristup koji integriše sve procese koji su prisutni u prirodi. Upotreba dostupnih hidroloških modela očekuje razumni nivo kvaliteta i kvantiteta ulaznih podataka kako bi se na pravi način predstavile karakteristike slivnog područja i kako bi se zadržale karakteristike lokalnog ekosistema. Jedan od modela koji ispunjava takve zahteve je model *SWAT* (engl. *Soil and Water Assessment Tool*). Cilj ovog rada je ispitivanje uticaja različite rezolucije ulaznih prostornih podataka i padavina na kreiranje slivnog područja primenom modela *SWAT* na primeru plavnog područja koje se nalazi u slivu reke Dunav u Srbiji. U radu je izvršeno istraživanje procene uticaja (1) digitalnih modela terena različite rezolucije, odnosno baza podataka *SRTM* i *ASTER* (30 m) i *TanDEM-X* (12.5 m), (2) baza podataka *CORINE* i *GlobCover* o načinu korišćenja zemljišta, (3) mape svetskog zemljišta *FAO/UNESCO* i digitalizovane pedološke mape Vojvodine na kreiranje slivnog područja. Istraživanje je sprovedeno uporedo sa analizom klimatskog parametra, padavina, koristeći podatke iz različitih izvora *CFSR*, *CarpatClim* i nacionalnih godišnjaka. Kada su u pitanju prostorni podaci, rezultati ukazuju da je podatke sa visokom rezolucijom (*TanDEM-X*) za ovo područje neophodno dodatno analizirati i prilagoditi, a sloj *ASTER* na prihvatljivom nivou odgovara za dalje zahteve modeliranja u modelu *SWAT*. Bolje je koristiti interpolisane podatke o padavinama, koji su izvedeni iz nacionalnih godišnjaka s obzirom na veću rezoluciju i heterogenu rasprostranjenost stanica.

Ključne reči: prostorni podaci, padavine, *SWAT*, hidrološko modeliranje.

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*Autor za kontakt: e-mail: senka.zdero@polj.uns.ac.rs

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INVESTIGATION OF THE NITROGEN AND PHOSPHORUS CONTENT IN ARABLE AGRICULTURAL LAND IN SERBIA

Divna Ž. Simić[1*], Snežana M. Janković[1], Vera M. Popović[2,3], Jela N. Ikanović[3,4], Sladjan R. Stanković[1], Sveto M. Rakić[3] and Petar R. Stevanović[5]

[1]Institute for Science Application in Agriculture, 68b Blvd despota Stefana, Belgrade, Serbia
[2]Institute of Field and Vegetable Crops, Maxim Gorky 30, Novi Sad, Serbia
[3]University of Bijeljina, Faculty of Agriculture, Pavlovića put bb, Bijeljina, B&H
[4]University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade-Zemun
[5]Technical Faculty, Agriculture, European University, Brčko District, Brčko, B&H

Abstract: The aim of this work was to determine the nitrogen and phosphorus content in arable agricultural land on farms in the Republic of Serbia. The sampling of soils was carried out in 2020 with the recording of GPS coordinates and entering the obtained results into the software platform. The analysis of the nitrogen content (N) was determined by the Kotzman method and calculated from the humus on a sample of 30,957 plots, with an area of 19,629.24 ha. The phosphorus content (P_2O_5) was determined by extraction, whereby the easily soluble part of the phosphorus was transferred into a solution and colorimetrically determined in the resulting extract, on a sample of 28,944 plots with an area of 18,459.96 ha. The results obtained show that 64.59% of the area, i.e., 12,678.81 ha, had a good nitrogen content (0.2–0.1%). A very high phosphorus content (400.1–500.0 mg kg^{-1} of the area) was found on 27.68% of the area, which is 5,108.92 ha. On 15.34% of the plots where a harmful value was found, additional analyses should be carried out to determine the cause of such a high phosphorus content in the soil. Based on data on the content of total nitrogen and readily available phosphorus in the soil, the type and amount of fertilizer can be adequately selected, which can lead to a reduction in ecosystem pollution. The results in this study represent the basis for soil management, biodiversity protection and optimal planning of cultivation of arable, vegetable and fruit crops. The obtained results can help agricultural producers and other entities to increase the competitiveness of their production.

Key words: soil, nitrogen and phosphorus content, GPS coordinates, resource conservation.

[*]Corresponding author: e-mail: dsimic@ipn.co.rs

Introduction

Soil is one of the most important natural resources of any country. As a basic means of agricultural production, its economic importance is determined by fertility, which represents the ability of the soil to provide the necessary nutrients, water, air, heat and other factors during the growing season. An important measure to preserve this resource is the control of soil fertility, which also plays a role in the proper use and reduction of the negative effects of uncontrolled fertilizer application, development and planning of agricultural production based on the principles of sustainable development and environmental protection.

The concept of a new generation of agricultural production requires soil protection and product quality improvement. For this reason, it is important to know the content of nitrogen and phosphorus and other macro and micro elements. Soil protection also significantly contributes to the preservation of agroecology (Ikanović and Popović, 2020). Moreover, the correct use of fertilizers is one of the most important factors because they contain necessary elements whose application (directly or indirectly) improves plant nutrition.

Fertilizers are any material of 1) natural or 2) synthetic origin (Manojlović and Čabilovski, 2019). Organic fertilizers are obtained from natural sources (livestock and poultry droppings, plant residues, biogas residues and agricultural by-products), while inorganic fertilizers are obtained from mineral deposits or produced from synthetic compounds.

Optimal and balanced nitrogen nutrition is important as a building block of plants (Bojović et al., 2014; 2022; Janković et al., 2021; Nožinić et al., 2022). Insufficient amounts negatively affect the yield and quality, and the first signs appear in older leaves, the formation and setting of fruits are reduced, the root system is longer, more elongated and less branched in the search for nutrients (Đekić et al., 2016; Popović et al., 2011; 2013; 2017; 2019; 2020; 2022; Lakić et al., 2018; 2020).

Plants take up nitrogen from the soil in the form of nitrate and ammonium ions, whereas legumes in symbiosis with nitrogen fixers can use elemental nitrogen from the atmosphere, transforming it into a form available for plants. It depends on soil fertility, plowed crop residues, organic matter content, preceding crops and nitrogen fertilization, yields, basic fertilization and climatic factors. It also depends on the type of soil, its use, temperature, humidity and water content in the soil. Due to its high mobility in soil, nitrogen can be lost very easily, mostly through evaporation and leaching. Some of the most important parameters that lead to nitrogen depletion from the soil are the form of nitrogen, soil properties, the timing of fertilizer application, improper fertilizer application and other factors (Simić et al., 2020).

Increased nitrogen efficiency can be achieved by applying appropriate fertilizers at the optimal time (Janković et al., 2020a). The needs of plants for nitrogen are different, and harmful consequences can arise from both nitrogen deficiency and nitrogen excess. An excess of nitrogen can cause more problems than a slight deficiency. Due to the excess of nitrogen, plants are less resistant to various stressful conditions. Large amounts of nitrogen can result in bad taste of fruits, but more importantly, those fruits are less safe for human health (Glamočlija et al., 2015; Ikanović et al., 2020). Large amounts of nitrogen in the soil can have a detrimental effect in the case of leaching, contaminating groundwater and disrupting biodiversity.

Phosphorus is a necessary element for the development of generative organs, plant growth, and cell division, better rooting of plants, seed and fruit development, early fruit ripening, and for increased plant resistance to diseases. The lack of phosphorus slows down the growth of the plant, the formation of fruit and leaf buds. Such leaves are initially dark green, and later they turn red purple. An excess of phosphorus rarely occurs, but when it does, it causes a reduced growth of plants, with dark brown spots on the leaves (Glamočlija et al., 2015). Phosphorus is a non-renewable resource, and modern research focuses on a more efficient use of phosphorus fertilizers, its recycling and the use of alternative sources (Dawson and Hilton 2011). The amount of remaining fossil phosphate resources in the world is uncertain, but practically finite. Thus, fossil P resources may become depleted by ongoing mining. Despite calls for resource conservation, fossil P resources are being depleted at an increasing rate. More than 90% of current phosphorus resources are still used as fertilizer in agriculture (Reijnders, 2014). Therefore, increasing the efficiency of using phosphorus from fertilizers and, accordingly, reducing the pressure on the environment, and decreasing the demand for fossil phosphorus is a primary goal. The mobility of phosphorus in the soil is limited and therefore the roots of plants can only take up phosphorus from their immediate environment (Glamočlija et al., 2015). Plants absorb a certain amount of phosphorus that they need at that moment, and the rest remains in the soil. Phosphorus ions that have not been adopted by the plants remain free and unstable in the soil and as such can quickly bind to manganese, iron and calcium ions, all depending on the pH value of the soil (Janković et al., 2020b). These reactions create insoluble compounds in the soil and thus phosphorus becomes unavailable to the plants. The leaching of phosphorus originating from mineral fertilizers and manure from the soil is the cause of water quality degradation and damage to biodiversity.

The aim of this study was to determine the content of nitrogen and phosphorus in arable agricultural land on farmers' plots.

Material and Methods

The soil samples for the determination of the nitrogen content were taken from 30,957 farm plots, comprising the area of 19,629.24 ha, and those for the phosphorus content from 28,944 plots, comprising the area of 18,459.96 ha.

The samples were taken from arable agricultural areas after the harvest of arable and vegetable crops (maize, wheat, alfalfa, clover, peppers, tomatoes, potatoes).

An Android application was used to record the GPS coordinates on the Android6+ operating system. It recorded the location coordinates, date and time, the cadastral municipality number and the plot number. The data were then automatically sent to the software. The data on the sampler, the soil class, the sampling depth and the preceding crop were also recorded in the software. The samples were prepared and delivered to accredited laboratories (SRPS ISO/IEC 17025 standard).

The nitrogen content (Table 1) was determined from the humus (Aksentijević et al., 2017; Džamić et al., 1996), and the readily available phosphorus (Table 2) according to the AL method of Egner and Riehm, 1960.

Table 1. Total nitrogen content (%) (calculated from humus).

Soil nitrogen	Of limited capability	Very poor	Poor	Medium secured	Well provided	Rich	Very rich
% Nitrogen	<0.02	0.03–0.02	0.06–0.03	0.1–0.06	0.2–0.1	0.3–0.2	>0.3

Table 2. The content of easily accessible phosphorus (P_2O_5) according to the AL method of Egner and Riehm.

Phosphorus in the soil	Very low	Low	Medium	Optimal	High	Very high	Harmful
P_2O_5 mg kg^{-1}	<50.0	50.1–100.0	100.1–150.0	150.1–250.0	250.1–400.0	400.1–500.0	>500.1

Statistical analyses: Statistical tests were carried out by the statistical package and descriptive statistics and presented in tabular and graphical form.

Results and Discussion

The study covered large areas of agricultural land of individual farmers. Modern methodologies and technologies were used to obtain the results presented in the study. The application of digital technologies, in addition to monitoring

functions, is an important tool in plant nutrition management, providing the necessary information on the nutritional status of the soil.

In the arable layer of cultivated soils, the content of total nitrogen is usually between 0.1 and 0.2%. Most of it (over 90%) is contained in organic compounds of the soil, while it is only a few percent in the inorganic form (mineral $NH4^{+}$, $NO2^{-}$, $NO3^{-}$), which is mostly taken up by the plants. Considering that it is not possible to precisely assess the supply of these nutrients in the crop based on the total amount of nitrogen in the soil, the value of this analysis is a useful indicator, especially when analyzing smaller agroecological units, on a farm and on the same type of soil.

The results of the N content on a sample of 30,957 plots comprising the area of 19,629.24 ha show that 64.59%, i.e., 12,678,81 ha, had a good level of nitrogen availability (0.2%–0.1%), calculated based on the humus content. Moreover, 23.39% of the examined plots, i.e., 4,590.34 ha, were rich in nitrogen (0.3%–0.2%), 7.73% of the examined areas, i.e., 1,518.26 ha had medium nitrogen levels (0.1%–0.06%), while 3.23% of the examined areas, i.e., 634.07 ha were very rich in nitrogen (> 0.3%). A poor nitrogen content was found on 0.81% of the surveyed plots, i.e., 159.01 ha, (0.06%–0.03%) and a very poor nitrogen content (0.03%–0.02%) on 0.14% of the examined plots, i.e., 28.31 ha. Furthermore, 0.10% of the examined plots, i.e., 20.54 ha, had a limited ability to provide nitrogen (<0.02%) (Table 3, Figure 1).

This indicates that on the examined farm plots, the largest percentage of plots, 64.59%, were well supplied with nitrogen, so farmers in these areas should use formulations with an appropriate nitrogen content when choosing fertilizer.

Table 3. Total nitrogen content (%).

Plot number	Area of analyzed plots (ha)	Nitrogen content from humus (%)	Nitrogen content in the total area (%)
PNo 1	634.07	> 0.3	3.23
PNo 2	4,590.34	0.3–0.2	23.39
PNo 3	12,678.81	0.2–0.1	64.59
PNo 4	1,518.26	0.1–0.06	7.73
PNo 5	159.01	0.06–0.03	0.81
PNo 6	28.31	0.03–0.02	0.14
PNo 7	20.54	< 0.02	0.10
Total	19,629.34	-	100
IV	-	-	64.49
Std. dev.	-	-	23.75

Phosphorus is a non-metal, usually found in the five-valent form in nature. Due to its reactivity, it is not available in free form, but as part of numerous organic and non-organic compounds. It originates from about 170 minerals (apatite, phosphorite, vivianite, amblygonite, monazite, wavellite, triplite), with apatite and

phosphorite accounting for the largest proportion of around 90% (Gudžić, 2015). Delgado and Torrent (1997) emphasize the importance of having the balance between the optimal phosphorus content in the soil for agricultural production and ecological and economic security, in which phosphorus fertilizers play a significant role.

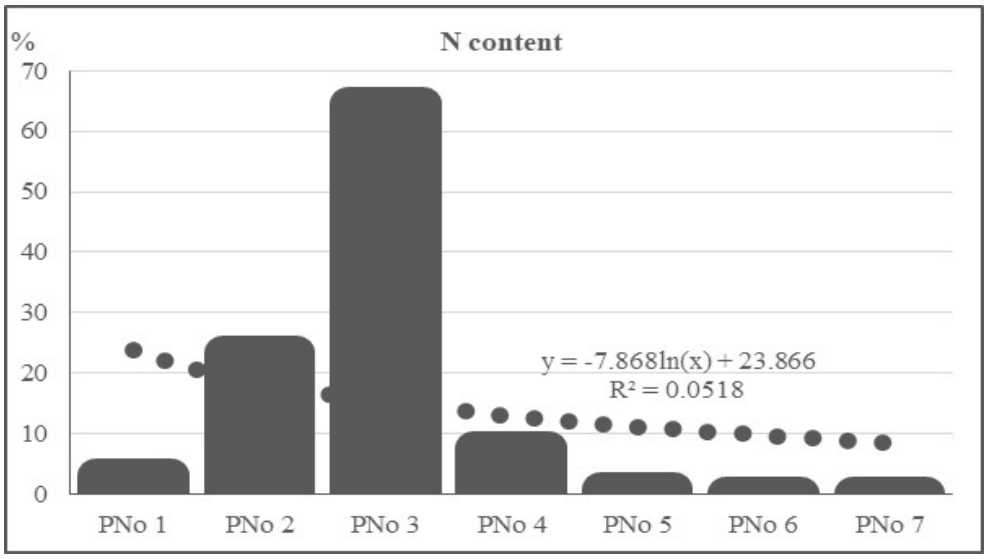

Figure 1. Nitrogen content (%) in the investigated soils in Serbia.

