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THE EFFECTS OF PRE-SOWING TREATMENTS WITH AQUEOUS ALLELOPATHIC PLANT EXTRACTS ON THE GERMINATION PARAMETERS OF AGED SOYBEAN SEEDS

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Abstract: The aim of the paper was to examine the allelopathic influence of aqueous extracts of herbs and medicinal plants on the quality parameters of aged soybean seeds. The research was carried out at the Institute of Field and Vegetable Crops in Novi Sad on aged seeds of two soybean varieties, NS simba and NS viseris. The seeds were aged for 20 months. In order to determine the allelopathic effect, the seeds were primed in aqueous plant extracts: geranium (*Geranium sinense*), dill (*Anethum graveolens*), andy everlasting (*Helichrysum arenarium*), creeping thyme (*Thymus serpyllum*), celery (*Apium graveolens*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), lemon balm (*Melissa officinalis*), rosemary (*Rosmarinus officinalis*), wormwood (*Artemisia absinthium*), peppermint (*Mentha x piperita*), sage (*Salvia officinalis*), and lavender (*Lavandula angustifolia*). The results show that it is not possible to talk about the universal application of a particular aqueous extract, because the effect of the allochemicals was significantly influenced by the variety. In the variety NS viseris, all aqueous extracts except *Melissa officinalis*, significantly reduced GE, GP, and VI. The most negative effect was achieved with the use of *Apium graveolens*, *Thymus serpyllum*, and *Ocimum basilicum*. *Apium graveolens* and *Thymus serpyllum* also had the greatest impact on quality reduction in the NS simba variety. However, in the NS simba variety, a significant increase in seed quality was achieved in addition to the reduction. The use of the aqueous extract of *Salvia officinalis* increased GE and GP by 13.7%, and VI by 10.21%. A positive effect was achieved with the use of *Melissa officinalis*. All aqueous extracts had a significant effect on T50 in both varieties, even the aqueous extracts that had a negative effect on GE and GP.

Key words: allelopathy, aqueous extracts, priming, soybean.

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Introduction

Seeds are the basic link to our agricultural past (Navazio, 2021). Seed trade has been a key component in the spread of crops since very early in the domestication process (Allaby et al., 2021). However, the commercial sale of seeds did not become common in North America and Europe until the second half of the 19^{th} century, and the seed trade of that era consisted almost entirely of small packets of vegetable, flower, or herb seeds that served as starter packets (Navazio, 2021). With the advent of commercial seed companies, farmers and gardeners bought speciality seeds and hard-to-find items. Sometimes they purchased seeds of a particular species that was difficult to produce in their climate (Fernandez-Cornejo and Just, 2007). Along with the growth of commercial seed production, different methods are also being developed to improve seed quality. Today, in many countries, there is a growing awareness that healthy and safe food is also important in addition to quantity (Dozet et al., 2017). For this purpose, scientists are increasingly studying the natural mechanisms by which plants communicate with each other and influence each other. One of the most important mechanisms is allelopathy. Although the term allelopathy is most often used to describe chemical interactions between two plants (allelopathy in the narrow sense), it can also be used to describe chemical communication between plants and microbes, plants and insects, and plants and herbivores (allelopathy in the broad sense) (Weir et al., 2004). Seed quality plays an important role in agricultural and animal husbandry production, the effective utilization of genetic resources, the conservation of biodiversity, and the restoration and reconstruction of plant communities (Han et al., 2022). Soybean seeds reach their maximum potential for germination and vigor in the phase of physiological maturity, while the value of these parameters decreases with aging (Ebone et al., 2020). The aging of seeds is a common physiological phenomenon during storage. It is a natural, irreversible process that occurs and develops along with the extension of seed storage time. It is not only related to growth, yield, and seed quality, but also has a significant impact on the conservation, utilization, and development of soybean germplasm resources (Rao et al., 2023).

The aim of the research is to examine the allelopathic potential effects of aqueous extracts of herbs and medicinal plants on the quality parameters of aged soybean seeds.

Material and Methods

In order to study the allelopathic effect of aqueous extracts of herbs and medicinal plants on the quality of aged seeds, two soybean varieties, NS viseris and NS simba, were chosen for the study. The varieties were selected at the Institute of Field and Vegetable Crops in Novi Sad. The seeds were produced at the Rimski Šančevi experimental field during 2022. The seeds were aged for 20 months during the analysis.

Seed treatment

Before being primed, the seeds were surface sterilized with a 3% solution of sodium hypochlorite (NaOCl) for two minutes and washed under a stream of distilled water. In order to determine the allelopathic effect, the seeds were primed in aqueous plant extracts: geranium (*Geranium sinense*), dill (*Anethum graveolens*), andy everlasting (*Helichrysum arenarium*), creeping thyme (*Thymus serpyllum*), celery (*Apium graveolens*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), lemon balm (*Melissa officinalis*), rosemary (*Rosmarinus officinalis*), wormwood (*Artemisia absinthium*), peppermint (*Mentha x piperita*), sage (*Salvia officinalis*), and lavender (*Lavandula angustifolia*).

In the preparation of all aqueous plant extracts, 100 g of plant material was used, which was cut into small pieces measuring 1–4 cm. After cutting, the plant material was poured into 1 liter of distilled water and left to ferment with daily stirring. After fermentation, the obtained aqueous plant extract was filtered through gauze and left to stand at a temperature of 18–22 °C in hermetically sealed glass bottles. Prior to foliar treatment of the soybean plants, the aqueous extract was diluted with water in a ratio of 1:15.

The seeds were primed for 6 hours. They were then dried naturally to their original moisture content. The working sample consisted of 4 × 50 seeds. Seeds were evenly distributed on sterile filter paper in 9-cm diameter Petri dishes. All Petri dishes were closed with paraffin tape to prevent moisture loss and contamination. The Petri dishes containing the seeds were placed in a germination chamber at a temperature of 25 °C for 8 days. Every day, the germination of the seeds was noted, assuming that seeds with radicles of 2 mm or longer were considered to have germinated. After five days, the germination energy was determined, and the germination percentage after eight days (ISTA, 2019).

- The coefficient of velocity of germination (CVG) was determined using the formula of Nikolas and Heydeker (1968):

$$CVG = 100 \times \sum Ni / \sum NiTi \quad (1)$$

where: Ni=number of germinated seeds per day, Ti=number of days from the start of the experiment (Nikolas and Heydeker, 1968).

- Time to 50% germination (T50) was determined using the formula of Coolbear et al. (1984):

$$T50 = ti + (N/2 - ni)(tj - ti) / (nj - ni) \quad (2)$$

where N is the final number of germinating seeds, nj and ni are the cumulative number of seeds germinated at times t j and t i, respectively, when $ni<N/2<ni$.

- Vigor index (VI) was calculated according to the following formula:

$$VI = GP \times SL, \quad (3)$$

where GP=germination percentage and SL=seedling length after 8 days (Abdul-Baki and Anderson, 1973).

The research results were processed by analyzing the variance of the two-factorial experiment (Hadzivukovic, 1991), and the significance of the differences was tested by the LSD test at the level of significance 0.01 and 0.05 (statistical program “Statistica 10.0”).

Results and Discussion

Germination energy and germination percentage

According to many scientists, priming is a pre-sowing treatment to improve the quality of soybean seeds (Murungu et al., 2005; Rouhi et al., 2011; Miladinov et al., 2015; Lewandowska et al., 2020). However, when aqueous plant extracts are used for priming, the allelopathic relationship must be taken into account. In recent years, allelopathy has been the subject of numerous studies by scientists who seek to find various alternative methods that can be successfully used in agriculture and thus reduce the use of synthetic compounds. Certain plants have the ability to secrete one or more biochemical substances that affect the growth, reproduction, and survival of other plants. These substances have an inhibitory or stimulating effect on other plants (Cheng and Cheng, 2015). Allelopathy offers a new alternative for the development of ecologically acceptable environmental measures that fulfil a dual purpose, on the one hand, increasing productivity and, on the other hand, maintaining the stability of the ecosystem (Scavo et al., 2019). At the very beginning, before applying any measure, in this case priming, it is necessary to determine the allelopathic compatibility between the plant from which the aqueous extract is produced and the donor, i.e., the plant whose seeds are treated.

When observing the influence of aqueous extracts on GE, it can be seen that most of them lead to a decrease in this parameter (Table 1).

The most significant reduction is seen with the application of aqueous extracts: *Apium graveolens* (22.86%), *Thymus serpyllum* (21.43%), *Ocimum basilicum* (18.57%), and *Artemisia absinthium* (17.14%). However, looking at the variety-aqueous extract interaction, it can be seen that the varieties reacted differently to most aqueous extracts. In the ns simba variety, the use of *Salvia officinalis* and *Melissa officinalis* increased GE by 13.70% and 8.70%, respectively. They had a greater effect on the reduction of GE. The reduction was caused by the use of *Thymus serpyllum* by 20.63%, Apium graveolens by 19.05%, and *Artemisia absinthium* by 15.87%. In the variety NS viseris, all aqueous extracts, except for *Melissa officinalis*, reduced GE. The reduction ranged from 8% to 24%. The use of aqueous extracts of *Ocimum basilicum* (24%), *Apium graveolens* (22.67%), *Thymus serpyllum* (21.33%), *Anethum graveolens* (18.67%), *Artemisia absinthium* (17.33%), *Helichrysum arenarium* (13.33%), and *Rosmarinus officinalis* and *Lavandula angustifolia* (12%) led to a significant reduction. When considering the influence of the aqueous extracts on seed germination, it can be seen that most of them influenced the reduction of GP. The most significant reduction was observed with the use of *Thymus serpyllum*, 21.42%; a positive effect was only observed with the use of *Salvia officinalis* and *Melissa officinalis*, but without a significant increase. As for GE, the varieties reacted differently to aqueous extracts. In the NS simba variety, even three aqueous extracts had a significant effect on the increase in GP, while three led to a significant decrease. The application of *Salvia officinalis* increased GP by 13.70%, *Melissa officinalis* by 11.27%, *Mentha x piperita* by 10%, *Anethum graveolens* and *Lavandula angustifolia* by 8.70%, and *Rosmarinus officinalis* by 7.35%. The reduction was caused using *Thymus serpyllum* by 20.63%, *Apium graveolens* by 15.87%, and *Artemisia absinthium* by 12.70%.

Table 1. The influence of the aqueous extract application on the germination energy and germination percentage of soybean seeds.

Treatment (B)	Germination energy (GE)			Germination percentage (GP)		
	NS simba	NS viseris	Average (B)	NS simba	NS viseris	Average (B)
Control	63	75	70	63	76	70
Geranium sinense	63ns	69*	65 ns	65 ns	69*	67 ns
Anethum graveolens	64ns	61**	63*	69*	61**	65*
Helichrysum arenarium	60ns	65**	63*	62 ns	66**	64*
Thymus serpyllum	50**	59**	55**	50**	60**	55**
Apium graveolens	51**	58**	54**	53**	58**	56**
Origanum vulgare	66ns	69*	68 ns	66 ns	69*	68 ns
Ocimum basilicum	57*	57**	57**	59 ns	60**	60**
Melissa officinalis	69*	75 ns	72 ns	71**	75 ns	73 ns
Rosmarinus officinalis	67ns	66**	67 ns	68*	67**	68 ns
Artemisia absinthium	53**	62**	58**	55**	63**	59**
Mentha x piperita	68ns	68*	68 ns	70*	69*	70 ns
Salvia officinalis	73**	69*	71 ns	73**	70*	72 ns
Lavandula angustifolia	68ns	66**	67 ns	69*	67**	68 ns
Average	62	66	-	64	66	-

Factor	$LSD_{0.05}$		$LSD_{0.01}$	
	GE	GP	GE	GP
A	3.31	1.97	4.11	2.94
B	5.45	4.89	9.83	7.12
A x B	6.37	5.84	11.48	7.13

*, ** Significant at the 0.05 and 0.01 probability levels, respectively (LSD test); ns = not significant.

In the ns viseris variety, all extracts led to a decrease in GP. The decrease ranged from 1.32% to 23.68%. The use of *Apium graveolens* (23.68%), *Thymus serpyllum* (21.05%), *Anethum graveolens* (19.76%), *Artemisia absinthium*

(17.11%), *Helichrysum arenarium* (13.16%), *Rosmarinus officinalis* (11.84%), and *Lavandula angustifolia* (11.84%) led to a significant reduction. Only the application of *Melissa officinalis* had no effect on GP in the ns viseris variety. The use of an extract of *Salvia officinalis* in the ns simba variety led to an increase in both parameters of germination. The results are in contrast to research conducted on barley and lettuce (Bajalan et al., 2013), where the application of an aqueous extract of *Salvia officinalis* significantly reduced seed germination in these plant species. Similar results were obtained using an extract of *Mentha x piperita*. In these studies, germination was increased by 10%. However, Shakir et al. (2021) found in the seeds of *Cicer arietinum* L. that the application of the aqueous extract of *Mentha longifolia* led to a decrease in seed germination. Możdżeń et al. (2019) determined the negative influence of *Mentha x piperita* extract on the germination of cereal seeds. This is attributed to the fact that *Mentha longifolia* and *Mente x piperite* extracts contain high concentrations of effective compounds, such as phenols, alkaloids, and tannins. These compounds have the ability to inhibit the germination and growth of plants (Shakir et al., 2021). An allelopathic effect is mainly referred to as a type of negative interaction (De Albuquerque et al., 2011), but positive interactions have also been reported, depending on the allelochemical considered, the target plant, and the concentration tested (Eichenberg et al., 2014), and in these studies also on the variety.

Coefficient of velocity of germination and time to 50% germination

Total percentage germination after a specific period of time does not give a full explanation of the dynamics of germination (Joosen et al., 2010). The timing of seed germination is one of the key steps in the plant life cycle, determining when plant growth begins in natural or agricultural ecosystems. In nature, many seeds exhibit various types and levels of dormancy (Baskin et al., 2000). Therefore, different parameters are used to determine, first of all, the time required for seed germination. This is particularly important in field conditions for uniform germination, emergence, and a uniform crop. The timing of seed germination is one of the key steps in the plant life cycle, determining when plant growth begins in natural or agricultural ecosystems (Miladinov, 2020). When observing the influence of the aqueous extracts on CVG, it can be seen that, on average, only the application of *Melissa officinalis* significantly increases the value of this parameter by 18.11% (Table 2).

However, if the variety x aqueous extract interaction is observed, it can be noted that the varieties reacted differently to most aqueous extracts. In the NS simba variety, all aqueous extracts had a significant effect on the increase of CVG, ranging from 12.5% to 30.28%. The greatest effect was achieved with the use of *Salvia officinalis* (30.28%) and *Melissa officinalis* (27.39%). In the NS viseris variety, only *Melissa officinalis* aqueous extract had a significant effect on increasing CVG by 9.02%. All other aqueous extracts significantly reduced their value. The reduction ranged from 25% for *Mentha x piperita* and *Salvia officinalis* to 49.59% for *Apium graveolens*.

Table 2. The influence of the aqueous extract application on the coefficient of velocity of germination and time to 50% germination of soybean seedllings.

Treatment (B)	Coefficient of velocity of germination (CVG)			Time to 50% germination (T50)		
	NS simba	NS viseris	Average (B)	NS simba	NS viseris	Average (B)
Control	17.5	22.2	19.9	3.30	4.2	3.75
Geranium sinense	22.2**	16.6**	19.4ns	2.10**	2.77**	2.44**
Anethum graveolens	23.8**	14.0**	18.9ns	1.54**	2.58**	2.06**
Helichrysum arenarium	22.1**	14.6**	18.4ns	1.93**	2.35**	2.14**
Thymus serpyllum	20.7**	13.6**	17.2ns	2.31**	2.81**	2.56**
Apium graveolens	20.9**	12.3**	16.2ns	2.37**	2.82**	2.60**
Origanum vulgare	23.8**	14.6**	19.2ns	1.49**	2.81**	2.15**
Ocimum basilicum	22.3**	13.3**	17.8ns	1.57**	1.81**	1.69**
Melissa officinalis	24.1**	24.4*	24.3**	1.32**	1.42**	1.37**
Rosmarinus officinalis	23.7**	15.4**	19.6ns	1.62**	1.79**	1.71**
Artemisia absinthium	21.1**	14.2**	17.7ns	1.98**	2.37**	2.18**
Mentha x piperita	23.9**	18.3**	21.1ns	1.79**	1.40**	1.60**
Salvia officinalis	25.1**	18.3**	21.7ns	1.31**	1.64**	1.48**
Lavandula angustifolia	23.7**	14.7**	19.2ns	1.65**	1.66*	1.66**
Average	22.49	16.18	-	1.88	2.32	-

Factor	$LSD_{0.05}$		$LSD_{0.01}$	
	CVG	T50	CVG	T50
A	2.11	0.57	3.97	0.84
B	3.15	0.89	3.38	1.18
A x B	2.17	0.74	4.18	0.98

*, ** Significant at the 0.05 and 0.01 probability levels, respectively (LSD test); ns = not significant.

On average, all aqueous extracts had a significant effect on the reduction of T50, ranging from 31.73% to 63.47%. The greatest effect was achieved using *Melissa officinalis* (63.47%) and *Salvia officinalis* (60.53%). The inhibitory effect on the process of seed germination can be explained by the presence of biologically active compounds in the donor plants that act on the components of the cell membranes of the recipients of the analyzed culture species (Harper and Balke, 1981). These substances cause, among other things, enzyme malfunction and disturbances in the absorption of water and mineral substances, which have a negative effect on seed germination (Siyar et al., 2017). In addition, compounds from the extract impede the breathing process (Keating, 1999), plant hormones and protein synthesis (John and Sarada, 2012), and the ion absorption process (Qasem and Hill, 1989). Moreover, the aqueous extract plays an indirect role in cell death by producing reactive oxygen, which causes lipid oxidation and damages the cell membranes (Mutlu et al., 2011). Consequently, this leads to stunted plant growth and death.

When observing the interaction of variety x aqueous extract, it can be seen that the varieties reacted similarly to most aqueous extracts. In ns simba, all extracts significantly reduced the T50 value. The decrease ranged from 28.18% to 60.30%. The most significant effect was achieved by using *Salvia officinalis* and *Melissa officinalis* (60.38% and 60%, respectively). In the NS viseris variety, the effect of the aqueous extracts on the reduction of the T50 parameter was even more significant, namely from 32.86% to 66.67%. The most significant effect was achieved with the use of *Mentha x piperita* (66.67%) and *Melissa officinalis* (66.19%). Seed priming with aqueous extracts in the ns simba variety significantly reduced T50, which may be a consequence of the initiation of metabolitic processes in primed seeds (Yaldagard et al., 2008). According to Mohamed et al. (2018), priming leads to the repair of aged seeds by resuming metabolic activity to restore cellular integrity by improving enzyme activities and protein synthesis, a repaired cell membrane, and increased antioxidant defense mechanisms, which enhances the seed and seedling performance compared with non-primed seeds.

The results show that all aqueous extracts had a significant influence on the germination rate in the initial period for both varieties. Even the aqueous extracts, which had a negative effect on GE and GP, had a stimulating effect on the germination rate, especially in the initial period, which can be seen from the value of the parameter T50, which shows when 50% of the seeds have germinated. Those aqueous extracts that had a favorable effect on these parameters reduced T50 the most, while aqueous extracts that significantly reduced GE and GP had the least impact on T50, but still significantly reduced germination time. The varieties reacted similarly, regardless of the fact that aqueous extracts have significantly different effects on their parameters, GE and GP. The results show that, in the ns simba variety, all aqueous extracts accelerated the entire germination period, regardless of whether they had a favorable or unfavorable effect on GE and GP. However, in the ns viseris variety, all aqueous extracts except *Melissa officinalis* increased CVG, i.e., extended the germination time compared to the control. Otherwise, in this variety, all aqueous extracts had a negative effect on GE and GP, except for *Melissa officinalis*, which had no significant effect.

Vigor index

When looking at the influence of the aqueous extracts on VI on average, only the application of *Melissa officinalis* led to a significant increase in the value of this parameter, namely by 10.91%. The other aqueous extracts either reduced or had no significant effect on the VI value. The reduction was greatest with the application of *Apium graveolens* and *Thymus serpyllum*, 27.51% and 27.30%, respectively. However, looking at the interaction of variety x aqueous extract, the varieties reacted differently to most aqueous extracts. In the variety NS simba, the use of *Mentha x piperita* increased VI by 10.54%, *Salvia officinalis* by 10.21%, and *Melissa officinalis* by 7.28%. Plant aqueous extracts had a greater effect on the reduction than on the increase of VI. The use of *Thymus serpyllum* and *Apium graveolens* led to a significant reduction of 16.64% and 15.67%, respectively. In the NS viseris variety, all aqueous extracts, except for *Melissa officinalis*, led to a decrease in VI, ranging from 5.78% to 20.96%. A significant decrease was caused by the use of the aqueous extracts of *Ocimum basilicum* (20.96%), *Apium graveolens* (20.93%), *Thymus serpyllum* (19.75%), *Helichrysum arenarium* (17.92%), *Anethum graveolens* (19.34%), and *Rosmarinus officinalis* (17.16%). *Melissa officinalis* increased VI by 13.53% (Table 3).

Table 3. Effects of the aqueous extract application on the vigour index of soybean seedlings.

Treatment (B)	Vigor index (VI)		
	NS simba	NS viseris	Average (B)
Control	510.19	658.12	584.11
Geranium sinense	520.1ns	610,12*	565.11ns
Anethum graveolens	525.18ns	530.84**	528.01*
Helichrysum arenarium	505.13ns	540.20**	522.67*
Thymus serpyllum	425.20**	528.12**	476.66**
Apium graveolens	430.18**	520.40**	475.29**
Origanum vulgare	507.40ns	605.15*	556.28ns
Ocimum basilicum	510.15ns	520.18**	515.17*
Melissa officinalis	550.18*	761.12**	655.65**
Rosmarinus officinalis	530.20ns	545.18**	537.69*
Artemisia absinthium	515.65ns	610.12*	562.89ns
Mentha x piperita	570.20**	615.10*	592.65ns
Salvia officinalis	568.12**	620.05*	594.09ns
Lavandula angustifolia	530.35ns	605.58*	567.97ns
Average	514.16	590.73	-

Factor	VI	
	$LSD_{0.05}$	$LSD_{0.01}$
A	67.12	84.09
B	41.77	51.37
A x B	2.17	0.74

Allelopathy research has traditionally focused on evaluating the phytotoxicity of plant residues or crude extracts (Weston, 1996). In recent years, when agricultural producers began to look for alternative methods, allelopathy or allelopathic relationships have been given great importance. By making appropriate use of allelopathic crops in agriculture, agricultural producers try to reduce the use of pesticides and thus reduce environmental and food pollution, decrease costs in agriculture, improve food security in poor regions and soil productivity, and increase biodiversity and sustainability in the agro-ecosystem (Islam et al., 2018). However, improving products using allelopathic compounds, which are mainly secondary metabolites, is not easy (Bhowmik and Inderjit, 2003). Furthermore, the use of allelopathic relations requires knowledge of the allelochemicals involved and fluctuations depending on environmental conditions and agricultural practices. However, as a pre-sowing seed treatment to improve seedling performance, the use of allelochemicals has not yet found significant application. One of the reasons for this is that there is not much research on this topic. Therefore, more attention should be paid to the application of allelopathy to improve seed quality in the future.