The phosphorus content was determined on a sample of 28,944 plots, comprising the area of 18,459.96 ha.

A very high phosphorus content (400.1–500.0 mg kg-1) was found on 27.68% of the examined plots, i.e., on 5,108.92 ha. On 18.22% of the plots, i.e., on 3,363.25 ha, an average and optimal phosphorus content was detected (100.1–250.0 mg kg^{-1}). A high phosphorus content (250.1–400.0 mg kg-1) was found on 17.37% of the surveyed plots, i.e., on 3,206.59 ha. Moreover, a low phosphorus content was determined (50.1–100 mg kg-1) on 17.25% of the plots, i.e., on 3.183,53 ha. A harmful phosphorus content (>500.0 mg kg^{-1}) was detected on 15.34% of the plots, i.e., 2,832.5 ha. Finally, 4.15% of the plots, i.e., 765.17 ha, were found to have a very low level of phosphorus (< 50.0 mg kg^{-1}) (Table 4, Figure 2).

The result shows that the agricultural soils on the largest percentage of plots (27.78%) had a very high phosphorus content, so farmers in these areas should pay special attention and choose fertilizer formulations without phosphorus or with a lower phosphorus content. Moreover, additional analyses should be carried out on

15.34% of the plots with harmful content in order to determine the cause of such a high phosphorus content in the soil.

Table 4. Content of readily available phosphorus (P_2O_5).

Plot number	Plot area (ha)	Content of readily available phosphorus P_2O_5 (mg kg^{-1})	Content of P_2O_5 in the total analyzed area (%)
PNo 1	765.17	< 50.0	4.15
PNo 2	3,183.53	50.1–100.0	17.25
PNo 3	3,363.25	100.1–250.0	18.22
PNo 4	3,206.59	250.1–400.0	17.37
PNo 5	5,108.92	400.1–500.0	27.68
PNo 6	2,832.5	>500.1	15.34
Total	18,459.96	-	100
IV	-	-	23.53
Std. dev.	-	-	7.52

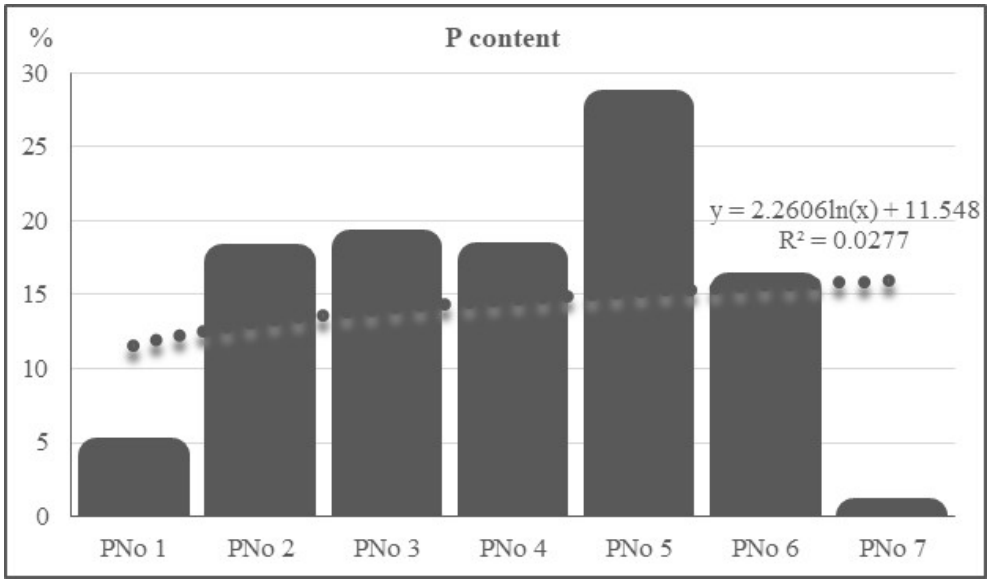

Figure 2. Phosphorus content (mg kg^{-1}) in the examined soils in Serbia.

Conclusion

Based on the obtained results, the following conclusions can be drawn:

The largest percentage of the examined farm plots, 64.59%, were well supplied with nitrogen, and farmers should choose fertilizer formulations with a correspondingly lower nitrogen content.

When it comes to phosphorus, the largest percentage of plots (27.78%) had a very high phosphorus content. On these areas, farmers should pay special attention and use formulations without phosphorus or with a lower phosphorus content when choosing fertilizers.

As for 15.34% of the plots on which harmful phosphorus levels were detected, some additional analyses should be done to determine the reason for such high phosphorus levels in the soil.

The obtained results can enable the optimal application of fertilizers as a prerequisite for the production of healthy and safe food for humans and animals, adaptation to climate change and environmental protection. The aforementioned is a basis for the sustainable development of farmers, the strengthening of environmental awareness and the preservation of biodiversity.

The obtained results can help farmers and other subjects engaged in agricultural production receive the right piece of affordable advice and therefore increase the competitiveness of their production.

Based on the data on the content of total nitrogen and readily available phosphorus in the soil, the type and amount of fertilizer can be adequately selected, which can lead to a reduction in nitrate and phosphate leaching and pollution of ecosystems.

The obtained results can contribute to the development and planning of agricultural production based on the principles of sustainable development and reducing the negative effects of uncontrolled fertilizer application.

The results represent the starting point for the introduction of good practices: rational land management, biodiversity protection, designing appropriate land use systems, improving soil quality, correct application of fertilizers and planning the cultivation of arable, vegetable and fruit crops.

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SADRŽAJ AZOTA I FOSFORA U OBRADIVOM POLJOPRIVREDNOM ZEMLJIŠTU SRBIJE

Divna Ž. Simić[1*], Snežana M. Janković[1], Vera M. Popović[2,3], Jela N. Ikanović[3,4], Sladjan R. Stanković[1], Sveto M. Rakić[3] i Petar R. Stevanović[5]

[1]Institut za primenu nauke u poljoprivredi,
Bulevar despota Stefana 68b, Beograd, Srbija
[2]Institut za ratarstvo i povrtarstvo Novi Sad, Maksima Gorkog 30, Novi Sad, Srbija
[3]Univerzitet u Bijeljini, Poljoprivredni fakultet, Pavlovića put bb, Bijeljina, B&H
[4]Univerzitet u Beogradu, Poljoprivredni fakultet, Nemanjina 6, Beograd – Zemun
[5]Tehnički fakultet, Poljoprivreda, Evropski Univerzitet, Brčko Distrikt,
Brčko, B&H

R e z i m e

Cilj ovog rada bio je da se utvrdi sadržaj azota i fosfora na obradivom poljoprivrednom zemljištu u Republici Srbiji na poljoprivrednim gazdinstvima. Uzorkovanje zemljišta sprovedeno je u 2020. godini uz evidentiranja GPS kordinata i evidencije dobijenih rezultata u softverskoj platformi. Analiza sadržaja azota (N) određena je metodom Kotzman i izračunata iz humusa, na uzorku od 30.957 parcela, površine 19.629,24 ha. Sadržaj fosfora (P_2O_5) određen je ekstrakcijom, prevođenjem lako rastvorljivog dela fosfora u rastvor i kolorimetrijski određen u dobijenom ekstraktu, na uzorku od 28.944 parcele površine 18.459,96 ha. Dobijeni rezultati su pokazali da 64,59% površina, odnosno 12.678,81 ha, ima dobar nivo azota (0,2–0,1%). Na 27,68% površina, što iznosi 5.108,92 ha, utvrđen je veoma visok nivo fosfora (400,1–500,0 mg kg^{-1} zemljišta). Na površinama od 15,34% na kojima je utvrđen štetni sadržaj potrebno je uraditi dodatne analize kako bi se utvrdio uzrok tako visokog sadržaja fosfora u zemljištu. Na osnovu podataka o sadržaju ukupnog azota i lako dostupnog fosfora u zemljištu, može se adekvatno odabrati vrsta i količina đubriva, što može dovesti do smanjenja zagađenja ekosistema. Rezultati u ovoj studiji predstavljaju osnovu za upravljanje zemljištem, zaštitu biodiverziteta i optimalno planiranje gajenja ratarskih, povrtarskih i voćarskih kultura. Dobijeni rezultati mogu pomoći poljoprivrednim proizvođačima i drugim subjektima da povećaju konkurentnost svoje proizvodnje.

Ključne reči: zemljište, sadržaj azota i fosfora, GPS kordinate, očuvanje resursa.

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[*]Autor za kontakt: e-mail: dsimic@ipn.co.rs

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PHENOTYPIC DIVERSITY ASSESSMENT OF MOROCCAN LOQUAT USING MULTIPLE CORRESPONDENCE ANALYSIS

Ghizlane Kabiri[1], Oussama Kodad[2], Francisca Hernandez[3], Fatima Zahra Lachkham[1], Said Ennahli[2] and Hafida Hanine[1*]

[1]Laboratory of Industrial Engineering and Surface Engineering, Faculty of Science and Techniques, Sultan Moulay Slimane University, Beni Mellal, Morocco
[2]Department of Horticulture and Viticulture,
National School of Agriculture, Meknes, Morocco
[3]Center for Agri-food and Agri-environmental Research and Innovation (CIAGRO-UMH) Miguel Hernández University of Elche, Alicante, Spain

Abstract: The loquat fruit has a very important commercial value due to its benefits for human health. However, there is very limited scientific research on this species in Morocco. In this regard, a set of 35 genotypes was collected from the Zegzel valley (Berkane). The phenotypic variability was evaluated using nine traits related to fruit and leaf. The results revealed a coefficient of variation ranging from 13.02 to 42.21%, implying a large phenotypic variation in Moroccan loquat, especially for the characteristics associated to the fruit shape. Regarding the multiple correspondence analysis, the first two axes explained 62.57% of the total variance. The major traits that made it possible to distinguish between the genotypes were those related to fruit size. Therefore, the Mekerkba genotype in the Zegzel region is not a single variety, but rather genotypes with a round fruit shape. In addition, the 35 genotypes studied were divided into three main groups regardless of their geographical origin. The results indicate that the geographical proximity did not play an important role in the structure of genotypes, implying a weak adaptation of the genotypes to the environment. The findings of this study could be used in conventional breeding and in situ conservation programs for Moroccan loquat.

Key words: *Eriobotrya japonica*, phenotypic variability, quantitative traits, coefficient of variation, multiple correspondence analysis.

Introduction

Loquat (*Eriobotrya japonica* Lindil.), belonging to the *Rosaceae* family, is an evergreen tree native to China. Since the 19th century, the loquat tree has been

*Corresponding author: e-mail: h.hanine@usms.ma

widely cultivated throughout the world for commercial and health purposes (Gariglio et al., 2002). The loquat fruit is consumed as fresh fruit or juice due to its excellent flavor and high nutritional values (Sun et al., 2020). Globally, China and Japan are the leading loquat producers, while Spain and Turkey have been the most important producers in the Mediterranean region over the last 20 years (Pinillos Villatoro and Hueso Martin, 2011).

In Morocco, the loquat is cultivated both as a commercial food crop and an ornamental crop for its yellow fruits (Hussain et al., 2011). This species is found in several areas throughout the country, but it is practically concentrated in the Berkane region, especially in the Zegzel valley, producing over 10,000 tons in 2021. The genotypes planted are unknown and the distinction between the most planted individuals in this region is based on the shape of the fruit, considering that the fruits of round shape and large size (called mkerkeba) are the most appreciated by the consumer (ORMVAM, 2021).

When evaluating plant germplasm, it is crucial to measure the extent of available genetic diversity (Zubair et al., 2007). In this regard, the application of various statistical tools is an efficient strategy for classifying germplasm and analyzing genetic association among breeding materials (Mohammadi and Prasanna, 2003). The multiple component analysis (MCA) was applied to study the qualitative characteristics of 35 loquat genotypes from the Zegzel valley in order to improve the identification of the loquat genotypes and contribute to their selection and conservation in Morocco.

Material and Methods

Plant material

During April and May 2016, a total of 5 healthy fruits and 10 well-developed leaves of 35 genotypes were randomly selected from the Zegzel valley, the main growing area of loquat in Morocco (Table 1, Figure 1).

Table 1. Plant material studied and corresponding codes.

Geographical origin	Genotype codes	Number of samples
Takerboust	T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12,	12
Taghsrout	TA1, TA2, TA5, TA6, TA7, TA8, TA9, TA13, TA14	9
Tazaghin	TZN1, TZN2, TZN3, TZN4	4
Zegzel	Z1, Z2, Z3, Z4, Z5, Z6, Z7, Z8, Z16, Z17	10
Total		35

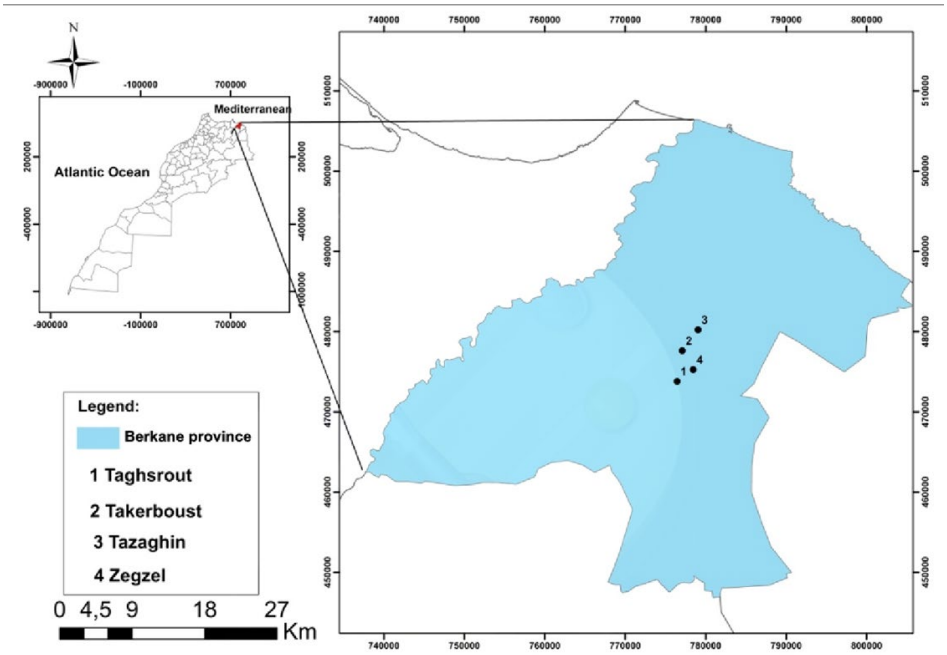

Figure 1. The sampling location of the studied loquat genotypes.