Conclusion

The results show that the phenomenon of allelopathy can play a significant role in the pre-sowing treatment of soybean seeds to improve quality parameters. In this way, it is possible to obtain a cheap and simple presetting measure that will find its place in organic production. However, before applying any treatment, it is necessary to carry out preliminary tests because, in addition to the positive influence of allelochemicals, they can significantly reduce the quality of the seeds. It is therefore not possible to speak of the universal application of a certain extract because the effect of allelochemicals is significantly influenced by the variety. In the variety ns viseris, all extracts, except *Melissa officinalis*, significantly reduced GE, GP, and VI. The most negative effect was obtained by using *Apium graveolens*, *Thymus serpyllum*, and *Ocimum basilicum*. *Apium graveolens* and *Thymus serpyllum* also had the greatest impact on quality reduction in the ns simba variety. However, in the ns simba variety, a significant increase in seed quality was achieved in addition to the reduction. The use of *Salvia officinalis* extract increased GE and GP by 13.7%, and VI by 10.21%. All extracts had a significant effect on the germination rate in both varieties, even the extracts that had a negative effect on GE and GP.

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UTICAJ PREDSETVENIH TRETMANA VODENIM EKSTRAKTIMA ALELOPATSKIH BILJAKA NA PARAMETRE KLIJANJA STAROG SEMENA SOJE

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R e z i m e

Cilj rada je bio da se ispita alelopatski uticaj ekstrakta začinskog i lekovitog bilja na kvalitet starog semena soje. Ispitivanje je obavljeno na Institutu za ratarstvo i povrtarstvo u Novom Sadu, na starom semenu dve sorte soje ns simba i ns viseris. Seme je bilo staro 20 meseci. Radi utvrđivanja alelopatskog uticaja seme je potapano u biljne vodene ekstrakte: geranijuma (*Geranium sinense*), mirođije (*Anethum graveolens*), smilja (*Helichrysum* arenarium), majčine dušice (*Thymus serpyllum*), celera (*Apium graveolens*), origana (*Origanum vulgare*), bosiljka (*Ocimum basilicum*), matičnjaka (*Melissa officinalis*), ruzmarina (*Rosmarinus officinalis*), pelina (*Artemisia absinthium*), nane (*Mentha x piperita*), žalfije (*Salvia officinalis*), i lavande (*Lavandula angustifolia*). Rezultati su pokazali da se ne može govoriti o univerzalnoj primeni određenog ekstrakta, jer na efekat alelohemikalija značajan uticaj ima sorta. Kod sorte ns viseris, svi ekstrakti, osim ekstrakta *Melissa officinalis*, značajno su smanjili GE, GP i VI. Najnegativniji efekat ostvaren je primenom *Apium graveolens*, *Thymus serpyllum* i *Ocimum basilicum*. *Apium graveolens* i *Thymus serpyllum* su najviše uticali na smanjenje kvaliteta i kod sorte ns simba. Međutim, kod sorte ns simba, pored smanjenja ostvareno je i značajno povećanje kvaliteta semena. Primenom ekstrakta *Salvia officinalis*, GE i GP su povećani za 13,7%, a VI je povećana za 10,21%. Dobar efekat je ostvaren i upotrebom *Melissa officinalis*. Svi ekstrakti, kod obe sorte, imali su značajan uticaj na brzinu klijanja, čak i ekstrakti koji su negativno delovali na GE i GP.

Ključne reči: alelopatija, vodeni ekstrakti, potapanje, soja.

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PERFORMANCE EVALUATION OF PROVITAMIN A MAIZE (*ZEA MAYS* L.) HYBRIDS FOR YIELD AND OTHER AGRONOMIC TRAITS IN SOUTHWESTERN NIGERIA

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Abstract: Maize is a vital dietary staple in Nigeria, offering crucial nutrients such as carbohydrates, proteins, fats, and micronutrients. However, conventional maize lacks enough of the nutritional precursor for vitamins, necessitating the cultivation of stable, high-yielding provitamin A maize hybrids. This is crucial for combating malnutrition, particularly in rural populations vulnerable to "hidden hunger". This study aimed to assess the agronomic performance and yield of newly developed maize hybrids over a two-year period in Ikorodu and Osogbo, Nigeria. Twenty-two improved maize hybrids, two commercial hybrids, and a reference check were evaluated. Hybrid grain yields ranged from 3.33 t/ha (Ife-hybrid 3) to 5.69 t/ha (LY1409-61) over the two years, averaging 4.03 t/ha. All hybrids, except Ife-hybrid 3 (3.33 t/ha), outperformed the reference check (3.92 t/ha), with four hybrids achieving yields exceeding 5 t/ha across the two years. The distribution of precipitation in 2019, with higher and well-distributed rainfall, significantly impacted grain yields compared to 2020. This effect was particularly notable during the flowering and grain filling stages from July to October. LY1409-61, AS1802-15, and LY1409-21 consistently performed well across varying weather conditions, indicating their adaptability to diverse agro-ecologies. Adopting these maize hybrids has the potential to enhance maize output and alleviate malnutrition in rural southwestern Nigeria. The study emphasises the vital role of promoting nutrient-enriched maize varieties to combat nutritional deficiencies, enhance food security, and benefit communities dependent on maize as a staple, which could notably contribute to sustainable agriculture and improved nutritional outcomes in the region.

Key words: adaptation, agronomic traits, grain yield, hidden hunger, malnutrition, food security.

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Introduction

Maize is a significant crop in sub-Saharan Africa, making up 40% of cereal production. It is mainly used as a food source, providing around 30% of the calorie intake in the region (Ekpa et al., 2019). Maize is a staple in Africa and Latin America, with varying daily consumption amounts. Additionally, maize is known for its health benefits, as it contains nutraceuticals that can enhance health and prevent diseases. These include phenolics, carotenoids, anthocyanins, phlobaphenes, dietary fibre, and lipids (Ekpa et al., 2019). Since maize constitutes a significant portion of the diet in tropical Africa and is a major feed ingredient in the livestock industry, the development of nutritionally balanced and vitamin-rich genotypes has become critical for its use by the African populace. Therefore, the development of high-yielding and well-adapted cultivars is the major goal of most maize breeding projects.

The interplay of genetic potential and environment determines grain yield. The variability of genetic potential among cultivars is a major factor in yield variation. Plant breeding uses multi-location trials to estimate and predict yield based on limited experimental data, yield stability, and genotype response patterns in different conditions. These provide trustworthy guidelines for selecting the optimum genotypes for planting at new places in future years (Crossa, 1990).

The extent of genetic variability for different traits among various maize hybrids is a key to crop improvement. The ability to develop high-yielding and stable cultivars is an ultimate goal of most breeding programmes. An ideal maize hybrid should have a high mean yield combined with a low degree of fluctuation under different conditions (Annicchiarico, 2002; Yousaf et al., 2021). However, drought stress influences the reduction of plant growth, development, and production (Mohammadai et al., 2012; Ansari et al., 2019). Therefore, understanding the environmental and agronomic responses of maize hybrids is fundamental to improving the efficiency of maize production. As a result, newly introduced hybrids must often be studied for several years before being approved for a specific site (Beiragi et al., 2011). Before making suggestions, the specificities of the hybrids and the growing conditions of the locations must be examined (Mitrovic et al., 2012).

Furthermore, the climatic conditions in these agro-ecologies fluctuate significantly across seasons and regions, which is a cause for concern. Inadequate crop management methods, diminishing soil fertility, the occurrence of unpredictable diseases and insect pests, and the escalation of climate variations are all issues of concern for maize production in Nigeria. The sensitivity of agro-ecosystems in which small-scale farmers in SSA grow maize to weather fluctuations is becoming increasingly worrisome as optimal production scenarios linked to uncertain climate change may become more widespread (Gaudin et al., 2015). As a result, the current study was designed to assess the adaptability of maize hybrids in southwestern Nigeria, with the goal of examining the adaptability and performance of new hybrid maize varieties as well as identifying high-yielding, disease resistant (tolerant) hybrid maize varieties.

Material and Methods

The experimental material for the present study comprises twenty-two single- and triple-crossed provitamin A hybrids, two commercial checks (Ife hybrid 3 and Ife hybrid 4), and one reference check (Table 1). The hybrids were evaluated along with checks in a randomized complete block design (RCBD) with three replications, having a plot length of 5 m and inter and intra row spacing of 75cm and 50cm, respectively, during the 2019 and 2020 growing seasons across two locations in different agro-ecological zones of Ikorodu (6.6194°N, 3.5105°E) in Ogun and Osogbo (7.7827°N, 4.5418°E) in Osun State.

Table 1. The list of genetic materials used in this study.

Entry	Hybrid	Origin
1	A1702-28	IITA
2	A1706-2	IITA
3	A1736-12	IITA
4	A1736-13	IITA
5	A1736-6	IITA
6	A1802-4	IITA
7	A1802-14	IITA
8	A1802-15	IITA
9	A1802-66	IITA
10	A1802-67	IITA
11	Ife Hybrid-3	IITA
12	Ife Hybrid-4	IITA
13	LY1001-18	IITA
14	LY1001-23	IITA
15	LY1409-14	IITA
16	LY1409-21	IITA
17	LY1409-61	IITA
18	LY1501-1	IITA
19	LY1501-5	IITA
20	LY1501-6	IITA
21	LY1501-7	IITA
22	LY1501-8	IITA
23	LY1501-9	IITA
24	Reference check	IITA
25	M1124-31	IITA

Three seeds were planted per hill and later thinned to two plants per hill to obtain a final plant density of about 53,333 plants/ha. Pre- and post-emergence herbicides were used to control weeds, followed by the application of a combination of the active ingredients, paraquat and atrazine, respectively, immediately after planting at a dosage rate of 2.5kg a.i./ha of atrazine and 0.75kg a.i./ha of paraquat. Supplementary hand weeding was carried out as needed to effectively control weeds during the growing period. NPK 20-10-10 fertiliser was applied at a rate of 80 kg N, 60 kg P_2O_5, and 60 kg K_2O ha^{-1} as a basal fertilizer at three (3) weeks after planting and later top-dressed with additional N (urea 46-0-0) at six (6) weeks after planting. The field perimeter surroundings were kept clean to minimise insect and rodent invasion. Other cultural and agronomic management practices were carried out according to the recommended package by Kamara et al. (2020).

Data collection

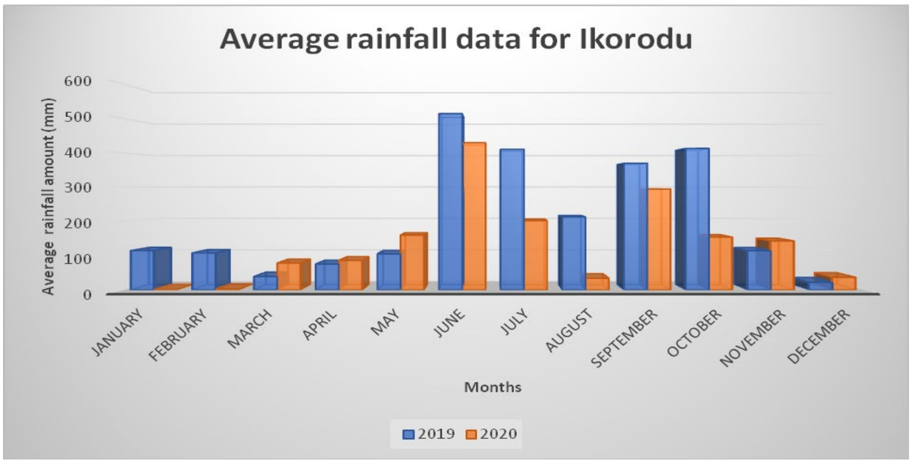
Data for grain yield and other agronomic traits were collected from the two inner rows. These traits included days to 50% anthesis, days to 50% silking, plant and ear heights (cm), root lodging (number of plants leaning more than 30^o from vertical), stalk lodging (stalks broken at or below the highest ear node), ear aspect, number of plants harvested, number of ears harvested and disease ratings using a scale of 1–5 (where 1=excellent, 2=very good, 3=good, 4=fair, and 5=very poor). All other data and ratings in this study were according to Badu-Apraku et al. (2012).

Statistical analysis

Data were analysed separately for each location and then combined across locations for grain yield (t/ha) and other measured traits using SAS (version 9.0) to determine the genotype × environment (G × E) interaction. In the combined analyses of variance, replications, year, location, and the year by location interaction were considered as random factors, while varieties were considered as fixed factors. The combined analysis of variance (ANOVA) was performed considering year-location combination as the environment to determine the effect of the environment (consisting of year [Y], location [L], and Y × L interaction), genotype, and all possible interactions among these sources of variation. Means were compared using New Duncan's Multiple Range Tests at the 0.05 and 0.01 probability levels when the F values were significant.

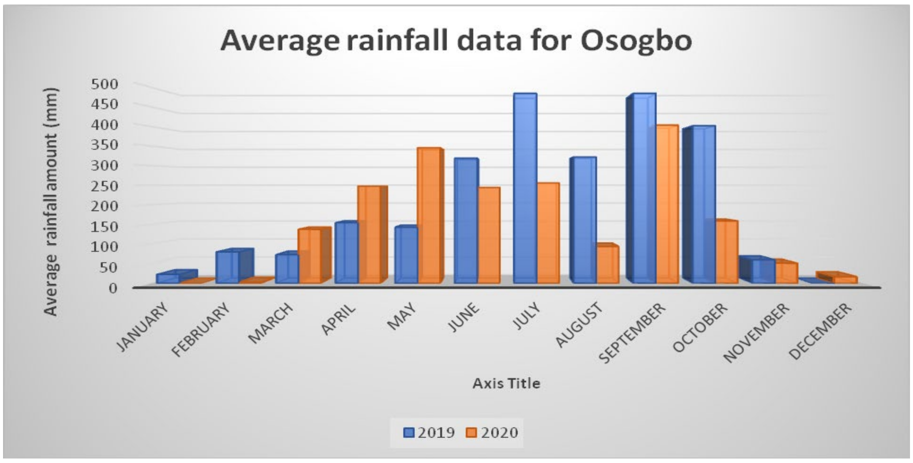
Results and Discussion

The amount and timing of rainfall during the flowering and grain-filling stages of the maize crop life cycle greatly affect plant growth and maturation. In this study, rainfall distribution and temperature were found to be the most influential factors on crop performance. The rainfall patterns in 2020 were not as favourable as in 2019, resulting in differences in agronomic and yield variables (Figures 1 and 2). Specifically, there was less rainfall during the critical flowering period in August 2020 compared to both 2019 and 2020 (Figures 1 and 2).



Source: www.worldweatheronline.com.

Figure 1. The monthly rainfall distribution pattern for Ikorodu in 2019 and 2020.



Source: www.worldweatheronline.com.

Figure 2. The monthly rainfall distribution pattern for Osogbo in 2019 and 2020.

The analysis of variance (ANOVA) showed that location and year had a significant effect on grain yield and other agronomic traits, except for ear aspect (Table 2). The interaction between genotype and year was significant for grain yield and ear height, while the interaction between genotype and location was significant for all traits except grain yield and ear height. The interaction between genotype, location, and year was significant for days to tasselling and silking, plant height, plant aspect, and ear height (Table 2). Genotype variations were highly significant for most traits, likely due to the different origins of the genotypes used. However, there was no significant interaction between genotype and environment for grain yield, ear height, and disease ratings, suggesting that these traits were consistent across the two research locations (Table 2). The findings highlight the importance of conducting multi-location trials to select genotypes with high yield and adaptability to different agro-ecological zones before releasing them as varieties (Badu-Apraku et al., 2010; Goa and Mohammed, 2013; Uba and Nwobi, 2022).

Table 2. Mean square of the agronomic characters of the provitamin A maize hybrids evaluated in 2019 and 2020 cropping seasons.

Source of var.	Df	Days to 50% tasselling	Days to 50% silking	Plant height	Ear height
Rep	2	10.44	1.84	157.43	350.94
Location	1	1434.45**	2766.40**	36278.00**	2151.60**
Genotype	24	17.83**	20.95**	380.67**	73.75
Year	1	1541.33**	552.16**	30947.36**	29333.74**
Genotype x loc.	24	19.41**	25.56**	173.92**	48.80
Loc. x year	1	2465.33**	3724.16**	660.08**	18825**
Gen. x year	24	6.47**	11.73**	380.82**	95.04*
Gen. x loc. x year	24	13.43**	17.56**	181.32**	48.24
Error	198	1.41	0.78	43.28	55.68
CV (%)		2.05	1.43	4.30	11.57
Means		57.89	62.04	153.07	64.48
Source of var.	Df	Plant asp.	Ear asp.	Husk cover	Grain yield (t/ha)
Rep	2	0.37	0.01	0.04	0.33
Location	1	42.56**	0.33	0.96**	85.47**
Genotype	24	0.52**	0.65**	0.31**	2.68*
Year	1	7.36**	12.81**	1.20**	131.14**
Genotype x loc.	24	0.42**	0.50**	0.17*	1.42
Loc. x year	1	19.76**	1.92**	0.56*	1.43
Gen. x year	24	0.46**	1.15**	0.13	2.78*
Gen. x loc. x year	24	0.37**	0.49**	0.12	1.31
Error	198	0.16	0.21	0.10	1.54
CV (%)		25.74	21.51	26.95	27.20
Means		1.54	2.11	1.16	4.56

*, ** significantly different at the 0.05 and 0.01 probability levels, respectively.

Table 3 displays the results of the ANOVA for disease scores across different locations and years. The location had a significant effect on the measured traits, except for root lodging. The interaction between location and year was significant for ear rot, curvularia, and rust, while root lodging was significant at a slightly lower significance level ($p<0.05$). The genotype did not have a significant effect on any of the measured traits. The year had a significant effect on ear rot, rust, and curvularia, and root lodging was also significant at a slightly lower significance level ($p<0.05$). Stalk lodging, streak, and blight did not have a significant effect on the different varieties. The genotype and its interactions with location and year were not significant for any of the disease ratings (Table 3).

Table 3. Mean square of disease/pest scores in provitamin A enriched maize hybrids.

Source of var.	Df	Root lodging	Stalk lodging	Ear rot	Streak	Rust	Blight	Curvularia
Rep	2	0.04	0.06	0.56	1.02	0.16	0.57	0.42
Location	1	0.56	0.65**	46.41**	17.76**	5.60**	9.01**	22.96**
Genotype	24	0.04	0.04	0.13	0.09	0.07	0.07	0.07
Year	1	0.16*	0.05	4.32**	0.16	5.60**	0.05	8.67**
Genotype x loc.	24	0.04	0.04	0.15	0.09	0.07	0.07	0.07
Loc. x year	1	0.16*	0.05	1.08**	0.16	5.60**	0.05	8.67**
Gen. x year	24	0.02	0.03	0.15	0.16	0.07	0.12	0.03
Gen. x loc. x year	24	0.02	0.03	0.12	0.16	0.07	0.12	0.03
Error	198	0.04	0.05	0.15	0.15	0.06	0.13	0.07
CV (%)		19.95	20.60	26.88	30.93	21.36	30.60	20.69
Means		1.04	1.05	1.45	1.24	1.14	1.17	1.28

*, ** significantly different at the 0.05 and 0.01 probability levels, respectively.

Table 4 displays the effects of genotype and location on days to tasselling and silking, as well as plant height. At Ikorodu, the days to tasselling ranged from 47 to 52 days in 2019 and 60 to 62 days in 2020, with an average of 55.71 days. At Osogbo, the range was 56 to 64 days in 2019 and 52 to 64 days in 2020, with an average of 60.08 days. The days to silking varied from 51 to 56 days in 2019 and 63 to 65 days in 2020, with an average of 59.00 days at Ikorodu and 61 to 73 days in 2019 and 57 to 66 days in 2020 at Osogbo. Plant heights ranged from 162.33 to 201.33cm in Ikorodu, with an average of 164.07cm. At Osogbo, the range was 126.67cm to 175.00cm in 2019 and 110.00cm to 150.00cm in 2020, with an average of 142.07cm.

Table 4. Interactive effects of location on the yellow hybrid variety for plant height and days to 50% tasselling and silking across two (2) years.

Varieties	Days to 50% tasselling				Days to 50% silking				Plant height (cm)			
	Ikorodu		Osogbo		Ikorodu		Osogbo		Ikorodu		Osogbo	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
A1702-28	50	62	63	58	54	65	71	62	178.50	153.33	150.00	130.00
A1706-2	51	61	61	61	54	64	65	64	183.83	156.67	156.00	110.00
A1736-12	52	60	64	58	55	63	72	61	178.33	156.33	170.00	135.00
A1736-13	51	61	53	57	54	64	63	60	162.33	147.67	140.00	135.00
A1736-6	50	61	62	58	54	64	68	61	168.50	149.33	140.00	140.00
A1802-4	51	61	59	58	55	64	64	61	186.67	152.00	150.00	140.00
A1802-14	50	61	65	64	55	63	73	67	173.33	149.33	126.67	110.00
A1802-15	51	60	63	60	53	64	68	63	172.00	149.00	152.00	130.00
A1802-66	51	61	58	59	55	64	63	62	177.50	153.33	160.00	125.00
A1802-67	51	60	56	58	53	63	63	63	168.17	151.00	165.00	120.00
Ife Hybrid-3	51	61	56	52	55	64	62	57	162.50	153.33	130.00	125.00
Ife Hybrid-4	51	61	62	58	55	63	71	62	201.33	151.67	161.33	140.00
LY1001-18	49	61	64	59	51	64	70	62	166.50	154.67	155.00	140.00
LY1001-23	50	61	58	59	54	64	62	62	180.00	150.00	153.00	130.00
LY1409-14	52	61	63	64	55	63	71	67	183.33	154.00	151.67	135.00
LY1409-21	49	61	61	58	55	64	70	61	172.00	156.67	135.00	150.00
LY1409-61	51	61	63	63	54	63	73	66	174.00	153.00	145.00	150.00
LY1501-1	51	62	64	59	55	65	72	63	174.67	154.33	155.00	120.00
LY1501-5	50	61	61	60	54	65	65	63	176.33	148.67	160.00	140.00
LY1501-6	52	62	63	60	56	65	68	63	183.67	155.67	160.00	125.00
LY1501-7	51	61	57	60	54	64	62	64	187.67	152.33	175.00	140.00
LY1501-8	52	60	57	61	54	63	62	65	174.00	147.00	155.00	135.00
LY1501-9	51	60	57	63	56	63	61	66	175.33	155.00	158.00	145.00
Reference check	49	61	63	62	54	64	73	65	168.17	150.00	131.67	135.00
M1124-31	47	61	64	58	51	63	72	63	164.00	156.33	133.33	150.00
Mean	55.71		60.08		59.00		65.07		164.07		142.07	
S.E (0.05)	0.21		0.24		0.41		0.33		1.26		1.20	

Table 5 illustrates the impact of location on plant aspect, ear aspect, and grain production for the yellow hybrid variety. Plant aspect varied from 1.00 to 2.67 and 1.67 to 2.67 in 2019 and 2020 at Ikorodu, respectively, whereas it ranged from 1.00 to 2.00 in both 2019 and 2020 at Osogbo. At Ikorodu, the ear aspect ranged from 1.00 to 3.00 in 2019 and 2020, with an average mean of 2.14 in both years. In the 2019 and 2020 cropping seasons, however, it varied from 1.00 to 2.00 and 2.00 to 3.00 in Osogbo. The grain yields in Ikorodu and Osogbo varied between 3.23 and 7.55 t/ha and 2.33 and 6.41 t/ha, respectively, in 2019. In the 2020 season, the grain yields ranged from 3.48 to 5.60 t/ha in Ikorodu and 2.75 to 3.87 t/ha in Osogbo. The average grain yield for the 2019 season was 5.09 t/ha, while for the 2020 season it was 4.03 t/ha. The hybrid LY1409-61 performed well in both seasons at Ikorodu, with yields of 7.55 t/ha in 2019 and 5.60 t/ha in 2020. AS1802-15 was the highest yielding variety in Osogbo in 2019 with a yield of 6.41 t/ha, while LY1409-21 was the top yielding variety in 2020 with a yield of 3.87 t/ha.