Phenotypic analysis

Nine qualitative traits related to fruit and leaf were analyzed according to the descriptor of the International Union for the Protection of Plants (UPOV, 1998) (Table 2). The selection of the studied traits was based on their economic importance as well as the possibility of their introduction in the improvement programs of this species.

Table 2. Phenotypic parameters analyzed in this study.

Traits	Code	Phenotype
Fruit shape in longitudinal section	FFL	1. Elliptic, 2. Broad elliptic, 3. Round, 4. Oblate, 5. Broad obovate, 6. Obovate
Fruit shape in transverse section	FFT	1. Round, 2. Slightly angular, 3. Strongly angular
Fruit shape at the stalk end	FPP	1. Acute, 2. Obtuse, 3. Round
Shape of the leaf apex	FS	1. Sharp acute, 2. Blunt acute, 3. Rounded
Shape of the seed	FG	1. Elliptic, 2. Round, 3. Obovate
Color of flesh	CCH	1. White 2. Yellowish white 3. Yellow 4. Orange yellow 5. Orange
Green color of leaf's upper side	CVFS	3. Light, 5. Medium, 7. Dark
Attitude of leaf in relation to shoot	PPP	1. Upwards, 2. Outwards, 3. Downward
Density of margin serrations	DD	3. Loose, 5. Medium, 7. Dense

Statistical analysis

The data obtained were subjected to several measurements, such as the general mean, standard deviation and coefficient of variation (CV). This latter was calculated using the following formula: CV (%) $=\frac{SD}{\bar{X}} \times 100$. Moreover, the multiple correspondence analysis (MCA) and the hierarchical cluster analysis were performed according to the quantitative traits related to fruits and leaves to determine the most discriminant variables and modalities and to assess the genetic structure of the loquat genotypes. This analysis was performed using the XLSTAT software.

Results and Discussion

Phenotypic variation

The mean values, standard deviation and coefficient of variation of nine traits related to loquat fruit and leaf are shown in Table 3. The CV parameter is independent of a unit of measurement, meaning that it is more powerful when comparing measured characteristics (Khadivi-Khub and Etemadi-Khah, 2015). The results obtained revealed a wide range of CV with the values ranging from 13.02 to 42.21%. Pomological characteristics, especially those related to the fruit shape, showed the greatest variation, with a CV of 42.21% for fruit shape at the stalk end (FPP) and 40.20% for fruit shape in transverse section (FFT). In contrast, the lowest variation in leaves was found in the density of the serration of margin (DD) (13.2%). The remaining traits showed a CV of over 17%, indicating high levels of phenotypic variation. In the Moroccan loquat, the acute and obtuse shapes were the most frequent for the fruit shape at the stalk end trait and the slightly angled shape was the most dominant trait in the fruit shape in transverse section. In addition, the color of the flesh varied between orange-yellow and orange. Similarly, the results of the morphological observation of loquat in Karo, Dairi and Simalungun districts showed uniform leaf morphology, while the fruit and seed showed diverse characters within the genotypes studied (Elimasni and Nasution, 2021). This variability could be explained by propagation by the seeds used by the farmers, which causes very high polymorphism. In addition, the woody species, the outcrossing reproductive system and wind dispersal show greater genetic diversity within the species and the population (Hamrick et al., 1991). The occurrence of the same characters between varieties is caused by the presence of genes composing the same phenotype, although they are influenced by different environments (Jayanti, 2018).

Table 3. The mean values, standard deviation and coefficient of variation of the traits measured in the 35 genotypes.

Genotypes	FFL	FFT	FPP	CCH	FG	PPP	CVFS	FS	DD
T1	6	2	1	5	1	1	5	2	7
T10	5	1	1	5	1	1	3	2	5
T11	4	1	2	3	2	1	5	1	7
T12	4	1	3	3	1	2	3	1	7
T2	6	1	2	4	1	1	3	1	7
T3	6	3	1	5	1	1	7	1	7
T4	6	2	1	3	1	1	5	1	7
T5	6	3	1	5	1	1	3	1	7
T6	6	1	1	4	2	1	5	1	7
T7	6	2	1	4	1	1	3	2	7
T8	6	3	2	5	1	1	5	2	7
T9	4	1	2	4	2	2	3	1	7
TA1	4	2	2	5	2	2	7	1	7
TA13	4	2	3	5	2	1	5	1	7
TA14	5	2	3	3	1	1	3	2	5
TA2	6	2	2	4	1	1	5	2	7
TA5	6	2	1	4	2	2	7	1	7
TA6	5	3	1	3	1	2	5	1	5
TA7	5	1	2	4	1	1	7	1	7
TA8	4	3	2	5	1	1	7	2	5
TA9	4	3	3	5	2	1	7	1	7
TZN1	5	2	1	4	1	1	7	1	7
TZN2	5	3	2	4	1	1	7	2	5
TZN3	5	2	2	3	1	1	7	1	5
TZN4	5	2	1	5	1	1	7	1	7
Z1	6	2	1	4	1	1	7	1	7
Z16	4	1	3	5	2	2	7	1	7
Z17	4	1	3	5	2	2	7	1	7
Z2	6	2	2	3	1	2	7	1	7
Z3	6	2	1	5	1	1	7	1	7
Z4	6	2	2	4	2	1	5	1	5
Z5	4	3	2	4	1	1	7	2	7
Z6	4	1	3	3	1	1	7	1	7
Z7	6	1	3	4	1	1	7	2	7
Z8	4	1	3	3	1	1	5	2	5
Mean	5.09	1.89	1.89	4.11	1.29	1.23	5.63	1,31	6.54
SD	0.89	0.76	0.80	0.80	0.46	0.43	1.59	0,47	0.85
CV (%)	17.44	40.20	42.21	19.35	35.65	34.68	28.28	35,84	13.02

Multiple correspondence analysis

Eigenvalues and percentage of variance

According to the MCA applied to the qualitative characters of the fruit and the leaf, the first two axes explained 45.02 and 17.54% of the variance, respectively, corresponding to a total explanation of 62.57% (Table 4). The contributions of each modality to the total variance are summarized in Table 5. The traits that mainly contributed to the formation of axis 1 were modalities 4 and 5 of the fruit shape on

longitudinal section (FFL), modality 1 of the fruit shape on transverse section (FFT), modalities 1 and 3 of the fruit shape at the stalk end (FPP), modality 2 of the seed shape (FG), modality 5 of the density of margin serrations, modalities 1 and 2 of the shape of the leaf apex (FS), and modality 2 of the leaf attitude in relation to the shoot (PPP). Regarding axis 2, it was mostly explained by modalities 5 and 6 of the shape of the fruit on longitudinal section (FFL), modality 2 of the shape of the fruit on transverse section (FFT), modalities 1 and 3 of the shape of the fruit at the stalk end (FPP), modality 3 of the color of the flesh (CCH), modality 2 of the shape of the leaf apex (FS), and modalities 5 and 7 of the density of margin serration (DD). According to this explanation, the characteristics related to fruit size were the major traits allowing the distinction between the loquat genotypes.

Table 4. The percentage of total variance explained by the two axes.

Axis	F1	F2
Eigenvalue	0.29	0.22
Variance %	45.02	17.54
Cumulative	45.02	62.57

Table 5. The contribution of the variables in the first two axes.

Modality	F1	F2
FFL-4	1.08	0.46
FFL-5	-0.79	0.77
FFL-6	-0.45	-0.78
FFT-1	0.72	0.39
FFT-2	-0.34	-0.5
FFT-3	-0.45	0.35
FPP-1	-0.57	-0.67
FPP-2	-0.1	0.19
FPP-3	0.97	0.7
CCH-3	0.15	0.87
CCH-4	-0.31	-0.41
CCH-5	0.21	-0.2
FG-1	-0.46	0.16
FG-2	1.14	-0.4
PPP-1	-0.34	0.02
PPP-2	1.15	-0.07
CVFS-3	-0.12	0.4
CVFS-5	-0.17	-0.05
CVFS-7	0.14	-0.12
FS-1	0.34	-0.28
FS-2	-0.74	0.62
DD-5	-0.66	1.31
DD-7	0.2	-0.39

Therefore, the Mekerkba genotype in the Zegzel region, which is highly appreciated by the consumer, is not a variety but a genotype with round fruits, yellow-orange flesh and a sweet taste. In contrast, many authors have noted that morphological and agronomic characteristics used to measure genetic diversity in some populations often do not allow the identification of discrete taxonomic groups since most plant characteristics are influenced by environmental factors and exhibit continuous variation and a high degree of phenotypic plasticity (Rabelo et al., 2020).

The multiple correspondence analysis (MCA) results showed a large dispersion of genotypes along axes 1 and 2. The bi-dimensional graph of the genotypes and the modalities (Figure 2) as well as the dendrogram (Figure 3) classified the genotypes studied into three main groups regardless of their geographical origin. The MCA results indicate that geographical proximity did not play an important role in the differentiation and structure of the genotypes, suggesting a weak adaptation of genotypes to their environment. The first group (G1) was divided into two subgroups. The first (G1.1) contained the following genotypes: T7, T2, T5, T10, T6, T4, T8, T1 and TA2. These genotypes, located on the negative part of axes 1 and 2, were characterized by fruits with a slightly angular, obovate shape, an acute fruit shape at the stalk end, orange-yellow flesh, and leaves of medium green color on the upper side. The second subgroup (G1.2) consisted of four genotypes such as TA13, T11, T12 and T9. The genotypes of this group were located on the positive part of axis 1. The fruits had an acute fruit shape at the stalk end and orange flesh. The leaves of these specimens were dark green with densely serrated margins. The second group (G2) included the genotypes TA14, Z8, TA6, Z4, TA8, TZN2 and TZN3. These genotypes were characterized by broad obovate and strongly angular fruits, with an obtuse fruit shape at the stalk end. The leaves were blunt acute, light green and directed upwards in relation to the shoot, while the shape of the seed seemed to be elliptical. The last group (G3) was bifurcated into two subgroups. The first set (G 3.1) included genotypes TA9, Z5, Z17, Z16, TA1, TA7, Z6 and Z7. The genotypes of this group were located on the positive part of axis 2 with round oblate fruits, yellow flesh and a rounded fruit shape at the stalk end. The second subgroup (G 3.2) consisted of TA5, Z2, Z3, Z1, T3, TZN4, TZN1. These genotypes were located on the negative part of axes 1 and 2 and were characterized by fruits oblate with a slight angular shape, an acute fruit shape at the stalk end, yellow-orange flesh and a medium green color of the upper side of the leaf. According to these results, the genotypes studied show a great differentiation, particularly in the shape and color of the fruit. In addition, the 35 Moroccan loquat genotypes were classified into three different groups even though they belonged to the sites which are geographically close to each other and have similar climatic conditions, indicating a weak effect of environmental conditions on the loquat genotypes. However, several studies have shown considerable

plasticity and adaptability of the species to various environmental conditions (Drobná, 2010). The genetic diversity observed in the Moroccan loquat could be due to the seed propagation practiced by farmers, which can generate a large polymorphism.

Figure 2. The graphical representation of the 35 genotypes and modalities.

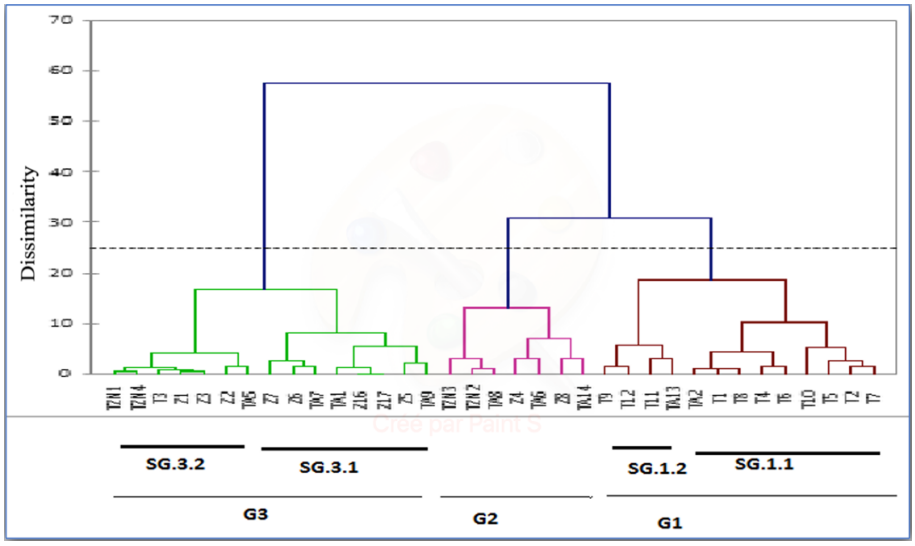

Figure 3. The dendrogram of the 35 genotypes based on nine qualitative traits.

Conclusion

Based on the results of the phenotypic characterization of Moroccan loquat, the studied genotypes showed a great diversity, especially in terms of fruit shape and color. In addition, the Mekerkba genotype in the Zegzel valley is just a set of genotypes with round fruits and orange-yellow flesh. The high phenotypic variability of the genotypes should be exploited to develop strategies for the conservation and improvement of the Moroccan loquat crop to satisfy the needs of farmers, producers and consumers.

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PROCENA FENOTIPSKE RAZNOLIKOSTI MAROKANSKOG LOKVATA KORIŠĆENJEM VIŠESTRUKE KORESPONDENTNE ANALIZE

Ghizlane Kabiri[1], Oussama Kodad[2], Francisca Hernandez[3], Fatima Zahra Lachkham[1], Said Ennahli[2] i Hafida Hanine[1*]

[1]Laboratory of Industrial Engineering and Surface Engineering, Faculty of Science and Techniques, Sultan Moulay Slimane University, Beni Mellal, Morocco
[2]Department of Horticulture and Viticulture,
National School of Agriculture, Meknes, Morocco
[3]Center for Agri-food and Agri-environmental Research and Innovation (CIAGRO-UMH) Miguel Hernández University of Elche, Alicante, Spain

R e z i m e

Plod lokvata ima veoma važnu komercijalnu vrednost zbog njegove koristi za ljudsko zdravlje. Međutim, postoje vrlo ograničena naučna istraživanja o ovoj vrsti u Maroku. S tim u vezi, skup od 35 genotipova prikupljen je iz doline Zegzel (Berkane). Fenotipska varijabilnost je procenjena korišćenjem devet osobina koje se odnose na plod i list. Rezultati su pokazali koeficijent varijacije u rasponu od 13,02 do 42,21%, što implicira veliku fenotipsku varijaciju kod marokanskog lokvata, posebno kada je reč o karakteristikama koje su povezane sa oblikom ploda. Koristeći metodu višestruke korespondentne analize, prve dve ose su objasnile 62,57% ukupne varijanse. Glavne osobine koje su omogućile razlikovanje između genotipova bile su one koje su povezane sa veličinom ploda. Dakle, genotip mekerkba u regionu Zegzel nije jedna pojedinačna sorta, već genotipovi sa okruglim oblikom ploda. Pored toga, 35 proučavanih genotipova podeljeni su u tri glavne grupe bez obzira na njihovo geografsko poreklo. Rezultati pokazuju da geografska blizina nije imala značajnu ulogu u strukturi genotipova, što implicira slabu prilagodljivost genotipova životnoj sredini. Nalazi ovog istraživanja mogli bi se koristiti u konvencionalnom oplemenivanju i programima *in situ* očuvanja za marokanski lokvat.