Table 5. Interactive effects of location on the yellow hybrid variety for plant aspect, ear aspect and grain yield across two (2) years.

Varieties	Plant aspect				Ear aspect				Grain yield (t/ha)			
	Ikorodu		Osogbo		Ikorodu		Osogbo		Ikorodu		Osogbo	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
A1702-28	1.00	1.67	1.00	1.00	1.33	2.67	2.00	2.33	6.71	3.49	4.24	3.39
A1706-2	1.67	2.33	1.00	2.00	2.00	2.33	2.00	2.00	4.76	4.41	5.37	3.25
A1736-12	1.67	2.33	2.00	1.00	1.67	2.33	3.00	2.00	5.40	4.20	4.63	3.06
A1736-13	1.67	2.33	1.00	1.00	1.67	2.33	2.00	2.00	5.35	4.61	3.21	3.35
A1736-6	2.67	2.67	1.33	1.00	3.00	2.67	2.33	2.33	4.23	4.03	4.12	3.75
A1802-4	1.00	2.33	1.00	1.00	1.33	2.33	1.00	2.33	6.17	3.80	4.07	3.17
A1802-14	1.00	2.33	2.00	1.67	1.33	2.00	2.33	2.00	6.61	5.43	4.27	2.89
A1802-15	1.00	2.00	1.00	1.00	1.67	2.00	2.00	2.00	5.71	5.47	6.41	3.40
A1802-66	1.67	2.00	1.00	1.00	2.67	2.00	2.00	2.00	5.72	5.52	3.19	3.01
A1802-67	1.67	2.33	1.00	1.00	2.67	2.33	1.00	2.00	5.14	3.82	6.36	2.75
Ife Hybrid-3	2.67	2.00	1.00	1.00	3.00	2.33	2.00	2.33	3.22	4.75	2.33	3.02
Ife Hybrid-4	1.33	2.33	1.00	1.00	1.67	2.33	2.00	2.33	5.78	4.47	5.34	2.97
LY1001-18	2.33	2.00	2.00	1.00	2.00	2.33	3.00	2.33	4.79	4.29	4.23	3.42
LY1001-23	1.00	2.67	1.00	1.00	1.00	3.00	1.00	2.67	7.06	3.71	6.40	3.18
LY1409-14	1.00	2.33	1.00	1.00	1.33	2.33	2.00	2.67	5.92	4.25	5.10	3.63
LY1409-21	1.33	2.33	2.00	1.00	1.67	2.33	2.00	2.00	6.06	4.33	4.72	3.87
LY1409-61	1.00	2.00	1.00	1.00	1.00	2.33	2.00	2.00	7.55	5.60	5.75	3.86
LY1501-1	1.33	2.33	2.00	1.00	2.00	2.33	2.00	2.00	5.82	5.00	4.21	3.3
LY1501-5	1.00	2.00	1.00	1.00	1.33	2.00	2.33	2.00	6.46	4.83	4.05	3.42
LY1501-6	1.33	2.33	1.00	1.00	2.00	2.33	2.00	2.33	6.27	3.48	4.28	3.44
LY1501-7	1.33	2.33	1.00	1.00	1.67	2.67	1.00	2.33	6.15	5.29	4.84	3.14
LY1501-8	1.67	2.67	1.00	1.00	1.67	2.67	1.00	3.00	5.40	4.00	5.33	3.69
LY1501-9	1.33	2.33	1.00	1.00	3.33	2.67	1.00	2.00	6.19	4.42	6.04	3.12
Reference check	2.67	2.67	1.33	1.00	1.33	2.67	3.00	2.00	3.23	5.01	4.28	3.16
M1124-31	1.33	2.67	2.00	1.00	1.67	2.67	2.67	2.00	6.43	4.30	6.20	3.13
Mean	1.92		1.67		2.14		2.07		5.09		4.03	
S.E (0.05)	0.06		0.03		0.06		0.05		0.12		0.12	

The weather conditions, specifically rainfall and temperature, have a notable influence on the yield of maize crops during important stages of their growth (Randjelovic et al., 2011; Petrović et al., 2023). In 2019, drought and high temperatures during the flowering stage resulted in less favourable rainfall, leading to a decrease in grain yield per hectare compared to 2020. Previous studies have shown that water deficiency stress at different stages of growth can significantly reduce grain yield (Naderi et al., 2009). Environmental factors also play a role in the genetic components of maize, as the location where the crops are grown can impact their yield. Other studies have shown that the interaction between genotype and location is influenced by environmental factors such as temperature and humidity (Butron et al., 2002; Adu et al., 2013).

The maize genotypes evaluated in this study had heterogeneous genetic compositions, which led to different results for the parameters examined. Plant height, days to tasselling, days to silking, and grain yield are all affected by varietal variances. The variance in the characteristics evaluated when compared to other locations, on the other hand, is due to environmental factors. Tahir et al. (2008) and Yang et al. (2021) have found that plant height is a genetically and environmentally determined factor; however, crop cultivar selection regulates the impact of the environment. Revilla et al. (2000) also discovered a difference in plant height across maize types owing to genotype by environment interactions.

The Duncan multiple range test was used in Table 6 to compare the means of the agronomic and yield-related characteristics and also to determine the variations between the genotypes for the characteristics examined. AS1804-14 exhibited the longest duration for both tasselling and silking, with values of 60.00 and 64.25, respectively. LY1409-14 followed closely behind with a tasselling duration of 59.29, while LY1409-61, LY1409-14, and reference check had a silking duration of 64.00. On the other hand, Ife Hybrid-3 (commercial control) had the shortest duration for both tasselling and silking, with values of 55.00 and 59.33, respectively. LY1409-61 was the highest-yielding variety with a grain yield of 5.69 t/ha. It was followed by AS1804-15 with an average yield of 5.35 metric t/ha across all environments in Table 6.

Table 7 shows that the varieties tested were generally resistant to streak, blight, rust, and curvularia leaf spot, with disease scores ranging from 1.17 to 1.33 for streak, 1.08 to 1.33 for blight, 1.08 to 1.25 for rust, and 1.17 to 1.42 for curvularia leaf spot. In contrast, M1124-31 and AS1802-4 received a higher rating of 1.33 (blight) and 1.42 (curvularia). There was no significant difference between the varieties in terms of streak resistance, blight and curvularia disease. Root and stalk lodging ranged from 1.00 to 1.17 among the genotypes, indicating that these factors played a role.

In plant breeding programmes, the genetic association between characteristics is critical for enhancing selection efficiency. Studies on the relationship between yield and associated characteristics might be a useful technique for crop development. The fact that grain yield and other yield components have a substantial positive correlation implies that any of the traits might be used for indirect selection for grain yield.

The correlation coefficient (r) for various characteristics of yellow hybrid maize is shown in Table 8. The study found that grain yield was positively and significantly (*p<0.001*) correlated with plant and ear heights. The flowering traits, specifically days to 50% anthesis and silking, were highly correlated with each other (*p<0.001*), suggesting that they were influenced by the same genes or a pleiotropic influence on one another, or that they have linkage genes in common (Brown and Caligari, 2008; Lobulu et al., 2021). However, these traits were negatively correlated with grain yield, plant height, and ear height, meaning that selecting for early flowering days may not be beneficial for these characteristics. Jayakumar et al. (2007) and Sabiel et al. (2014) also found a negative relationship between flowering time and grain yield.

Table 6. Mean performance of agronomic and yield related characters of the provitamin A enriched maize hybrids evaluated in 2019 and 2020.

Hybrids	Days to 50% asselling	Days to 50% silking	Plant height (cm)	Ear height	Plant aspect	Ear aspect	Husk cover	Grain yield (t/ha)
A1702-28	58.33defghi	63.00bc	152.96cde	66.88abc	1.42cde	2.08cd	1.00c	4.46bcd
A1706-2	58.50cdefgh	61.83efgh	151.63def	70.00a	1.75abc	2.08cd	1.25bc	4.45bcd
A1736-12	58.58cdefg	62.75cd	159.92ab	67.63ab	1.75abc	2.25bcd	1.25bc	4.32bcd
A1736-13	55.50mn	60.25l	146.25fg	61.71bc	1.50bcde	2.00cd	1.17bc	4.13bcd
A1736-6	57.58ghijk	61.67fghi	149.46ef	63.04abc	1.92a	2.58ab	1.25bc	4.03bcd
A1802-4	57.08jkl	60.83jkl	157.17bcd	66.71abc	1.33de	1.83d	1.08c	4.30bcd
A1804-14	60.00a	64.25a	139.83h	64.42abc	1.75abc	2.00cd	1.00c	4.80abc
A1804-15	58.67cdefg	62.50cde	150.75def	64.42abc	1.25e	1.92d	1.42ab	5.35ab
A1804-66	57.25ijkl	61.00ijkl	153.96bcde	62.00bc	1.42cde	2.17bcd	1.08c	4.36bcd
A1804-67	56.42lm	60.67kl	151.04def	60.71bc	1.50bcde	2.00cd	1.17bc	4.52abcd
Ife Hybrid-3	55.00n	59.33m	142.71gh	59.63c	1.67abcd	2.42abc	1.25bc	3.33d
Ife Hybrid-4	57.83efghijkl	62.00defg	163.58a	66.54abc	1.42cde	2.08cd	1.00c	4.64abc
LY1001-18	58.17defghi	61.83efgh	154.04bcde	64.00abc	1.83ab	2.42abc	1.17bc	4.18bcd
LY1001-23	57.00kl	60.50kl	153.25cde	64.46abc	1.42cde	1.92d	1.08c	5.09abc
LY1409-14	59.92ab	64.00a	156.00bcd	64.58abc	1.33de	2.08cd	1.17bc	4.71abc
LY1409-21	57.25ijkl	62.33cdef	153.42cde	65.33abc	1.67abcd	2.00cd	1.08c	4.75abc
LY1409-61	59.50abc	64.08a	155.50bcde	65.08abc	1.25e	1.83d	1.08c	5.69a
LY1501-1	58.92bcde	63.37ab	151.00edf	62.33bc	1.67abcd	2.08cd	1.00c	4.59abc
LY1501-5	57.92efghijk	61.67fghi	156.25bcd	65.38abc	1.25e	1.92d	1.17bc	4.69abc
LY1501-6	59.08abcd	62.92c	156.08bcd	64.46abc	1.42cde	2.17bcd	1.08c	4.37bcd
LY1501-7	57.17jkl	61.00ijkl	163.75a	66.08abc	1.42cde	1.92d	1.08c	4.86abc
LY1501-8	57.67ghijk	61.08hij	152.75cde	61.79bc	1.58abcde	2.08cd	1.00c	4.61abc
LY1501-9	57.75fghij	61.50ghij	158.33abc	64.83abc	1.42cde	1.83d	1.42ab	4.94abc
Reference check	58.83cdef	64.00a	146.21fg	61.67bc	1.92a	2.75a	1.67a	3.92cd
M1124-31	57.42hijkl	62.25cdefg	150.92def	68.21ab	1.75abc	2.25bcd	1.08c	5.02abc

Table 7. Mean disease score and lodging resistance of the provitamin A enriched maize hybrids evaluated in 2019 and 2020.