Ključne reči: *Eriobotrya japonica*, fenotipska varijabilnost, kvantitativne osobine, koeficijent varijacije, višestruka korespondentna analiza.

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[*]Autor za kontakt: e-mail: h.hanine@usms.ma

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STRUCTURAL MODELING, EXPRESSION AND PURIFICATION OF CHIMERIC CHITINASE 42 CONTAINING HIS-TAG IN *NICOTIANA TABACUM* HAIRY ROOT SYSTEM

Faranak Soleimani, Mostafa Motallebi*, Mohammadreza Zamani and Esmat Jourabchi

Plant Molecular Biotechnology Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), P.O. Box: 14965-161, Tehran, Iran

Abstract: Chimeric chitinase42 (Chit42 containing ChBD) has great potential as a candidate for digesting and recycling chitin as a beneficial nutrient, which can be produced in bioreactors. The plant is one of the most efficient bioreactors that can produce the eukaryotic proteins in active forms. With the plant hairy root system, it is possible to express a variety of recombinant proteins cost-effectively, easily, and quickly. Due to the huge amount of proteins in plants, protein purification can be facilitated by the use of the His-tag. In this research, different computer programs were used for the three-dimensional structural analysis of Chimeric chitinase42 containing His-tag. The results showed that these comparative modeling approaches had a remarkable degree of accuracy in predicting the fused protein structure. The Z-score of -9.38 and -3.64 predicted for Chit42 and ChBD by ProSA represents the good quality of the model. In addition, bioinformatic observations showed that the His-tag was exposed and can be used to purify the Chimeric chitinase42. The Chimeric chitinase42 containing a His-tag was expressed in *Nicotiana tabacum* hairy roots, and the role of the His-tag in the detection by Western blot and purification using a Ni-NTA column was investigated. The presence of the Chimeric chitinase42 was confirmed by analyzing root extracts using SDS-PAGE and Western blot. The purification step was achieved using the His-tag and the Ni-NTA column. The plant-derived Chimeric chitinase42 was confirmed to be biologically active by measuring the chitinase activity of the purified protein on a media plate containing colloidal chitin.

Key words: Chimeric chitinase 42, His-tag, tobacco hairy root, structural modeling.

*Corresponding author: e-mail: Motalebi@nigeb.ac.ir

Introduction

Chitin, a polymer found in abundance in nature, is made up of β-(1,4)-linked N-Acetyl-D-glucosamine (GlcNAc) units and is the primary structural element of the cell walls of numerous fungi and arthropod exoskeletons (Akeed et al., 2020; Bhattacharya et al., 2007). During the past decades, more attention has been paid to the degradation of chitin in order to recycle it as a nutrient in nature (Deng et al., 2019). Chitinases (EC.3.2.1.14) are enzymes that catalyze the hydrolysis of chitin, a polysaccharide found in the cell walls of fungi, plants, bacteria, insects, viruses, and vertebrates. These enzymes are present in a wide variety of organisms and play an important role in the breakdown of chitin (Duo-Chuan, 2006; Bhattacharya et al., 2007; Churklam and Aunpad, 2020). Fungal chitinases have been described as more efficient enzymes compared to other chitinases. They offer a promising and sustainable solution for industrial production (Poria et al., 2021). ChBDs are important for the recognition and binding of chitin, and the linker region is thought to be important for the efficient transfer of the substrate to the active site (Arakane et al., 2003; Kowsari et al., 2014). To further improve the efficiency of chitin degradation, researchers have developed a novel approach that combines the presence of ChBD domains with the use of engineered chitinases. This approach has been shown to significantly increase the rate of chitin degradation, resulting in improved efficiency and productivity (Matroodi et al., 2013; Ataei et al., 2016). Among Trichoderma chitinases, Chit42, which lacks a ChBD, is important due to its role in biocontrol (Guthrie et al., 2005; Kowsari et al., 2016). Using protein engineering, the activity of this enzyme can be increased by the adding of a ChBD (Matroodi et al., 2013; Kowsari et al., 2014). Due to the various applications of chitinase, many attempts have been made to improve its activity and produce it in many organisms (Fan et al., 2007; Matroodi et al., 2013; Karthik et al., 2014).

Plants are one of the safest, most scalable, and economic systems for the production of recombinant proteins (Lico et al., 2008). Hairy roots (HRs), among plant tissues, are considered a bioreactor for large-scale production without the requirement for expensive hormones and light, which is cost-effective (Srivastava et al., 2018; Varasteh-Shams et al., 2020). In addition, HRs have genotypic and phenotypic stability, and they are capable of secreting expressed proteins into the culture media (Pham et al., 2012; Gutierrez-Valdes et al., 2020).

The goal of this study is to express the Chimeric chitinase42 containing ChBD and His-tag in the C-terminal end to facilitate the detection and purification of this enzyme, using the *Nicotiana tabacum* hairy root expression system.

Material and Methods

Structural analysis

The ExPASy website (https://web.expasy.org/translate/) was used to translate the nucleotide sequences of Chimeric chitinase42 and ChBD into amino acid sequences. The three-dimensional structures of Chimeric chitinase42 and ChBD were modeled by the comparative modeling approach using Modeller v9.18. The quality and reliability of the models were then evaluated through model validation using the ProSA web server (https://prosa.services.came.sbg.ac.at/prosa.php) as recommended by Nezafat et al. (2016).

Microorganisms, plasmids, and plants

The *E. coli* strain DH5α and the pJET 1.2 cloning vector from Novagen were used for cloning, while the pARM2 expression vector containing a His-tag from the National Institute of Genetic Engineering and Biotechnology in Iran was used for the plant expression experiments. The transformation of the plants was done using the *Agrobacterium rhizogenes* strain ATCC 15834 from Invitrogen. Seeds of *Nicotiana tabacum* cv. *xanthi* were kindly provided by Dr. T. Lohrasebi.

Construction of recombinant plasmids

A Chimeric chitinase42 DNA was used in this study (Ghiasi-sis, 2016). This recombinant construct contained the *chit*42 from *T. atroviride* joined with the ChBD of *Rhizopus oligosporus chi*1. The amplification of the *chit*42-ChBD was performed using PCR and the *Pfu* DNA polymerase (Fermentas, Germany), along with specific primer pairs, (*Xba*I-F*chit*42f, 5'GCTCTAGAATGTTGGGCTTCCTCGGAAAG3' and *Xho*I-R*chit*42f, 5'CCGCTCGAGCAG ACAGTGCCGGAGGG3'), which were designed with *Xba*I and *Xho*I sites at their 5′ ends, respectively. The amplified fragment, purified with the PCR Product Purification Kit (Roche, Germany), was then inserted into the pJET 1.2 cloning vector. The *chit*42-ChBD sequence was digested from the pJET 1.2 vector using *Xba*I and *Xho*I restriction enzymes and inserted into the corresponding sites of the pARM2 expression vector. The expression of the recombinant construct was driven by the CaMV 35S promoter. The recombinant construct (pARMFS) contains a polyhistidine tag at its C-terminus (Figure 1). For comparison, a control was established using tobacco hairy roots transformed with an empty pARM2 vector. The pARM2 is an empty vector containing a multiple cloning site under the CaMV35S promoter in which the gene of interest can be cloned.

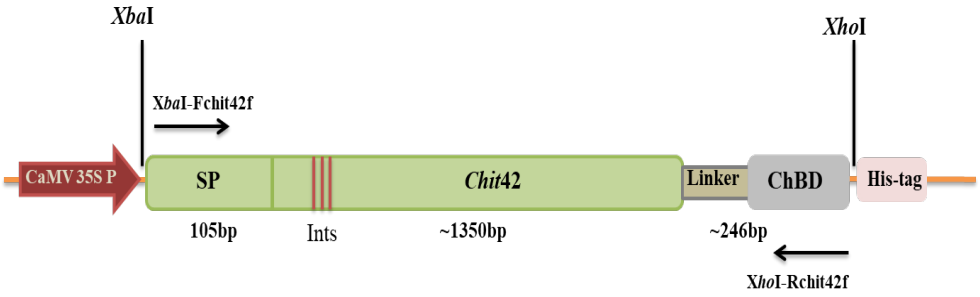

Figure 1. A representation of the pARMFS construct is shown, which includes the *chit*42 gene with three short introns, the ChBD domain, a linker region, and a His-tag sequence. The arrows show the specific primers (*Xba*I-F*chit*42f and *Xho*I-R*chit*42f) of the Chimeric chitinase42. SP: Signal peptide

Transgenic tobacco hairy root establishment by recombinant *Agrobacterium rhizogenes*

Tobacco seeds were cultured in sterile conditions, and leaf discs (2 cm^2) were prepared as explants. These explants were co-cultured with *A. rhizogenes* cultures containing pARMFS expression vectors for 7 minutes under shaking. The leaf discs were dried on the sterile filter papers and placed on the Murashige and Skoog (MS) solid medium (pH 5.6) for 2–3 days under dark conditions. After inoculation, the explants were transferred to MS medium containing antibiotics (400 mg/L cefotaxime and 50 mg/L kanamycin) and incubated for two weeks under a 16/8-hour photoperiod at 24°C. Engendered hairy roots were transferred to fresh liquid MS medium without antibiotics every two weeks at 24°C and 120 rpm for further growth. Negative control hairy roots were produced by leaf disc transformation with *A. rhizogenes* carrying an empty pARM2 vector.

Extraction of genomic DNA and confirmation of transgenic hairy roots

Genomic DNA was extracted from the transgenic tissue samples using the CTAB method (Doyle and Doyle, 1987). The presence of the *chit*42-ChBD in the hairy roots was confirmed via multiplex PCR amplification of the transgenic samples. The absence of *A. rhizogenes* infection was also detected by *Vir*G-specific primers (F-*Vir*G, 5'GGTCGCTATGCGGCATC3' and R-*Vir*G, 5'CCTGA GATTAAGTGTCCAGTCAG3').

Protein extraction and detection of recombinant protein in hairy roots

For the immunological detection of the expressed recombinant protein, the obtained hairy roots were ground in liquid nitrogen. One ml of protein extraction

buffer (1:1, w/v) (200mM Tris−HCl, pH 8.0, 10mM EDTA, 400mM sucrose, 14mM 2-mercaptoethanol, 100mM NaCl, 1mM phenylmethyl sulfonyl fluoride (PMSF), 0.05% Tween-20) was added to each sample. The total soluble protein (TSP) was measured by the Bradford method (1976) after centrifugation (15 min, 16,000×g, 4°C). Bovine serum albumin (BSA) was used as the standard protein.

The total soluble protein samples (70 µg) were loaded onto a 12% SDS-PAGE. The protein bands were then visualized by Coomassie Blue R250 staining. For the Western blot, the treated samples were separated using 12% SDS-PAGE and then transferred to a polyvinylidene fluoride (PVDF) membrane (Roche, Germany) using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad, the USA). The transfer was performed at 4°C for 4 hours with a transfer buffer consisting of 40 mM glycine, 50 mM Tris-base, 0.04% SDS, and 20% methanol, at a pH of 8.3, and at a voltage of 100 V. The protein was detected using anti-His-tag antibody as per the manufacturer's instructions (Qiagen, Catalogue No. 34460).

Purification of protein under native conditions

The enzyme was purified by a Ni-NTA column (Qiagen, Catalogue No. 30210). The column was prepared by loading 600 µL of lysis buffer (50 mM NaH2PO4, 10 mM imidazole, 300 mM NaCl, pH 8.0). One ml of the transgenic hairy root-extracted protein containing the 6×His-tagged Chimeric chitinase42 was then loaded onto the column, and the flow-through was collected. Then, 600 µL of wash buffer (50 mM NaH2PO4, 20 mM imidazole, 300 mM NaCl, pH 8.0) was used for washing the column two times. In the end, the protein was eluted with 200 µL of elution buffer (50 mM NaH2PO4, 300 mM NaCl, 500 mM imidazole, pH 8.0). The concentration of the purified protein was measured using the Bradford method and stored at -70°C.

Enzymatic activity test

The activity of the purified expressed protein was determined using a chitinase-detection agar (CHDA). CHDA was prepared using (g/L): 0.5 NH4Cl, 0.65 NaHPO4, 0.25 NaCl, 1.5 KH2PO4, 0.005 CaCl2, 0.12 MgSO4, and 20.0 agar-agar at a pH of 6.5, with 10% (w/v) colloidal chitin as described by Barboza-Corona et al. (1999). The method of Roberts and Selitrennikoff (1988) with some modifications was used for preparing the colloidal chitin. Five g of commercial chitin (Sigma-Aldrich, USA) was slowly added into 100 ml of cold concentrated HCl and kept overnight at 4◦C with gentle agitation on a magnetic stirrer. Then, 500 ml of ice-cold 96% ethanol was added, and the mixture was stirred rapidly for 24 hours at 4°C. The precipitate was centrifuged at 10,000 g for 20 minutes at 4°C

and then washed repeatedly with sterile distilled water until colloidal chitin reached a pH of 7.0 and was cleared. It was then stored at 4°C for future use.

Results and Discussion

Analysis of the structural modeling

The BLASTp webserver was employed to match the Chimeric chitinase42 of *T. atroviride* and the ChBD domain of *R. oligosporus chi*1 against the PDB database. This was to determine the template sequences that had the greatest sequence identity. A structural model was then predicted by selecting Chit42, which had an 82.7% sequence identity with a template (PDB code: 6EPB_A), and the ChBD domain, which had a 100% sequence identity with a template (PDB code: P29026.1) for protein model building.

The 3D structures of the Chit42 and ChBD domains were modeled by the comparative modeling approach using Modeller. Using the ProSA server, the "Z" scores of Chit42 and ChBD were -9.38 (Figure 2a) and -3.64 (Figure 2b), respectively. The results show that the models were of good quality and had a remarkable degree of accuracy in predicting the fused protein structure. The 3D model of the Chimeric chitinase42 and His-tag sequence was visualized using the Discovery studio 2020 client software (Figure 3). Bioinformatic observations demonstrated that the tertiary structure of Chimeric chitinase42 and the His-tag was exposed and can be used for the purification procedures of Chimeric chitinase42.

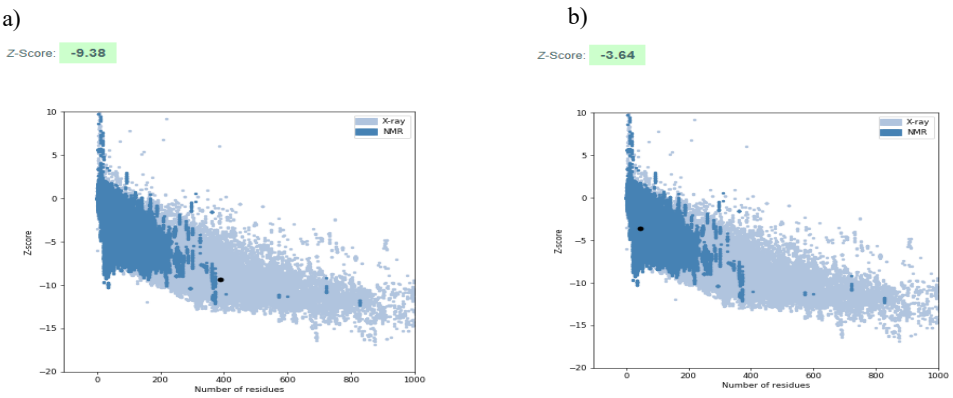

Figure 2. "Z" scores of the models in the ProSA server, (a) "Z" score plot of the Chit42 model (−9.38), (b) "Z" score plot of the ChBD model (-3.64).