Hybrids	Stalk lodging	Root lodging	Ear rot	Streak	Blight	Rust	Curvularia
A1702-28	1.00a	1.08a	1.42ab	1.33a	1.17a	1.25a	1.17a
A1706-2	1.00a	1.17a	1.33ab	1.17a	1.17a	1.08ab	1.42a
A1736-12	1.00a	1.00a	1.50ab	1.33a	1.08a	1.17ab	1.33a
A1736-13	1.00a	1.08a	1.58ab	1.17a	1.25a	1.17ab	1.33a
A1736-6	1.00a	1.08a	1.50ab	1.33a	1.17a	1.00b	1.25a
A1802-4	1.00a	1.17a	1.50ab	1.17a	1.25a	1.17ab	1.42a
A1804-14	1.00a	1.00a	1.50ab	1.33a	1.08a	1.08ab	1.17a
A1804-15	1.08a	1.00a	1.33ab	1.17a	1.25a	1.25a	1.33a
A1804-66	1.00a	1.08a	1.50ab	1.17a	1.17a	1.08ab	1.33a
A1804-67	1.00a	1.08a	1.33ab	1.33a	1.08a	1.17ab	1.33a
Ife Hybrid-3	1.08a	1.00a	1.67a	1.33a	1.17a	1.08ab	1.33a
Ife Hybrid-4	1.08a	1.00a	1.50ab	1.33a	1.25a	1.08ab	1.33a
LY1001-18	1.17a	1.00a	1.50ab	1.33a	1.17a	1.25a	1.33a
LY1001-23	1.00a	1.00a	1.50ab	1.33a	1.17a	1.25a	1.17a
LY1409-14	1.08a	1.00a	1.50ab	1.33a	1.25a	1.17ab	1.25a
LY1409-21	1.00a	1.00a	1.33ab	1.33a	1.25a	1.17ab	1.25a
LY1409-61	1.00a	1.08a	1.58ab	1.33a	1.08a	1.08ab	1.17a
LY1501-1	1.08a	1.08a	1.50ab	1.17a	1.08a	1.17ab	1.17a
LY1501-5	1.08a	1.00a	1.33ab	1.33a	1.17a	1.08ab	1.33a
LY1501-6	1.17a	1.00a	1.33ab	1.33a	1.17a	1.00b	1.33a
LY1501-7	1.00a	1.08a	1.50ab	1.33a	1.17a	1.25a	1.33a
LY1501-8	1.08a	1.00a	1.50ab	1.33a	1.25a	1.08ab	1.25a
LY1501-9	1.08a	1.08a	1.58ab	1.33a	1.17a	1.17ab	1.17a
Reference check	1.08a	1.08a	1.50ab	1.17a	1.08a	1.08ab	1.25a
M1124-31	1.00a	1.00a	1.33ab	1.17a	1.33a	1.08ab	1.25a

The association between days to tasselling and ear height as well as days to tasselling and plant height, days to silking and plant height, days to silking and ear height, days to tasselling and curvularia, and days to silking and curvularia was negative and highly significant ($P<0.001$). Days to tasselling positively and significantly correlated with 50% silking (0.93**), plant aspect (0.16**), and ear aspect (0.60**), suggesting that there is a strong relationship between days to tasselling and yield attributes such as plant aspect and ear aspect. In addition, there was a positive and highly significant correlation between plant height and ear height (0.79**), as well as root lodging (0.20**), ear rot (0.25**), streak (0.28**), rust (0.51**), blight (0.26**), curvularia (0.62**), and grain yield (0.53**) (Table 8). Consequently, the result also showed that ear height correlated positively and significantly with the above-listed traits as well as plant height except for ear rot, which had a weak positive correlation ($p<0.005$), suggesting the usefulness of indirect selection (Kapoor et al., 2022). There was a negative and significant correlation between grain yield and plant aspect (-0.14**) and grain yield and ear aspect (-0.46**). The significant negative correlations show that these pairs of variables may not influence each other in any way and may not negatively affect grain yield.

Table 8. The Pearson correlation matrix of the provitamin A maize hybrids evaluated in 2019 and 2020 cropping seasons.

	50% tasse.	50% silking	Plant height	Ear height	Root lodg.	Stalk lodg.	Husk cover	Plant asp.	Ear asp.	Ear rot	Streak	Rust	Blight	Curv.	Grain yield
50% tassel.	-	0.93**	-0.58**	- 0.77**	- 0.20**	- 0.01	-0.21**	0.16**	0.60**	-0.13*	-0.29**	-0.58**	-0.25**	-0.69**	-0.31**
50% silking		-	- 0.55**	- 0.72**	- 0.20**	- 0.05**	-0.19**	0.08	0.27**	-0.26**	-0.34**	-0.57**	-0.29**	-0.69**	-0.27**
Plant height			-	0.79**	0.20**	0.10	0.14*	0.05	-0.25**	0.25**	0.28**	0.51**	0.26**	0.62**	0.53**
Ear height				-	0.20**	0.05	0.21**	-0.08	-0.27**	0.13*	0.27**	0.64**	0.29**	0.72**	0.44**
Root lodg.					-	- 0.05	0.09	0.05	-0.06	0.06	0.18**	0.30**	0.08	0.16**	0.06
Stalk lodg.						-	0.04	0.15**	0.06	0.24**	0.02	0.05	0.06	0.04	0.03
Husk cover							-	0.15**	0.10	0.06	0.15**	0.20**	0.02	0.20**	0.02
Plant asp.								-	0.57**	0.42**	0.27**	-0.09	0.17**	0.16**	-0.14**
Ear asp.									-	0.06	0.05	-0.25**	-0.07	-0.07	-0.46**
Ear rot										-	0.38**	0.18**	0.36**	0.29**	0.21**
Streak											-	0.22**	0.20**	0.34**	0.18**
Rust												-	0.25**	0.47**	0.34**
Blight													-	0.28**	0.24**
Curv.														-	0.34**
Grain yield															-

*, ** significant at $p<0.05$ and 0.01, respectively.

Conclusion

The differences in the provitamin A maize hybrids can be attributed to changes in the environment caused by varying amounts of rainfall in the years when the hybrids were evaluated. Additionally, the variations among the hybrids were influenced by factors such as grain yield, time taken for tasselling and silking, plant height, ear height, plant appearance, and ear appearance. In this study, it was found that the exceptional hybrids, namely LY1409-61, AS1802-15, and LY1409-21, possessed desirable agronomic traits and had the potential to increase maize yield and address malnutrition issues. Consequently, these outstanding hybrids, which displayed consistent performance over the course of two years, have the ability to withstand moisture stress and are therefore recommended for sustainable production in the agro-ecology of southwestern Nigeria.

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ZNAČAJ HIBRIDA KUKURUZA (*ZEA MAYS* L.) OBOGAĆENIH PROVITAMINOM A ZA PRINOS I AGRONOMSKE OSOBINE U JUGOZAPADNOJ NIGERIJI

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R e z i m e

Kukuruz je vitalna namirnica u ishrani u Nigeriji, kojom se u organizam unose ključne hranljive materije kao što su ugljeni hidrati, proteini, masti i mikronutrijenti. Međutim, standardnim hibidima kukuruza nedostaje dovoljno nutritivnog prekursora za vitamine, što zahteva gajenje stabilnih, visokoprinosnih hibrida kukuruza obogaćenih provitaminom A. Ovo je važno u ishrani, posebno kod ruralnih populacija koje su podložne „skrivenoj gladi” (engl. *„hidden hunger”*). Ovo istraživanje je imalo za cilj da proceni agronomski učinak i prinos novorazvijenih hibrida kukuruza tokom dvogodišnjeg perioda na lokalitetima Ikorodu i Osogbo u Nigeriji. Ocenjivana su 22 poboljšana hibrida kukuruza, dva komercijalna hibrida i jedna kontrolna varijanta. Prinosi zrna hibrida su se kretali od 3,33 t/ha (ife-hibrid 3) do 5,69 t/ha (LY1409-61) tokom dve godine, prosečno iznoseći 4,03 t/ha. Svi hibridi, osim ife-hibida 3 (3,33 t/ha), nadmašili su prinose na kontroli (3,92 t/ha), pri čemu su četiri hibrida ostvarila prinose preko 5 t/ha tokom dve godine. U 2019. godini, količine padavina su bile veće i dobro raspoređene, što je značajno uticalo na prinose zrna u odnosu na 2020. godinu. Ovaj uticaj je bio posebno primetan u fazama cvetanja i nalivanja zrna od jula do oktobra. LY1409-61, AS1802-15 i LY1409-21 najbolje su reagovali na lošije meteorološke uslove, što se može smatrati njihovom sortnom karakteristikom. Uvođenje ovih hibrida u proizvodnju ima potencijala da poveća proizvodnju kukuruza i ublaži neuhranjenost u ruralnoj jugozapadnoj Nigeriji. Ovim istraživanjem se naglašava uloga unapređenja u stvaranju i gajenju hibrida kukuruza obogaćenih hranljivim materijama u borbi protiv nedostataka u ishrani, poboljšanja prehrambene sigurnosti i koristi zajednicama koje zavise od kukuruza kao osnovne namirnice. To bi moglo značajno da doprinese održivoj poljoprivredi i poboljšanim ishodima ishrane u regionu.

Ključne reči: adaptacija, agronomske osobine, prinos zrna, skrivena glad, neuhranjenost, prehrambena sigurnost.

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EFFECTS OF VERMICOMPOST ON THE GROWTH AND YIELD OF SPRING ONION (*ALLIUM FISTULOSUM* L.)

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Abstract: Spring onion (*Allium fistulosum* L.) is a popular salad vegetable produced widely over the world, including in Vietnam. Thanks to its flavor and aroma, it is an indispensable ingredient used to flavor soups and other dishes. Vermicompost is a natural and environmentally friendly fertilizer used widely to increase crop production and maintain the sustainability of agrosystems. Consequently, this study was conducted to investigate the efficiency of vermicompost at different application rates in promoting the growth and yield parameters of spring onion. The results show that adding vermicompost to spring onion production had significant positive effects on plant height, number of leaves, number of tillers, individual plant weight, and plot yield. Particularly, the application of vermicompost at 40 t ha^{-1} showed the highest performance in the observed parameters, increasing the number of leaves, number of tillers, individual plant weight, and plot yields to 64.78, 21.18, 302.96 g $plant^{-1}$, and 4.86 kg m^{-2}, respectively. The plot yields in the treatments of the highest and lowest vermicompost application increased by 49.1% and 3.9%, respectively, in comparison to the control. Consequently, there was a strongly positive relationship between the application rate of vermicompost and the plot yield.

Key words: *Allium fistulosum*, onion, organic fertilizers, vermicompost, Welsh onion.

Introduction

Vermicomposting is an environmentally and economically friendly decomposition of organic matter by oligochaete worms. Specifically, vermicompost is produced by the action of the earthworms *Lampito mauritii*, *Eudrilus eugeniae*, *Perionyx excavatus*, and *Eisenia foetida,* utilizing their capacity to convert organic matter such as manure, coffee husks and plant residues into a

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compost by the action of their digestive systems (Pattnaik and Reddy, 2010; Dominguez and Edwards, 2011; Getachew et al., 2018). Consequently, this technology increases the bioavailability of essential nutrients by converting raw plant and animal agricultural by-products that can be used as bio-fertilizers (Pattnaik and Reddy, 2010).

Using inorganic fertilizers in crop production is essential to enhance productivity in order to ensure food security for the increasing human population (Musafiri et al., 2023). However, the intensive use of chemical fertilizers in crop cultivation causes degradation of the soils to which they are applied, leading to negative impacts on the environment, the soil microbial community, and human health (Savci, 2012). Sole inorganic or organic fertilizer applications cannot both maintain the status of soil organic matter and sustain crop productivity. A proper combination of both organic and inorganic fertilizers is therefore required to effectively maintain soil health and to enhance nutrient efficiency (Hati et al., 2006; Gentile et al., 2008).

Organic fertilizers, including vermicompost, can act as crucial sources of nutrients for plant growth. Furthermore, organic fertilizers can improve soil quality by maintaining soil structure, supplying more beneficial microorganisms, increasing soil water holding capacity, increasing microfauna density, improving soil pH, and improving the stability of earthworm communities (Zhou et al., 2022). Consequently, agricultural practices with organic fertilization can improve the growth, productivity and quality of crops (Zhou et al., 2022; Ye et al., 2022).

It is well-acknowledged that vermicomposts are reliable bio-fertilizers thanks to their slow-release properties, which allow crops to absorb the nutrients more effectively, alongside their capacity to improve soil physical, chemical, biological, and microbiological characteristics. Besides the benefits of enhancing growth and improving the production of crops (Rekha et al., 2018), vermicomposts have also shown their potential for suppressing diseases and pests in agricultural crops. Such suppression may be related to many mechanisms, such as a higher density of useful microbes or total microbiological activity, which prohibits pathogens or pests, induces systemic resistance in plants against pests/diseases, improves soil quality and provides essential nutrients for pest control, and increases the competition of useful microbes for food/space/water with harmful microbes (Che Sulaiman and Mohamad, 2020).