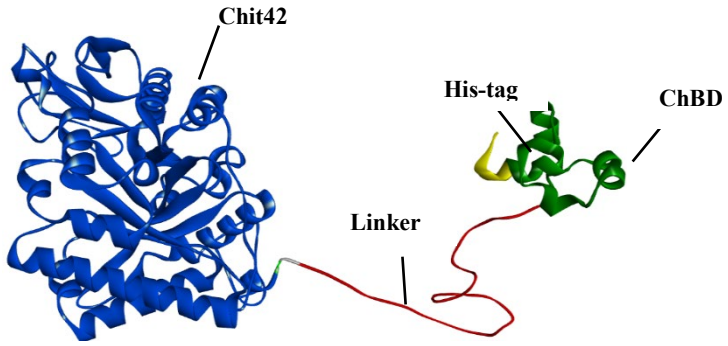

Figure 3. "Z" scores of the models in the ProSA server, (a) "Z" score plot of the Chit42 model (-9.38), (b) "Z" score plot of the ChBD model (-3.64).

Expression analysis in *N. tabacum*

The purpose of this study was to produce a new Chimeric chitinase42 enzyme, which contains a chitin-binding domain (ChBD) and a histidine-tag (His-tag), using a plant expression system. Since the T-DNA was transferred to the plant by *Agrobacterium*, the genomic DNA of *chit*42, containing three short introns, was used to express it only through the plant expression system (not via *Agrobacterium*). Chimeric chitinase 42 DNA (Ghiasi-sis, 2016) was sub-cloned in the pARM2 expression vector containing the His-tag sequence and designated as pARMFS. The chimeric sequence was confirmed through sequencing. This construct expressed a Chimeric chitinase 42 protein tagged at the C-terminal end with a 6xHis tag that can be easily detected (using Western blotting) and purified.

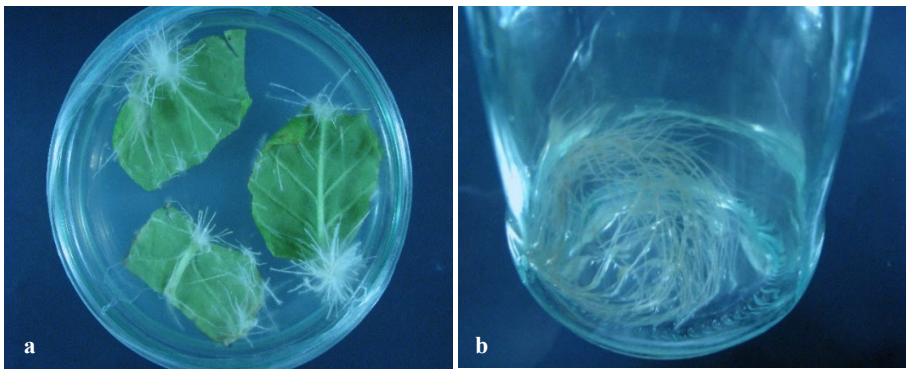

Figure 4. (a) A view of explants demonstrating the growth of hairy roots. (b) An observation of the propagation of hairy roots in MS medium at 24°C and 120 rpm.

The transformation via *A. rhizogenes* was used to transfer the construct to *N. tabacum*. The results of the experiment show that out of 25 inoculated leaf discs, 21 explants produced hairy roots with an average of three roots per explant (Figure 4), suggesting an 84% transformation efficiency. Based on the growth conditions of the hairy roots, ten explants were chosen for further molecular analysis. PCR amplification with *VirG* primers did not detect any *A. rhizogenes* contamination in the hairy roots. The cloned fragment (1699 bp) was confirmed using chimeric chitinase 42-specific primers, which showed the expected size of *chit*42-ChBD following the electrophoresis (Figure 5).

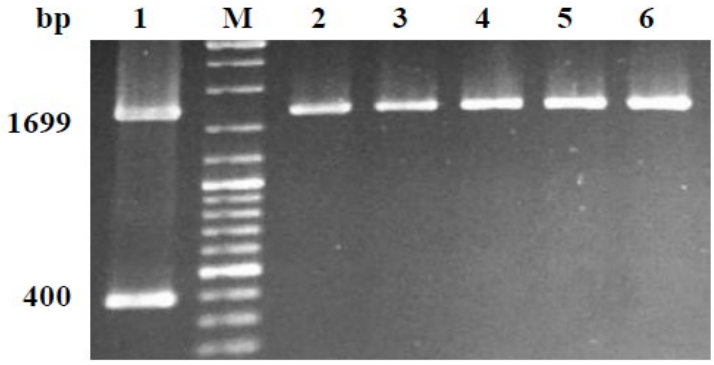

Figure 5. Screening transgenic hairy roots by multiplex PCR. The presence of a 1699-bp fragment of *chit*42-ChBD was detected by PCR amplification using specific primers and DNA extracted from tobacco hairy roots. *Vir*G specific primers also confirmed the *A. rhizogenes* infection elimination. 1) PCR on *A. rhizogenes* DNA harboring pARMFS, which shows a 1699-bp fragment of *chit*42-ChBD and a 400-bp fragment of *Vir*G. 2,3,4,5 and 6) The amplified fragment from transgenic hairy root explants. M) DNA ladder m.

Fifteen days after the growth of the transgenic hairy roots in a liquid medium, the total soluble protein was extracted and used to analyze the expression of the recombinant protein. The molecular weight of the expressed protein, estimated to be near 56 kDa, was confirmed by the analysis of SDS-PAGE (Figure 6a). Western blot analysis using an anti-His-tag antibody was performed for further validation. The results confirmed the presence of the recombinant protein with a 6×His tag and with a molecular weight of 56 kDa, which matched the expected molecular weight of the expressed protein (Figure 6b). The total cell protein of the hairy root harboring the empty pARM2 vector was used as a negative control.

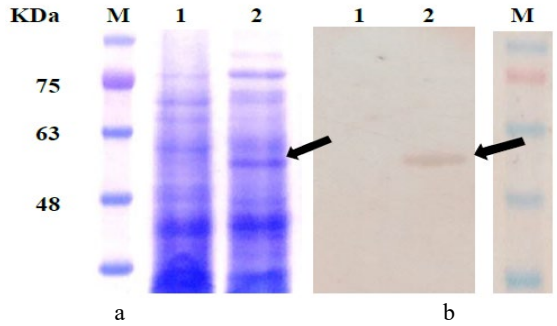

Figure 6. a) SDS-PAGE analysis of the total extracted proteins. b) Western blot analysis of the recombinant protein using the anti-His-tag antibody. 1) Extraction of proteins from hairy roots containing the empty pARM2 vector (negative control). 2) Proteins extracted from the transgenic hairy root. The arrows indicate the presence of a band of approximately 56 kDa in the samples of the transgenic hairy roots. M) Protein molecular weight marker.

Enzyme purification

After confirming the presence of the expressed protein in the hairy roots, the 6xHis/Ni-NTA purification system was carried out under native conditions to determine the role of the addition of His-tag for purifying the recombinant enzyme. The His-tagged protein was purified from the total hairy root protein extract using a Ni-NTA column. The expected 56-kDa band was detected by SDS-PAGE and confirmed through Western blot analysis (Figure 7).

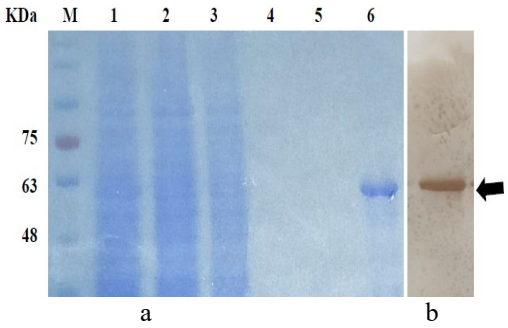

Figure 7. Purification of the Chit42-ChBD-6His protein by native chromatography on a Ni-NTA column. a) The protein was visualized on an SDS-PAGE gel. 1) Protein sample extracted from the hairy root; 2) Flow-through; 3, 4 and 5) Wash with 20 mM imidazole (pH 8); 6) Elution by 500 mM imidazole (pH 5.6); M) Protein molecular weight marker; b) Western blot analysis of the purified Chit42-ChBD-6His using anti-His-tag. The arrow indicates the 56-kDa band of the purified enzyme.

Chitinase activity assay

To verify the enzyme activity of the purified expressed enzyme, the Chitinase detection agar assay was used. Seven µg of the purified protein was loaded into each well, and after 24–48 h, the activity of the enzyme was assessed by observing the creation of a clear zone around the well on the media plate with colloidal chitin. The clear zone indicates the degradation of chitin by the enzyme, and its size can be used to quantify the activity of the enzyme (Figure 8).

a b

Figure 8. Chitinase detection of hairy root protein extract by agar assay. a) The well was loaded with the purified Chimeric chitinase 42 sample at a concentration of 7 µg. The enzyme activity was detected by a clearing zone around the well on the media containing the colloidal chitin. b) The well was loaded with buffer as a negative control. No clearing zone was observed around the well.

Chitinase [EC3.2.1.14] enzymes have the capacity to digest and recycle chitin as a beneficial nutrient. In this study, Chitinase42 from *T. atroviride* containing ChBD of *R. oligosporus chi1* (Kowsari et al., 2016) was used. In their study, adding ChBD resulted in a 1.56-fold increase in the chitinase activity of Chtinase42. Studies have demonstrated the impact of ChBD on enhancing the chitinase activity of Chimeric chitinases. Matroodi et al. (2013) showed that combining a ChBD from *Serratia marcescens Chi*B with Chit42 increased the activity of the resulting Chimeric enzyme. The same finding was also reported by Kowsari et al. (2014), who showed that the addition of a ChBD from *T. atroviride* Chitinase 18-10 to Chit42 of *T. harzianum* increased 1.7-fold of the enzyme specific activity. Furthermore, Limón et al. (2001) demonstrated that incorporating a ChBD from *N. tabacum* into Chit42 of *T. harzianum* increased the enzyme activity.

The purification of proteins by conventional affinity chromatography procedures has disadvantages, such as high cost and time consumption, thus,

affinity chromatography methods are alternative procedures for recombinant protein purification. A protein affinity tag provides an essential mechanism for protein purification. There are a number of tags such as streptavidin-binding tags, calmodulin-binding tags, polyarginine or histidine tags, which facilitate the purification of the various proteins (Amarasinghe and Jin, 2015; Oliveira and Domingues, 2018; Terpe, 2003).

In this study, prior to the experimental analysis of the protein expression using His-tag, different computer programs were used for the analysis of the structural three-dimension of Chimeric chitinase42 containing His-tag. The results showed that these comparative modeling approaches exhibited a remarkable degree of accuracy in predicting the fused protein structure. In addition, bioinformatic observations showed that the His-tag was exposed and can be used for the purification of Chimeric chitinase42. We used the polyhistidine tag (6×His-tag) which is a useful tool for easy, efficient, and high-quality protein purification to facilitate the detection (using Western blot) and purification of Chimeric chitinase. Using the enzyme activity assay, we have confirmed that the His-tag does not affect the activity of Chimeric chitinase. Some reports indicate that the His-tag does not change protein properties in most cases (Hung et al., 2002; Aslantas and Surmeli, 2019; Zhou et al., 2020). Kowolik and Hengstenberg (1998) reported that the location of the His-tag on the lactose enzyme II of *Staphylococcus aureus* (either at the C-terminal or N-terminal end) had no impact on its surface affinity. Nevertheless, it can sometimes influence the biological functions of the protein (Mohanty and Wiener, 2004; Kenig et al., 2006). It has been reported that one of the His-tagged subunits of the bacteriophage λ terminase enzyme demonstrated a change in its interaction with DNA (Hang et al., 1999). In a study by Zayakina et al. (2009), the addition of a polyhistidine tag to the coat protein of potato virus X resulted in the loss of its ability to form helical nucleoprotein virus-like particles with viral RNA.

In the present work, we utilized an expression vector containing the His-tag sequence (pARM2), in which the Chimeric chitinase 42 containing the His-tag at its C-terminal can be produced, and the plant expression system was used as a bioreactor. Protein purification from biochemically complex plant tissues containing too many different biomolecules and proteins is usually an important and challenging task in biochemistry (Sánchez-Ferrer et al., 1994; Borisjuk et al., 1999). The time required and the method of purification depend on the specific protein and the aim of using this protein.

The various recombinant protein production systems have some limitations, such as the inability of the bacterial systems to produce functional complex proteins or the high risk of contamination when producing recombinant proteins with toxic molecules in mammalian cells (Cardon et al., 2019). It has been reported that the recombinant protein production using a plant expression system as a

bioreactor has several advantages over other expression systems, including the production of a large amount of products, the sustainability of the molecules, high safety issues, and low upstream costs (Gutierrez-Valdes et al., 2020). In comparison to other plant-based expression systems, hairy root cultures have been recommended as a promising method for the production of recombinant proteins such as enzymes (Woods et al., 2008) and antibodies (Sharp and Doran, 2001; Martínez et al., 2005) due to their genetic stability and high productivity (Gutierrez-Valdes et al., 2020). In our study, we employed a hairy root of the tobacco plant as a platform for producing the recombinant Chimeric chitinase 42 protein, which was verified through SDS-PAGE and Western blot analysis.

Conclusion

In conclusion, our study has shown that a Chimeric chitinase 42 can be produced using a hairy root expression system. Additionally, it was shown that the His-tag did not affect the activity of Chimeric chitinase 42, making it a viable option for purifying the active recombinant protein.

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STRUKTURNO MODELIRANJE, IZRAŽAVANJE I PREČIŠĆAVANJE HIMERNE KITINAZE 42 SA HIS-OZNAKOM U ŽILIČASTOM KORENOVOM SISTEMU BILJKE *NICOTIANA TABACUM*

Faranak Soleimani, Mostafa Motallebi*, Mohammadreza Zamani i Esmat Jourabchi

Plant Molecular Biotechnology Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), P.O. Box: 14965-161, Tehran, Iran

R e z i m e

Himerna hitinaza 42 (Chit42 koji sadrži ChBD) ima veliki potencijal kao kandidat za razlaganje i recikliranje hitina kao korisne hranljive materije, koja se može proizvesti u bioreaktorima. Biljka je jedan od najefikasnijih bioreaktora koji mogu proizvesti eukariotske proteine u aktivnim oblicima. Pomoću žiličastog korenovog sistema biljke, moguće je ekonomično, lako i brzo dobiti različite rekombinantne proteine. Zbog velike količine proteina u biljkama, prečišćavanje proteina može biti olakšano korišćenjem His-oznake. U ovom istraživanju, korišćeni su različiti kompjuterski programi za trodimenzionalnu strukturnu analizu himerne hitinaze 42 koja sadrži His-oznaku. Rezultati su pokazali da su ovi pristupi uporednom modeliranju imali izuzetan stepen tačnosti u predviđanju strukture fuzionisanog proteina. Z-skor od -9,38 i -3,64 za Chit42 i ChBD koji je ProSA predvideo predstavlja dobar kvalitet modela. Pored toga, bioinformatička posmatranja su pokazala da je His-oznaka bila ekspresivna i da se može koristiti za prečišćavanje himerne hitinaze 42. Himerna hitinaza42 koja sadrži His-oznaku izražena je u žiličastim korenovima biljke *Nicotiana tabacum*, a istražena je i uloga His-oznake u detekciji putem analize Vestern blot i prečišćavanju pomoću kolone Ni-NTA. Prisustvo himerne hitinaze 42 potvrđeno je analizom ekstrakata korena korišćenjem tehnike SDS-PAGE i analize Vestern blot. Prečišćavanje je postignuto korišćenjem His-oznake i kolone Ni-NTA. Potvrđeno je da je dobijena himerna hitinaza 42 biološki aktivna merenjem hitinazne aktivnosti prečišćenog proteina na podlozi koja sadrži koloidni hitin.

Ključne reči: himerna hitinaza 42, His-oznaka, žiličast koren duvana, strukturno modeliranje.

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*Autor za kontakt: e-mail: Motalebi@nigeb.ac.ir

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DYNAMICS OF THE POULTRY MARKET IN GHANA

Edward Ebo Onumah[1*] and Selorm Ayeduvor[2]

[1]Department of Agricultural Economics and Agribusiness, University of Ghana
[2]CSIR – Science and Technology Policy Research Institute, Ghana

Abstract: This paper examines the dynamics of the poultry market in Ghana using secondary data and a field study in four regions including Greater Accra region, Western region, Ashanti region, and Northern region. Secondary data on prices, per capita consumption, import, and export quantities was obtained from the Food and Agriculture Organization (FAO) and the Ministry of Food and Agriculture (MoFA). Primary data on shopping malls, supermarkets, cold stores, and open markets as well as on imported and local chicken meat prices and brands was also obtained using surveys and focus group discussions. The study employs trend and content analyses to highlight specific imported products, their origins, drivers, and the potential of domestic poultry as a substitute for imported chicken meat. The paper confirms that Ghana imports about 80% of its poultry meat, mostly in the form of branded cut parts (thighs, wings, legs, back, and offal) from high-income countries including Belgium, the US, Brazil, Poland, and the Netherlands. Despite a 35% increase in tariffs, imported poultry meat tends to be 27–30% cheaper than locally produced chicken. The findings further show that although there is some preference for domestic poultry meat, this does not translate into purchase decisions, as people prefer more convenient and ready-to-use products. The paper recommends the prioritization of policies to boost local production through investments in processing (cut parts), branded packaging, and marketing facilities such as cold vans. Furthermore, policies to reduce chicken meat import volumes may be focused on other non-tariff measures such as licenses, allotments, trade embargoes, foreign exchange restrictions, and import depositories.

Key words: poultry meat, cut parts, imports, trade, packaging, Ghana.

Introduction

Food demand in less developed countries is rapidly changing toward the consumption of higher-value products such as meat (Pingali, 2015). In Africa, a similar transition toward meat products is emerging. Recent reports reveal that

*Corresponding author: e-mail: eeonumah@ug.edu.gh

poultry meat accounts for a larger part of meat consumption in the region (FAO, 2018). According to Hollinger and Staatz (2015), poultry consumption in West Africa has been stimulated by the availability of low-cost alternatives for consumers through imports. The influx of imported poultry meat products, mainly frozen cut parts, has become popular with urban consumers. These products are often sold below production cost because producers in exporting countries make their profits from the sale of high-value parts such as breasts, which are demanded by consumers in those countries (Hollinger and Staatz, 2015).

Like the global trend, poultry meat is increasingly becoming an important source of animal protein in Ghana (MoFA, 2020; Asante-Addo and Weible, 2020). The higher demand for imported poultry meat is attributed to increasing income growth, rapid population growth, and the high rate of urbanization (Sumberg et al., 2016). In Ghana, poultry meat consumption is dominated by chicken meat, but guinea fowls, ducks, turkeys, and ostriches are also consumed. It is estimated that the growth in consumer demand for poultry meat in Ghana is expected to increase further in the future (MoFA, 2020).

The rising consumer demand for poultry meat provides new market opportunities for growth and investment in the value chain of the sector in Ghana. However, local production lacks the capacity to meet this growth in consumer demand, resulting in the country currently depending on imports (Ashitey, 2017; Asante-Addo and Weible, 2020). The high import is attributed to the high cost of production (feed and drugs), production technology inefficiencies, lack of adequate processing facilities, as well as cheap import products resulting in low productivity of the poultry sector in Ghana (MoFA, 2020). Other operational constraints include high energy prices, which continue to increase production costs by over 60% (MoFA, 2020). This leads to a higher price of domestic broiler meat, making it uncompetitive. The situation has prompted local farmers to call for policy interventions to support domestic production (Sumberg et al., 2016).

In response, the government implemented measures to protect local producers by introducing import tariffs of about 35%, reducing tariffs on inputs (additives, feeds, medicines, and vaccination drugs), providing efficient veterinary services, and delivering subsidized inputs through the rearing for the food and job program (Andam et al., 2017). However, these strategies do not seem to be yielding the desired effect as the volume of poultry imports keeps increasing astronomically. Competition from imported chicken meat has put pressure on broiler production in Ghana as prices of imported chicken meat are 30–40% lower than locally produced chicken. This has led to a producer shift from the production of broiler (meat) to the production of layer birds for table eggs (Ashitey, 2017).

Stakeholders seeking to intervene in Ghana's poultry sector face a dearth of policy-relevant research to guide their decision process. Unfortunately, reliable, and up-to-date information on trends in domestic poultry production and trade is

hard to find (Sumberg et al., 2016). Existing research consists mostly of assessments at the production levels, and qualitative assessments of the impacts of disease outbreaks, such as avian flu on production (Diao, 2009). The ability of local producers to remain competitive requires an in-depth understanding of the dynamics of the poultry market in Ghana.

The main purpose of the paper is to explore evidence of the growing demand for poultry meat in Ghana by analyzing the dynamics of the poultry market in terms of production, consumption, trade, and how local poultry can be used as a substitute for imports. The paper provides a comprehensive overview of the development of domestic production, consumption, and imports of poultry products. It further uses secondary and primary data to analyze prices, sales points, packaging, and branding of local and imported chicken meat. It also highlights the country of origin of the imported products, whilst accounting for the extent of domestic and international trade among partner countries. The findings of this study will assist policy makers to formulate appropriate production and trade policies related to the poultry sector that are critically important in the advancement of commercial production, processing, and marketing of local poultry in Ghana. The rest of the paper is structured as follows: Section 2 provides an overview of the development of the poultry sector in Ghana, Section 3 describes the data used in the analysis, Section 4 presents the empirical results, while the conclusion of our paper is presented in the final section. The market for poultry meat in Ghana is segmented into imported and domestically produced chicken meats. The latter is sold typically in live bird markets where the consumers purchase the birds for slaughter at home.

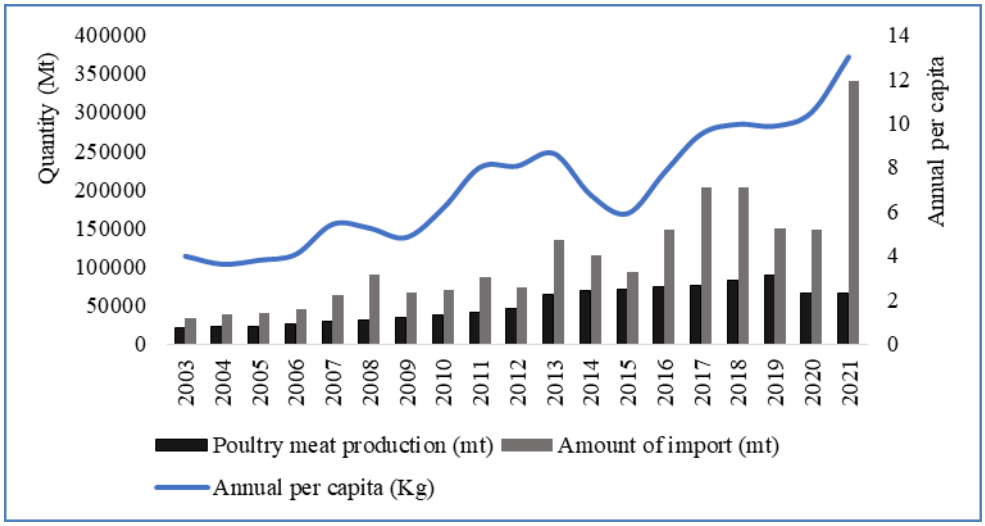
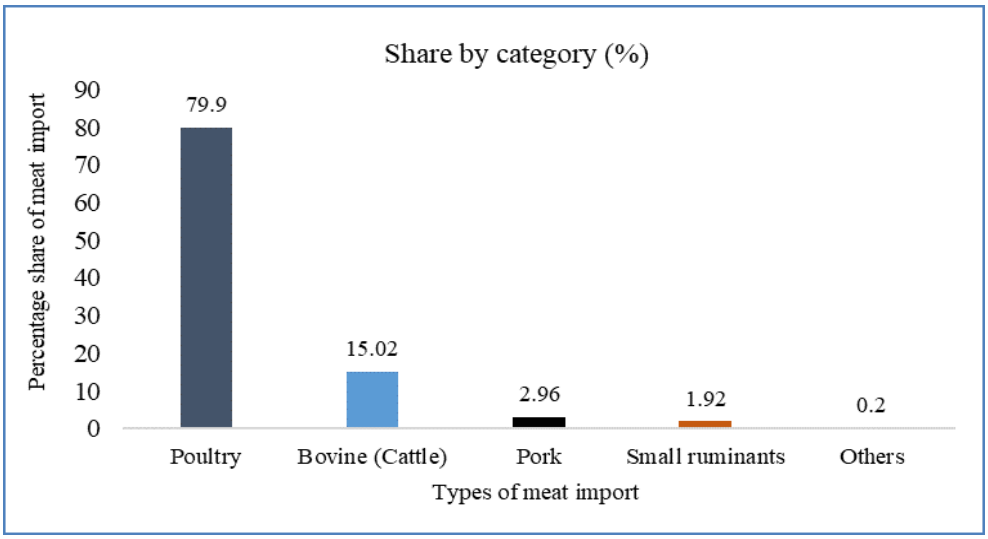


Figure 1. Poultry production and consumption in Ghana. Source: MoFA, 2020.

Retailers also provide slaughtering and primary processing services and sell them on the local markets. The imported meat is sold frozen and cut into parts (Asante-Addo and Weible, 2020). The popularity of frozen chicken parts in the market suggests that live birds are less preferred (Andam et al., 2017). Per capita consumption of chicken meat in Ghana considerably increased from 3.98 to 9.87 kg annually between 2003 and 2019 (Figure 1). Ghana's per capita consumption of poultry meat is relatively higher than the average in Africa (6.76) and West Africa (8.0). According to the FAO (2020), the daily consumption of poultry meat in Ghana has risen from 2 calories per day to 20 calories within the last two decades.

Even though substantial investments have been made in national poultry production, production is still not able to keep up with the growing demand for poultry meat in Ghana. Although local poultry production has recently seen 10.5% annual growth from 20,588 metric tons (Mt) in 2003 to 89,210 metric tons (Mt) in 2019, Ghana still imported about 150,039Mt of poultry meat in 2019 (Figure 1).

Although import constituted only about 12% of the total domestic supply of poultry meat in 1961, it increased to 44% in 1990 and reached about 76% in 2018. However, the share of domestic supply of poultry meat decreased from 80% in 2000 to 24% in 2018 (FAO, 2020).



Source: MoFA, 2020.

Figure 2. The import of frozen meat in Ghana.

This review section notes that the share of poultry meat in the overall domestic meat supply has increased drastically over the last six decades. Specifically, it increased from 11% in 1969/1970 to 30% in the late 1990s and 66% in 2010/2011. Total meat imports between 2015 and 2019 amounted to 910,604Mt,

of which poultry is estimated to be 728,386Mt, pork (26,962Mt), cattle (136,930Mt), sheep and goat (2,041Mt), and others including ducks, geese and guinea fowls were noted to be 1,010Mt. Total chicken meat import within the five years (2015–2019) was valued at US$700 million. In addition, MoFA (2020) noted that poultry meat imports accounted for nearly 79.9% of total meat imports in Ghana in 2019 (Figure 2). This was followed by bovine (2.96%), pork (15.02%), small ruminants (1.92%), and others (ducks, geese, and guinea fowls – 0.11%).

Price and non-price factors are believed to be the key drivers of demand for imported poultry meat in Ghana. Egyir et al. (2012) analyzed the "poultry made in the USA" label and consumer choice in Ghana and revealed that consumers preferred imported chicken meat because of packaging, meat quality, and food safety. They also established that consumers who prioritized country of origin as a factor influencing demand for imported poultry meat did not necessarily choose the US poultry label. Their results further showed that in 2012, about 71% of consumers preferred domestically produced meat, followed by imports from the United States (56%), the European Union (48%), and Brazil (40%). Thus, the Ghana poultry label can also be advanced if a repute for quality and food safety can be gained. This finding agrees with Asante-Addo and Weible (2020) and Ragasa et al. (2020), who also reported that some consumers preferred domestically produced poultry meat to imported chicken meat and might be willing to pay a premium for it.

Consumers bought imported poultry meat because of its availability, convenience, affordability, packaging, and proximity of access, while domestic poultry meat is preferred for its high quality, freshness, palatability, and tenderness (Atuahene et al., 2014; Asante-Addo and Weible, 2020). Kwadzo et al. (2013) found that the price of the meat remained an important attribute influencing consumer preference when purchasing broiler meat in Ghana. This finding is also in agreement with Makanyeza and Du Toit (2016) and Boimah and Weible (2023), who reported price as a driver of purchasing decision for imported poultry meat in Zimbabwe and Senegal, respectively.

Demand for chicken meat is also driven by health, nutrition, and food safety concerns. According to Asante-Addo and Weible (2020), consumers perceived local chicken meat as fresh, antibiotic- and hormone-free. Additionally, Ragasa et al. (2019) investigated consumer demand and willingness to pay for safe food in Accra and found that consumers were willing to pay a premium for chicken meat certified with a food safety management system. Consumers preferred HACCP-certified and antibiotic residue-free chicken over non-certified chicken. Consumer perception of chicken meat safety can be an important driver of purchasing decision. Recent findings revealed that nearly 50% of chicken meat consumers were health and safety conscious and willing to pay 15% percent more for chicken products (Ragassa et al., 2019). Thus, producers and processors could expand their market opportunities by having their meat tested for HACCP and the absence of

antibiotic residues. However, since food safety certification is done at production and processing levels, it requires some changes in the management and administration of farms.