Vermicompost contains useful amounts of NPK. The typical content in vermicompost is 2–3% nitrogen, 1.85–2.25% potassium and 1.55–2.25% phosphorus, but these levels can be up to 7.37% nitrogen and 19.58% phosphorus (P_2O_5) (Sinha et al., 2010). Vermicompost has other useful components, including micronutrients, and beneficial soil microorganisms such as nitrogen-fixing bacteria, actinomycetes and mycorrhizal fungi. Vermicompost also contains plant growth-regulating hormones and enzymes such as amylase, lipase, cellulase, and chitinase, which play an important role in breaking down organic matter in the soil (Sinha et al., 2010; Rekha et al., 2018). The ability of vermicompost to effectively suppress pests and plant diseases has also been shown in several studies (Sinha et al., 2010; Che Sulaiman and Mohamad, 2020).

Consequently, there is increasing research interest in the effect of vermicompost on plant growth and yields (Yang et al., 2015; Kumar and Gupta, 2018; Khan et al., 2019; Jankauskienė et al., 2022). Yang et al. (2015) conducted a glasshouse pot experiment to assess the effects of vermicompost on tomato yield under different soil water regimes. The results showed that under an irrigation level of 60–70% field capacity, vermicompost treatment increased tomato yield by 16.30, 9.63, 51.99 and 69.30% when compared to chicken compost, horse compost, chemical fertilizer, and no fertilizer, respectively (Yang et al., 2015). In the study by Khan et al. (2019), it was found that under field conditions, the growth, yield, and quality parameters of chili (*Capsicum annum* L.) increased with increasing vermicompost levels. Particularly, the treatment with vermicompost at an application rate of 2.5 t ha^{-1} had the highest yield (8.26 t ha^{-1}) compared to the treatments with 2.0 t ha^{-1} vermicompost (yield of 6.8 t ha^{-1}) and the control without vermicompost addition (yield of 3.6 t ha^{-1}) (Khan et al., 2019). The results of Rekha et al. (2018) demonstrated that a treatment of 50% vermicompost + 50% soil increased the growth parameters of the chili plants and improved the soil quality. In addition, chili plants grown in vermicompost-amended soil showed enhanced growth rates when compared to plants treated with the two plant growth hormones gibberellic acid (GA) and indole acetic acid (IAA). Although there are studies on the effects of organic fertilizers on the growth of spring onion (Afa, 2016; Kuroda et al., 2020), the effect of vermicompost on spring onion growth is yet to be established.

Allium fistulosum L. is a type of vegetable that is widely cultivated across the globe. *A. fistulosum* belongs to the *Liliaceae* family and is popularly known as spring onion, scallion, stone leek, Chinese onion, Welsh onion, and Japanese bunching onion. The spring onion is known for its flavor and aroma and is consumed fresh or cooked (Padula et al., 2022). In Vietnam, it is an indispensable ingredient to flavor soups and other dishes. Besides having a high content of fiber and sugar, protein, vitamins K, C, A and B6, and minerals, the spring onion has also been used in traditional Chinese medicine to treat a wide range of diseases thanks to its content of bioactive phytochemicals (Padula et al., 2022; Mandey et al., 2022).

To sum up, it is well-evidenced that vermicompost has great potential as a natural fertilizer for crops and can increase production and maintain the sustainability of agrosystems. More studies on the effect of vermicompost on a variety of crops, cultivation conditions and soil types are necessary to determine its effectiveness under different propagation conditions.

Consequently, the present study investigated the growth-promoting effect of vermicompost on the growth and yield of the plant *Allium fistulosum* L. The results of this study could prove the efficiency of vermicompost in enhancing crop yields while recycling nutrients from waste products, thereby benefiting farmers through decreased input costs and increasing the environmental sustainability of farming.

Material and Methods

The present study was conducted under field conditions between April and June 2023 in Tra On hamlet, Huyen Hoi commune, Cang Long district, Tra Vinh province, Vietnam. This area is an intensive spring onion production center. The experimental soil belongs to the Arenosols group and had pH values ~ 6.0 and the sandy loam textural class.

Field preparation and fertilizer application

The field was plowed, levelled and transplanting ridges were formed. Each ridge was 20 cm higher than the field surface and 1 m wide. A 20-cm wide furrow was made between two adjacent ridges to facilitate drainage and maintenance of the experiment (Figure 2). The experiment area was divided into plots of 1 m^2.

The spring onion production procedure of local farmers was applied in this experiment. Particularly, in all treatments, including the control, 500 kg ha^{-1} of cow manure was applied to the soil in the preparation period. Lime was also applied to adjust the soil pH to a range of 6.5–7.0. Before seedling transplantation, the plots were covered by a thin layer of dry paddy straw in order to retain soil moisture and control weeds. A basal dose of inorganic fertilizers (58.5 kg ha^{-1} N, 114.5 kg ha^{-1} P_2O_5 and 37.5 kg ha^{-1} K_2O) was applied at the same rate to all treatments. In addition to this basic fertilization, vermicompost was applied at rates between 10 and 40 t ha^{-1}.

Preparation of the transplants

The onion transplants used in this study were uniform, healthy, vigorously growing, and free of diseases. Roots and dry leaves covering the onion tillers (stems) were removed to ensure that new baby roots and sprouts were able to grow well after planting. Each onion seedling included two tillers (Figure 1).

Figure 1. The spring onion transplants used in this experiment.

Experimental design

The one-factor experiment was designed in a Completely Randomized Design (CRD) to evaluate the effect of vermicompost on the growth and yield of spring onions (Figure 2). The present experiment included five treatments, including the control, as follows:

T1: Control (no vermicompost application);
T2: Vermicompost application 10 t ha^{-1} (1 kg m^{-2});
T3: Vermicompost application 20 t ha^{-1} (2 kg m^{-2});
T4: Vermicompost application 30 t ha^{-1} (3 kg m^{-2});
T5: Vermicompost application 40 t ha^{-1} (4 kg m^{-2}).

The treatments were replicated three times. Each replication was a plot of 1 m^2 and each plot was planted with 20 seedlings (with a spacing between plants of 30 cm x 20 cm).

Vermicompost application

Vermicompost produced from vermicomposting of cow manure using *Perionyx excavatus* was bought from a local worm farm in Tra Vinh province. On each plot where vermicompost was applied, the application was divided into three times to slowly add nutrients during the plant growth time. The applications were made as follows: 50% of each application was applied before planting (by spreading it evenly over the plot before covering it with paddy straw), another 25% of the treatment was applied 15 days after planting, and the remaining 25% was applied 30 days after planting as a top dressing around the plant base.

Cow manure and inorganic fertilizers were applied equally in all treatments, including the control treatment (as described in the section on fertilizer application).

All growth parameters were measured every 10 days after planting. The yield parameters were determined on the harvest day (50 days after planting). Nine of the 20 plants in each plot were selected randomly to collect data. The growth parameters of plant height (cm), the number of leaves and the number of tillers (stems) were recorded at 10, 20, 30, 40 and 50 days after planting. In terms of yield parameters, the fresh weight of the individual plants and the total yield of each plot (plot yield calculated by the total weight of all 20 plants) were also evaluated on the harvest day (50 days after planting). Before investigating the yield parameters, all yellow leaves and soil/sand clinging to the plant roots were removed. The diameter of a single tiller was measured on the harvest day to determine if the production of multiple tillers affected tiller size.

Figure 2. The experimental area during spring onion growth showing the ridge and furrow system of cultivation.

Data analysis

Statistical analysis was conducted with SPSS *vs.* 22 (IBM Inc.). The data were statistically analyzed to determine the significance of the differences between the treatments in the growth and yield parameters of spring onion.

The data sets were analyzed for homogeneity of variance with the Levene's test to ensure that all comparison groups had the same variance prior to comparing means by a one-way ANOVA. The significance of the effects of vermicompost on the growth and yield of spring onion plants among treatments was determined by the Tukey's HSD *post-hoc* test at $p < 0.05$.

Results and Discussion

Effects of vermicompost on the growth parameters of spring onion

There were differences in the plant height, the number of leaves and the number of tillers per spring onion plant among the treatments during the experiment period (Tables 1, 2, 3).

The plant heights in the treatments with vermicompost were not significantly different from the control in any of the samplings during the growing period (Table 1). At the period of 10 days after planting, the plant height was higher than after 20 and 30 days. This was due to the fact that the plants were in adaptation phase 10 days after planting and old leaves were still present, but then new tillers and leaves developed, and the old leaves died. Spring onion plants of treatments T4 and T5 reached their greatest height 40 and 50 days after planting (Table 1).

Table 1. Effects of vermicompost on the height of spring onion plants (cm).

Treatments	Plant height (cm) in different growth periods				
	10 days	20 days	30 days	40 days	50 days
T1 (control)	41.55 ± 0.50	37.78 ± 0.84	35.17 ± 0.89	42.67 ± 2.24	48.48 ± 2.36
T2	43.08 ± 0.69	35.71 ± 1.28	34.21 ± 1.48	41.11 ± 1.70	48.00 ± 1.53
T3	43.02 ± 1.41	38.06 ± 0.78	34.70 ± 2.26	41.76 ± 1.64	48.96 ± 2.97
T4	41.61 ± 1.28	37.33 ± 0.29	36.24 ± 1.14	46.48 ± 1.69	52.40 ± 1.48
T5	42.52 ± 1.61	36.15 ± 1.30	35.81 ± 0.60	45.49 ± 0.60	51.41 ± 1.00
$F_{(4,10)}$	*0.398*	*1.123*	*0.344*	*2.014*	*0.944*
Sig.	*ns*	*ns*	*ns*	*ns*	*ns*

Values are mean ± S.E. of three replications; ns: non-significant; T1: no vermicompost application; T2: vermicompost application of 10 t ha^{-1}; T3: vermicompost application of 20 t ha^{-1}; T4: vermicompost application of 30 t ha^{-1}; T5: vermicompost application of 40 t ha^{-1}.

A positive effect of vermicompost on the number of leaves produced by the spring onion plants was shown in the present study (Table 2). The total number of leaves per plant increased with increasing application rates of vermicompost. The best performance for the number of leaves was recorded in the T5 treatment with vermicompost, an application of 40 t ha^{-1}. At harvest (50 days after planting), the mean number of leaves on the plant in the T5 treatment reached 64.78, an increase of 28.5% compared to the control (Table 2).

The results of a one-way ANOVA analysis showed that there were no significant differences in the number of leaves among treatments for 10, 20 and 30 days after planting, but significant differences were found after 40 and 50 days. Specifically, at harvest, plants in the T5 treatment had a significantly higher number of leaves compared to the control and all other treatments, but the number of leaves in the treatments with lower vermicompost application rates (10, 20 and 30 t ha^{-1}) did not differ significantly from each other or from the control (Table 2).

Table 2. Effects of vermicompost on the number of leaves of spring onion plants.

Treatments	The number of leaves per plant in different growth periods				
	10 days	20 days	30 days	40 days	50 days
T1 (control)	9.78 ± 0.70	18.74 ± 1.32	33.85 ± 2.29	44.89 ± 1.33[b]	50.41 ± 1.22[b]
T2	9.30 ± 0.45	18.11 ± 0.80	30.89 ± 0.71	44.78 ± 2.71[b]	51.00 ± 2.10[b]
T3	9.89 ± 0.22	19.00 ± 1.79	34.44 ± 2.89	47.11 ± 3.45[b]	53.34 ± 1.79[b]
T4	8.81 ± 0.35	20.30 ± 1.99	34.85 ± 3.54	47.97 ± 0.70[ab]	54.22 ± 0.11[b]
T5	9.52 ± 0.51	19.33 ± 1.50	38.18 ± 2.26	57.44 ± 0.99[a]	64.78 ± 1.22[a]
$F_{(4,10)}$	*0.813*	*0.275*	*1.078*	*6.057*	*16.00*
Sig.	*ns*	*ns*	*ns*	$p < 0.05$	$p < 0.05$

Values are mean ± S.E. of three replications; ns: non-significant; Means with different letters are significantly different at the $p < 0.05$ level according to the Tukey's significant difference test; T1: no vermicompost application; T2: vermicompost application of 10 t ha^{-1}; T3: vermicompost application of 20 t ha^{-1}; T4: vermicompost application of 30 t ha^{-1}; T5: vermicompost application of 40 t ha^{-1}.