From the above, consumer demand for chicken meat in Ghana is becoming more differentiated: on the one hand, there are many low-income consumers whose main focus remains on obtaining chicken meat at low cost, and on the other hand, there is an emerging middle class that is placing increasing importance on the quality, diet diversity, and safety of chicken meat. Thus, the ability of local producers to capture these markets will depend on their capacity to be consistent and reliable suppliers of quality local chicken meat at competitive prices. Currently, consumers believe that imports meet these standards in terms of price, quality, and availability.

Material and Methods

Data from both secondary and primary sources were used for the study. Price and trade data were collected from the Ministry of Food and Agriculture (MoFA) and the Ghana Statistical Service (GSS) over a period of sixteen years between 2003 and 2019. Data from the Food and Agriculture Organization (FAO) on export quantities and per capita consumption were also used. The information from these multiple sources was validated using survey data between September and November 2021 from shopping malls, supermarkets, cold stores, and open markets on imported and local chicken meat prices and brands in four regions (Greater Accra region, Western region, Ashanti region, and Northern region). These regions were selected to account for the urban coastal effect, proximity to the port, the rising cost of living emerging from the oil industry, and availability of chicken substitutes from other livestock.

Two (2) separate focus group discussions (FGDs) were conducted among chicken wholesalers and retailers in each of the four regions, making a total of eight (8). The participants selected for the FGDs were managers and traders from shopping malls, supermarkets, cold stores, and local markets in Ghana. The participants for this study were purposively recruited from four major cities in Ghana (Kumasi, Accra, Takoradi and Tamale) with high chicken consumption. A total of 96 managers and traders participated in the focus group discussion, of whom 47.9% were males and the remaining 52.1% were females (see Table 1).

The FGD participants were relatively young with age ranging between 25 and 65 years old, with the majority (61.4%) within the age group of 25–45 years. About 24% have primary education, while 33.3% of them have no formal education (illiterate) and are traders, public servants, and the retired, while 16.7% have university education. The average household size of the participants was 6 persons, and the average household income was GH₵9,675.00.

Table 1. Profile of respondents in the focus group discussion.

Characteristics of respondents	Wholesalers		Retailers		Total	
	Freq	%	Freq	%	Freq	%
Gender						
Male	21	58.3	25	41.7	46	47.9
Female	15	41.7	35	58.3	50	52.1
Age (years)						
25–35	10	27.8	10	16.7	20	20.8
36–45	12	33.3	27	45	39	40.6
46–55	10	27.8	15	25	25	26.04
56–65	4	11.1	8	13.3	12	12.5
Experience in trading (in years)	10.4	-	12.6	-	11.5	-
Education						
None	12	33.3	20	33.3	32	33.3
Basic (primary) education	8	22.3	15	25	23	24.0
Secondary education	6	16.6	7	11.7	13	13.5
Post-secondary (diploma, certificate)	4	11.2	8	13.3	12	12.5
University	6	16.6	10	16.7	16	16.7
Household size (mean)	4.8		6.5		5.5	
Average reported monthly income (GHC)	15,600		4,350		9,675	
Average reported monthly income (US$)	2,600		725		1,612.5	

Source: Market survey, 2021.

Information on the price of chicken, the places of purchase and consumer quality indicators was collected during the focus group discussions. Trend and content analytical investigations, and descriptive statistics using tables, graphs and bar charts were employed to analyze the data obtained.

Results and Discussion

This paper notes that in addition to price, product form (packaging, labeling and country of origin) and the place of sale of chicken meat have been found to be important factors that drive consumers' purchasing decisions. This section discusses the differences in the price of chicken meat, the brands, and the country of origin of chicken meat in various sales points in the study regions.

Product prices and differentials

Data from the field survey of selected markets in the four regions revealed that the mean retail price of imported chicken meat in 2021 was GHS 26.6/kg, while the mean retail price of domestic chicken was GHS 34/kg (Table 2).

The price difference of imported chicken meat ranged between 28% and 30%. Also, the data showed that 10 kg of imported chicken (soft, usually for stew) and

(hard, usually for soup) cost GHS 126 and GHS 147, respectively in the study area. Specifically, the average price of local chicken meat was Gh34.00/kg in Accra, GH₵30.00/kg in Tamale and Gh35.00/kg in Takoradi and Kumasi. The imported whole chicken meat was priced at GH₵26.60, GH₵18.00, GH₵22.00, and GH₵17.80 in Accra, Takoradi, Kumasi, and Tamale, respectively. Similarly, the imported chicken thigh was priced at GH₵18.00, GH₵19.00, GH₵18.99, and GH₵13.30 in Accra, Kumasi, Takoradi, and Tamale, respectively. The relatively high price of domestic poultry compared to imported poultry could be attributed to the high production costs, especially feed and medication expenses (Naggujja et al., 2020). Whilst imported formulated feeds are expensive due to high taxes, some ingredients used for locally prepared feeds such as yellow corn, soybean, fish meal, and vitamin-mineral premixes are equally imported and taxed, so that feed costs account for about 70% of the cost of chicken production in the country (Banson et al., 2015). Etuah et al. (2020) also noted that poor and inadequate local infrastructure, including poor rural and urban road networks, added to distribution cost. Additionally, poultry production is heavily subsidized in the USA and the EU, making imported chicken meat comparatively cheaper than domestic poultry meat.

Table 2. Average retail prices (GH) of imported and local chicken meat.

Chicken parts	Prices per kg (GHC)							
	Accra		Kumasi		Takoradi		Tamale	
	Import	Local	Import	Local	Import	Local	Import	Local
Whole chicken	26.60	34.00	22.00	35.00	18.00	35.00	16.75	30.00
Chicken thighs	20.00	-	19.00	23.00	18.99	22.99	13.30	-
Chicken wings	18.00	-	19.00	20.00	18.00	23.99	16.30	-
Gizzards	16.00	-	20.00	22.00	13.50	16.99	14.20	-

Source: Market survey, 2021.

The study also revealed that imported chicken meat of the same weight was cheaper in Tamale than in other locations. Although such price differences are contrary to expectation due to the cost of transporting the product over a long distance, the participants attributed this to the high demand for substitute meat products such as guinea fowls in the northern region, which could force retailers and wholesalers of imported chicken meat to reduce prices to remain competitive. Also, imports of beef and mutton from Burkina Faso to northern Ghana could also be responsible for the low meat prices in the region (Sumberg et al., 2016; Rich and Wane, 2021).

The study identified two local companies, Everyday chicken, and Master meal chicken, which process chicken into cut parts. These companies are in Kumasi and Takoradi. The study revealed that the local chicken parts (thighs, wings, and gizzards) in these two locations were relatively more expensive than the imported

ones. For example, the price of local chicken thighs ranged between GHC22.99 and GHC23/kg in Kumasi and Takoradi, respectively compared to GHC18.99 and GHC20.00 of imported chicken thighs in the same locations. It is also evident from the above that the average retail price of imported chicken meat in 2021 was relatively lower than the retail price of local chicken meat in all the regions visited. In addition to the factors stated earlier, the high price of local chicken parts could be due to the high cost of processing and inadequate processing technologies (MoFA, 2020; Andam et al., 2017).

Sale points of imported and local chicken meat

Generally, prices of chicken meat at supermarkets and shopping malls were higher than at other selling points in all the locations surveyed. Imported whole chicken meat was priced between GH₵20.00 and GH₵26.00 at supermarkets while it was priced between GH₵21.50 and GH₵26.90 at shopping malls across the country (Table 3). The survey results show that differences in quality and country of origin may account for some of the price differences. It is believed that these modern markets (supermarkets and shopping malls) also operate in a more improved shopping environment in terms of space, hygiene, air conditioning, food safety and security, which comes with additional costs. Ashraf et al. (2017) noted that products from the mall came with a premium.

Table 3. Average retail price (GHC) of whole imported chicken meat at various selling outlets.

Market outlets	Locations (prices/kg (GHC))							
	Accra		Kumasi		Takoradi		Tamale	
	Import	Local	Import	Local	Import	Local	Import	Local
Traditional	20.00	28.50	18.00	32.00	18.00	30.00	20.00	30.00
Supermarkets	20.20	-	22.00	28.00	26.00	-	21.10	-
Shopping mall	26.00	-	26.90	35.00	23.00	-	21.50	-
Cold store	17.00	-	20.00	30.00	18.00	-	16.00	-

Source: Market survey, 2021.

However, the study observed that though consumers purchased household food items such as staple food (cereals, tubers, and roots), vegetables, fish, and eggs in traditional markets, supermarkets and shopping malls were emerging as the major sale outlets for imported chicken meat, in addition to cold stores. Also, the prices at various cold stores were relatively lower than at other selling points.

These cold stores were mostly located in traditional open markets. For instance, the price of a kg of imported chicken meat on average cost between GHC16.00 and GHC20.00 in the four regional markets surveyed. This finding is

consistent with the results of Amfo and Ali (2020), who noted that frozen meat at cold stores was usually priced lower as the product was preserved and not processed.

The survey of the major markets, supermarkets, cold stores, and shopping malls in the study area revealed that local chicken meat was not available in these major selling points. These supermarkets, shopping malls and cold stores offered various qualities at different prices of imported chicken meat, including cut pieces and whole frozen birds. This could be due to unbranded and low-quality packaging materials for local chicken meat. Shopping malls usually cash in on products differentiated by brand, packaging, and country of origin, as noted by Ashraf et al. (2017).

Product packaging, branding, and country of origin

Branding and packaging of food stuff usually boost marketing and profit making (Yeboah, 2016). In this study, local chicken meat was found to be mostly sold unbranded in all the markets surveyed except for some shopping malls where three (3) branded products (Master-meal, Every-day, Seladels) were identified (Table 4). The reason for this is that branded chicken meat may be more expensive and therefore can only be sold in the shopping malls. Additionally, all imported chicken meat was sold as branded and well packaged in sizes of 1 kg, 2 kg, 2.5 kg, and 10 kg, which were available in all the outlets visited.

Table 4. The brand and country of origin of chicken meat at various selling outlets.

Major brands of chicken meat	Country of origin
Golden Dutch, Sadia, Seara, Perdix, Nature Al, Qualiko,	Brazil
Alpi, Frangosul, Lezita	Italy
Mountaire, Quickfrozen, Lamex, Mountaire, Crider	USA
Doux	France
Mastermeal, Every day, Seladels	Ghana
Miki, Ayekoo, Plukon, Vd-Bor, Remkes	Holland
Nana, Cedrob	Poland

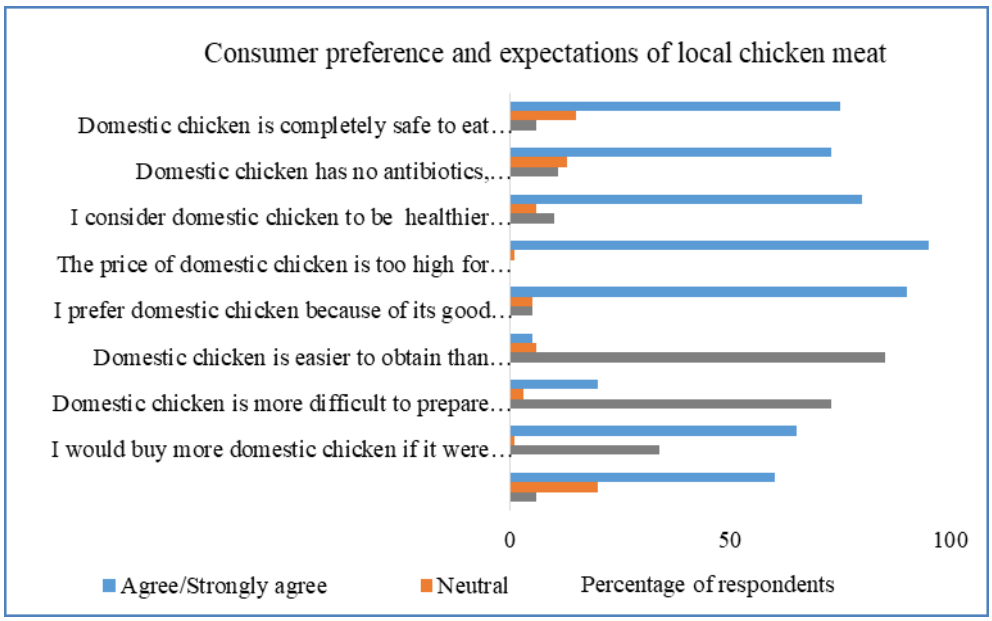
Sources: Market survey, 2021.

The identified brands of imported chicken meat included Sadia, Golden Dutch, Seara, Nature Al, Qualiko and Perdix from Brazil. Other brands imported from major European countries (Italy, France, Holland, and Poland) and the US are reported in Table 4. These brands of chicken meat were found in all outlets. The branded products were mostly cut parts, packaged in cartons and frozen with labels providing information such as the country of origin of the products, the expiry dates, nutritional and health information, and the weight of the products.

Unfortunately, the local branded chicken products did not have such packaging details. Ragasa et al. (2020) noted that packaging and branding were major challenges for local chicken meat in Ghana. Recent findings by Hollinger and Staaz (2015) reveal that urban consumers in Ghana are concerned about the lack of packaging and reliable labeling and other information about the health concerns of local chicken meat they consume. Furthermore, urban consumers in Accra often prefer international branded products to local alternatives because they trust the quality of these brands (Hollinger and Staaz, 2015). This perceived mistrust in the safety of domestically processed and branded food drives consumers towards international brands, putting local producers at a disadvantage. For local producers to compete with imports, the domestic poultry meat industry may have to focus on differentiating its product from the imported chicken in packaging brands, with a major advertising push.

Consumer preferences and expectations of local chicken meat

The perceptions of traders, both wholesalers and retailers, were used to assess the main factors consumers consider when purchasing local chicken meat. The discussants generally agreed that the consumers considered the price of the local chicken to be very high compared to the imported chicken (Figure 3).



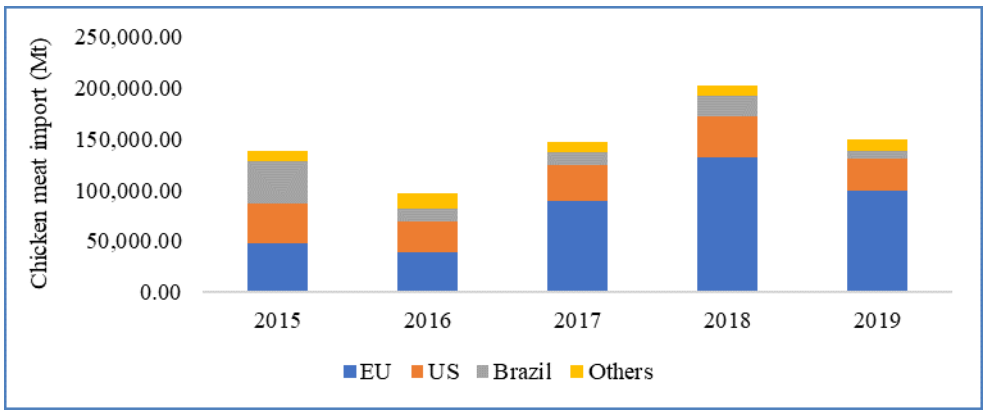
Source: Market survey, 2021.

Figure 3. Consumer preferences and expectations about local chicken in Ghana.

In addition, the preference for local chicken products was associated by the participants with (i) domestic chicken meat being perceived as natural and tasty and containing no antibiotics, hormones, or additives, (ii) domestic chicken meat being completely safe compared to imported chicken meat, and (iii) domestic chicken being generally considered healthier than imported chicken meat. Despite the perceived preference for local chicken, difficulty in obtaining local chicken meat and the time spent in processing deterred consumers from buying local chicken meat in both traditional open markets and modern supermarkets.

Domestic and international trade

According to the FAO (2020), the imports of chicken meat to Africa amounted to 1.75 million tonnes in 2018, with South Africa accounting for 515,000 tonnes (29.5%), followed by Angola with 335,000 tonnes (19%), while Ghana ranked third with 205,000 tonnes (11.7%). Figure 3 summarizes the trends of import market shares for chicken meat between 2015 and 2019. Chicken imports into the country were dominated by products from the EU between 2015 and 2019. Figure 4 shows that the dominance of the EU was followed by imports from the USA, Brazil, and other countries (Canada, Australia, and Asia), respectively.



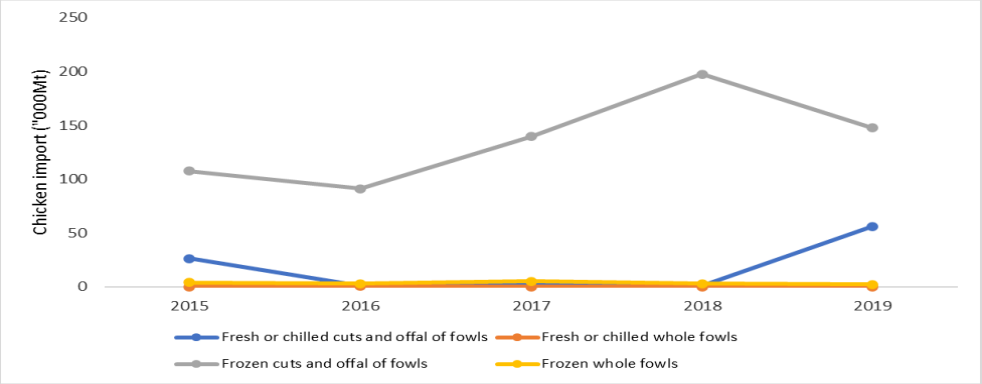
Source: MoFA (2020).

Figure 4. The import of poultry meat into Ghana.

For instance, in 2018, out of the total chicken meat imports of 201,936Mt, nearly 131,427Mt (65%) were supplied by the EU, followed by the US with 41,031Mt (20%), Brazil with 19,318Mt (10%) and other countries with 10,158Mt (5%). Although this trend was quite consistent between 2015 and 2019, Figure 4 further shows that Brazil and the USA had a reduction in chicken meat supply into the country within the same period. The ensuing deficit was made up for by increasing poultry meat exports from EU countries.

For example, the USA's share of Ghana's poultry imports declined from 27.89% in 2015 to 20.88% in 2019 (GSS, 2018). Similarly, the data also revealed that the share of chicken meat imports from Brazil decreased from 30.16% in 2015 to 5.52% in 2019. However, the EU share of chicken meat imports increased from 34.8% in 2015 to 66.08% in 2019. The emerging increase in the import of chicken meat from the EU may be due to the strong relationship between importers and exporters and loyalty to EU products. However, the decline in poultry exports from the USA may be due to perceived distinguishing attributes of products from other countries, existing non-tariff barriers, and the bureaucracy involved in the process of acquiring licenses for poultry meat exports (Ashitey, 2017; Asante-Addo and Weible, 2020). However, as noted by Zamani et al. (2021), USA products still enjoy a price advantage over commodities from Brazil, the EU, and other parts of the world.

In Ghana, most imported poultry meat comes in the form of frozen chicken and cut portions. Figure 5 shows the top four forms in terms of quantity and products in which chicken meat was imported into the country. The category of forms included fresh or chilled cuts and offal of poultry, frozen cuts and offal of poultry, fresh or chilled whole chickens, and frozen whole chickens. The imported cut parts included chicken backs, necks, rumps, wings, and feet, while the offal was composed of gizzards, hearts, and livers.



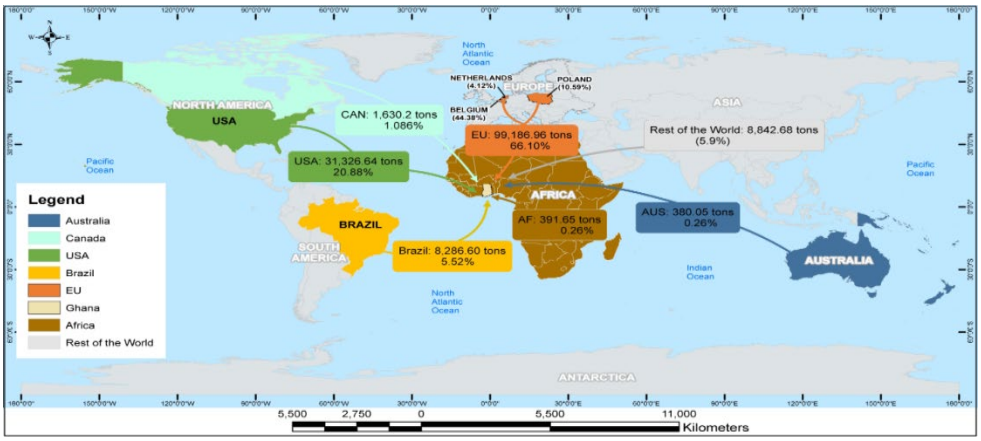
Source: GSS (2018).

Figure 5. Trends in imports of poultry products to Ghana.

Figure 5 reveals that between 2015 and 2019, nearly 98% of all chicken meat imports into Ghana were frozen cuts and offal of poultry. For example, in 2015, frozen cut parts and offal constituted 78.0% (107,719Mt) out of 138,056Mt of chicken meat imported. Similarly, frozen cuts and offal of poultry made up 94.5% (91,343.2Mt) of 96,634Mt in 2016, while it constituted 94.7% (139,722Mt) in 2018. In 2019, 98.05% (147,806Mt) out of 150,044Mt of frozen cuts and offal were imported into the country. Other products such as fresh or chilled cuts and

offal of fowls, fresh or chilled whole fowls, and frozen whole fowl constituted about 2% of imports. This also reflects the high demand for the cut products, as these can usually be used immediately by the consumer.

Figures 4 and 5 show a significant decrease in poultry meat imports in 2016. This was due to the broiler revitalization project which was implemented to stimulate and increase domestic production to about 40% of the total chicken meat demand in Ghana. Although the overall goal of the project was laudable, it could not achieve its aim due to inadequate production and processing logistics and heavy import competition (Asante-Addo and Weible, 2020). Further analysis of Figure 6 reveals that 66.10% of the chickens imported to Ghana originated from European Union countries (of which *44.38%* came from Belgium, *10.59%* from the Netherlands, *4.12%* from Poland, and the rest from Spain, and France).



Source: Authors' illustration, adapted from GSS (2018).

Figure 6. Global poultry trade flows to Ghana in 2019.

The other major importing countries included the US accounting for 20.88%, Brazil taking up 5.52%, while Asia, Canada, and Australia accounted for the rest. In 2019, Ghana imported chicken meat amounting to 392 million USD of which 61.14% came from the EU (239 million USD). Chicken meat imports from the US and Brazil were valued at 89 million USD and 28 million USD accounting for 22% and 7.38% of the total import value to Ghana in 2019. Imports from other countries, including some Asian countries, Canada, and Australia, were valued at 34 million USD, accounting for 7.49%. Ghana's trade with other African countries, including Kenya (0.45Mt), Namibia (50Mt), South Africa (214.2Mt), Togo (74.8Mt), and Tanzania (53Mt), accounted for only 0.26% of total poultry imports to Ghana between 2015 and 2019. Official data from FAO revealed that about 27Mt of chicken meat was re-exported from Ghana to other African countries such as Togo and Ivory Coast in 2019.

This ensuing argument reveals that Ghana is heavily dependent on poultry imports to meet demand. Boschloo (2020) has confirmed that Ghana spends about $374 million annually on the importation of chicken and chicken products. However, Zamani et al. (2022) have opined that the amount of money spent by the government of Ghana on poultry import annually can be relatively reduced if attention is focused on domestic production through input subsidies, improvement in processing technologies, marketing strategies, and reforms in the poultry and livestock import policy.

Conclusion

The exponential increase in poultry meat imports in Ghana due to urban population growth, increase in per capita income, changes in consumer preferences over the last three decades has been well documented in the literature. However, the links between global imports and local poultry markets have rarely been studied. This paper explored the evidence for the growing demand for poultry meat in Ghana by analyzing the dynamics of the poultry market in terms of production, consumption, imports, trade, and how local poultry can be used as a substitute for imports. Descriptives, trend, and content analyses were applied to secondary and primary data obtained from four regions (Greater Accra region, Western region, Ashanti region, and Northern region) of Ghana.

This study has confirmed that there is a high demand for chicken meat in Ghana, 80% of which is currently being met by imports from high-income countries. The study has revealed that 66.10% of these imports come from EU countries (Belgium, the Netherlands, and Poland), while about 20.88% come from the US, 5.52% from Brazil and the rest from Asia, Canada, and Australia. These chicken imports are nicely packaged, branded, and mainly come in the form of cut parts such as rumps, wings, legs, backs, and offal, making them cheap and ready to use. Despite the imposition of about a 35% increase in tariffs, the study has revealed that imported chickens and chicken parts are 27–30% cheaper than locally produced chicken meat. However, prices differed from region to region due to transportation costs and the availability of substitute products. The study also revealed that prices of chicken meat at supermarkets and shopping malls were higher than at other selling points in all the locations surveyed.

The paper shows that domestic chickens throughout the state were sold primarily on the live market. Although few companies were found to process chicken into cut parts, the products were not branded with proper packaging materials and tended to have a relatively higher price than imported products. The cheap imported chicken meat has negatively affected domestic production, making it uncompetitive for-profit gains. Findings further show that although there was some preference for domestic poultry meat, this did not translate into actual

purchase decisions due to the preference for handier chicken and ready-to-use products.

The paper recommends that poultry sector development policies aimed at boosting local production must prioritize investments in poultry meat processing infrastructure (slaughterhouses), storage (refrigeration systems), and marketing. In addition, policies meant to reduce chicken meat import volumes that jeopardize local poultry meat prices may additionally be focused on non-tariff measures such as licenses, allotments, trade embargoes, foreign exchange restrictions, and import depositaries. However, a gradual ban on imports would be necessary, while boosting local production through logistic support in ensuring the processing of local meat into cut parts with good, branded packaging. Additionally, improving the marketing system for local chicken meat through the development and modernization of wholesaling and retailing industry to enhance the availability and quality of products at various selling points including shopping malls and supermarkets is paramount.

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DINAMIKA TRŽIŠTA ŽIVINSKOG MESA U GANI

Edward Ebo Onumah[1*] i Selorm Ayeduvor[2]

[1]Department of Agricultural Economics and Agribusiness, University of Ghana
[2]CSIR – Science and Technology Policy Research Institute, Ghana

R e z i m e

Ovaj rad ispituje dinamiku tržišta živinskog mesa u Gani koristeći sekundarne podatke i terensko istraživanje u četiri regiona uključujući Veliku Akru, Zapadni region, Ašanti i Severni region. Sekundarni podaci o cenama, potrošnji po glavi stanovnika, količinama uvozne i izvozne robe dobijeni su od Organizacije za hranu i poljoprivredu (engl. *Food and Agriculture Organization – FAO*) i Ministarstva za hranu i poljoprivredu (engl. *Ministry of Food and Agriculture – MoFA*). Primarni podaci o tržnim centrima, supermarketima, hladnjačama i otvorenim pijacama kao i o cenama i brendovima uvezenog i lokalnog pilećeg mesa takođe su prikupljeni putem anketa i diskusija u fokus grupama. U istraživanju su se koristile analize trendova i sadržaja kako bi se istakli određeni uvozni proizvodi, njihovo poreklo, pokretači i potencijal domaćeg živinskog mesa kao zamene za uvezeno pileće meso. U radu se potvrđuje da Gana uvozi oko 80% živinskog mesa, uglavnom u obliku brendiranih sečenih delova (bataka, krilaca, nogu, leđa i iznutrica) iz zemalja sa visokim prihodima, uključujući Belgiju, SAD, Brazil, Poljsku i Holandiju. Uprkos povećanju tarifa za 35%, uvezeno živinsko meso obično je 27–30% jeftinije od lokalno proizvedenog pilećeg mesa. Rezultati dalje pokazuju da se, iako postoji određeno preferiranje domaćeg živinskog mesa, to ne prenosi na odluke o kupovini, jer ljudi više vole praktičnije i već spremne proizvode. U radu se preporučuje davanje prioriteta politikama za podsticanje lokalne proizvodnje kroz ulaganja u preradu (sečene delove), brendirano pakovanje i marketinške objekte poput rashladnih kombija. Štaviše, politike za smanjenje obima uvoza pilećeg mesa mogle bi se fokusirati na druge netarifne mere kao što su licence, dodele, trgovinski embargo, devizna ograničenja i uvozni depoziti.

Ključne reči: živinsko meso, sečeni delovi, uvoz, trgovina, pakovanje, Gana.

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[*]Autor za kontakt: e-mail: eeonumah@ug.edu.gh

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Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

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

Book chapter

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.

Proceedings
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, France.

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

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

Summary
The summary in Serbian is given at the end of the paper and should comprise 200 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

Tables
Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

Illustrations
All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

diagram should be given. The photographs must be of high quality so that they can technically be well reproduced. They should be submitted in "TIF" or "JPG" format, and they will be printed in black and white. The title of the illustration should be justified, with a full stop at the end, single spaced from the illustration and given below it. Each illustration should be mentioned in the text.

Abbreviations and units
Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature
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.

Formulae
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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Editorial Board of the Journal
Journal of Agricultural Sciences

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Za obradu teksta treba koristiti program MS-Word. Rukopise treba slati u jednom od sledećih formata .doc, .docx, koristiti font Times New Roman, veličina 12, jednostruki prored, margine 2,5 cm. Strane ne treba numerisati.
Originalan naučni rad – Rad koji sadrži prethodno neobjavljivane rezultate sopstvenih istraživanja. Obim ovog rada treba da iznosi od 6 do 12 strana.
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Obavezna poglavlja svakog originalnog naučnog rada i prethodnog saopštenja su sledeća: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, materijal i metode, rezultati i diskusija, zaključak, zahvalnica, literatura i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Pregledni rad mora da sadrži: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, analizu-diskusiju određene teme, zaključak, literaturu i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Ako su radovi na engleskom jeziku, prednost se daje britanskoj varijanti ovog jezika.

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Ključne reči
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

tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod

Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode

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.

Rezultati i diskusija

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.

Zaključak

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

Zahvalnica

Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

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Poglavlje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van

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Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

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

Rezime
Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 200 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmaknuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod

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Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *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* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation". Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

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