The number of tillers and the tiller diameter are important parameters that are closely related to the yield of spring onion (Tendaj and Mysiak, 2011). In the present study, the tiller number for each onion plant differed among treatments. Between 10 and 20 days after planting, there were slight differences in the number of tillers among treatments as the transplants were in an adaptation phase and starting to grow. After 30 days, new tillers started to be produced by the onion plants; thus, the differences in the number of tillers among treatments were more apparent. At 50 days after planting, the number of tillers in treatments T4 and T5 was the highest in comparison to the control and the other treatments, with 18.92 and 21.18 tillers plant $^{-1}$, respectively, an increase of 29.9% and 45.5% compared to the control (Table 3).

Subsequent statistical analysis confirmed that there were no significant differences in the number of tillers among the treatments at 10 to 30 days after planting, but significant differences occurred 40 and 50 days after planting. Particularly, the number of tillers in treatments T4 and T5 was significantly higher than that in the control and in the treatment with the lowest vermicompost application (10 t ha^{-1}; Table 3).

Previous work has shown that the more tillers that were made by an onion plant, the smaller the weight of each tiller was (Tendaj and Mysiak, 2011). Although the diameter of individual tillers was not investigated during the plant growth periods, this parameter was recorded at harvest (50 days after planting) in order to investigate if the diameter of the individual tillers of a plant that had more tillers was smaller than that of a plant that had fewer tillers. The result showed that the average diameter of a single tiller in the control, treatments T1, T2, T3, T4, and T5 was 9.33, 9.89, 10.67, 10.22, and 10.44 mm, respectively. Consequently, the highest diameter was found in the treatment T5 (10.44 mm) and the lowest diameter was observed in the control (9.33 mm), indicating that the positive effect of the vermicompost treatment T5 on the tiller number did not result in a reduction but in an increase of the tiller size.

Table 3. Effects of vermicompost on the number of tillers per spring onion plants.

Treatments	The number of tillers in different growth periods				
	10 days	20 days	30 days	40 days	50 days
T1 (control)	3.14 ± 0.07	4.55 ± 0.11	8.56 ± 0.45	11.26 ± 0.39[b]	14.56 ± 0.65[b]
T2	3.18 ± 0.04	5.00 ± 0.19	8.18 ± 0.16	11.11 ± 0.6[b]	14.92 ± 0.52[b]
T3	3.15 ± 0.04	4.52 ± 0.35	8.48 ± 0.60	12.41 ± 0.93[ab]	17.81 ± 1.16[ab]
T4	3.04 ± 0.04	4.70 ± 0.35	9.07 ± 0.58	13.00 ± 0.00[ab]	18.92 ± 0.59[a]
T5	3.11 ± 0.06	4.52 ± 0.10	9.22 ± 0.27	14.26 ± 0.52[a]	21.18 ± 0.61[a]
$F_{(4,10)}$	1.150	0.685	0.936	4.90	14.00
Sig.	*ns*	*ns*	*ns*	$p < 0.05$	$p < 0.05$

Values are mean ± S.E. of three replications; ns: non-significant; Means with different letters are significantly different at the $p < 0.05$ level according to the Tukey's significant difference test; T1: no vermicompost application; T2: vermicompost application of 10 t ha^{-1}; T3: vermicompost application of 20 t ha^{-1}; T4: vermicompost application of 30 t ha^{-1}; T5: vermicompost application of 40 t ha^{-1}.

Previous studies demonstrated that vermicompost had positive influences on the crops, soil parameters, and the environment (Rekha et al., 2018; Kumar and Gupta, 2018; Khan et al., 2019; Che Sulaiman and Mohamad, 2020; Jankauskienė et al., 2022). It is well-established that vermicompost has positive effects on promoting plant growth (Rekha et al., 2018; Kumar and Gupta, 2018; Khan et al., 2019). For example, Rekha et al. (2018) found that the treatment of 50% vermicompost + 50% soil increased the length of shoots, average intermodal length and the number of leaves and branches of *Capsicum annum* (Linn.) Hepper. Similarly, Jankauskienė et al. (2022) showed that mixing vermicompost with peat increased the plant height and the number of leaves of cucumber plants. In the present study, the growth of plants was also found to be significantly increased with increasing vermicompost application rates.

Effects of vermicompost on the yield parameters of spring onion

There were large differences in the individual mean plant weight at harvest and in the total yield of spring onions in the $1m^2$ plots among treatments (Table 4, Figure 3). The results of the present study demonstrated that individual weight and plot yields increased when doses of vermicompost application were raised. The highest average weight of a single plant was found in treatment T5 with a mean of 302.93 g, which corresponds to an increase of 48.5% and 38.2% compared to the control (T1) and the lowest vermicompost treatment (T2), respectively. In terms of the total yield of the plots, the highest yield of 4.86 kg m^{-2} was again found in the treatment with the highest vermicompost application (T5), and the lowest yield of

3.26 kg m^{-2} was recorded in the control (no vermicompost application). The increases in plot yields compared to the control ranged from 3.9% in T2 to 49.1% in T5, clearly indicating the effectiveness of vermicompost application in increasing yields (Table 4). As a result, a strongly positive relationship between the vermicompost rate and the plot yield was observed in this study (Figure 4).

Table 4. Effects of vermicompost on the individual weight and plot yield of spring onion plants.

Treatments	The individual weight and plot yield of spring onion (fresh weight)	
	Individual weight (g plant^{-1})	Plot yield (kg m^{-2})
T1 (control)	204.07 ± 0.74^{e}	3.26 ± 0.08^{c}
T2	219.26 ± 0.24^{d}	3.39 ± 0.08^{c}
T3	247.04 ± 0.93^{c}	3.66 ± 0.20^{bc}
T4	265.93 ± 1.96^{b}	4.16 ± 0.11^{b}
T5	302.96 ± 1.96^{a}	4.86 ± 0.14^{a}
$F_{(4,10)}$	831.77	25.77
Sig.	$P < 0.05$	$P < 0.05$

Values are mean ± S.E. of three replications; Means with different letters are significantly different at the $p < 0.05$ level according to the Tukey's significant difference test; T1: no vermicompost application; T2: vermicompost application of 10 t ha^{-1}; T3: vermicompost application of 20 t ha^{-1}; T4: vermicompost application of 30 t ha^{-1}; T5: vermicompost application of 40 t ha^{-1}.

Figure 3. Sizes of spring onion plants in different treatments. T1 (control): no vermicompost application (A); T2: vermicompost application of 10 t ha^{-1} (B); T3: vermicompost application of 20 t ha^{-1} (C); T4: vermicompost application of 30 t ha^{-1} (D); T5: vermicompost application of 40 t ha^{-1} (E).

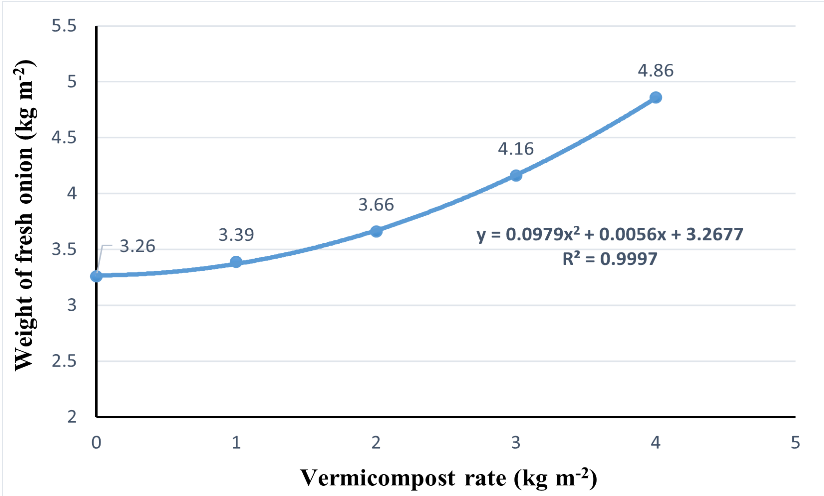

Figure 4. The relationship between the application rate of vermicompost and the yield of spring onion.

Consequent statistical analysis confirmed that there were significant differences in the mean weight of single plant among treatments, with the individual weights of spring onions significantly higher in all vermicompost treatments than in the control (Table 4, Figure 3). A similar tendency was found in the plot yields. The plot yields of treatments T4 and T5 increased significantly compared to the control (Table 4).

Regarding the potential use of vermicompost in the promotion of crop yields, several studies have demonstrated that the addition of vermicompost increased the yield of tomato (Yang et al., 2015), radish (Kumar and Gupta, 2018), chili (Khan et al., 2019), cucumber (Jankauskienė et al., 2022), and lettuce (Papathanasiou et al., 2012). The results of this study were in agreement with the results of these previous studies and confirmed the positive effects of vermicompost on alliums; adding vermicompost promoted the individual plant weight and the yield of spring onion (Table 4, Figures 3 and 4). Furthermore, the higher plant growth and yield depended on the amount of vermicompost added to the soil. The research by Khan et al. (2019) also demonstrated that the addition of vermicompost at an application rate of 2.5 t ha^{-1} (fruit yield of 8.27 t ha^{-1}) gave a higher yield compared to the application of 2.0 t ha^{-1} (fruit yield of 6.88 t ha^{-1}) and 1.5 t ha^{-1} (fruit yield of 5.50 t ha^{-1}) and to the control (fruit yield of 3.61 t ha^{-1}). Similarly, Jankauskienė et al. (2022) found that the treatment with peat + 30% vermicompost produced a higher cucumber yield than the treatments with peat + 10% and 20% vermicompost. In the present study, the growth and yield parameters of spring onion improved by adding vermicompost, with an application of 40 t ha^{-1} yielding the best performance on all parameters. Noticeably, a strongly positive relationship between vermicompost doses and the growth and yield parameters of spring onion was observed in the present study (Figure 4).

There is no doubt that vermicompost benefits crop yield in a range of different crops. This reflects the fact that vermicompost contains macronutrients (N, P, K), micronutrients, beneficial soil microbes, plant growth-regulating hormones, and enzymes such as amylase, lipase, cellulase, and chitinase (Sinha et al., 2010). As a result, the application of vermicompost in agricultural cultivation promoted plant growth and increased the yield of crops.

Conclusion

Vermicompost had significant positive effects on the growth and yield of spring onion. These positive effects increased with the level of the vermicompost application. At the highest vermicompost application rate (40 t ha^{-1}), onion plants had the highest performance in terms of plant height, number of leaves, number of tillers, individual weight, and plot yield. Noticeably, an increase in plot yield of 49.1% was found in the treatment of the highest vermicompost application compared to the control. Consequently, the findings of this study demonstrate that vermicompost has great potential for use as a bio-fertilizer in agricultural cultivation in general and in spring onion cultivation in particular.

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UTICAJI GLISTENJAKA NA RAST I PRINOS ALJME (*ALLIUM FISTULOSUM* L.)

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R e z i m e

Aljma (*Allium fistulosum* L.) je popularno povrće za salatu koje se proizvodi širom sveta, uključujući i Vijetnam. Zahvaljujući svom ukusu i aromi, nezamenjiv je sastojak koji se koristi za aromatizovanje supa i drugih jela. Glistenjak je prirodno i ekološki prihvatljivo đubrivo koje se široko koristi za povećanje prinosa useva i očuvanje održivosti agrosistema. Shodno tome, ova studija je sprovedena da bi se ispitala efikasnost glistenjaka pri različitim količinama primene u unapređivanju parametara rasta i prinosa aljme. Rezultati pokazuju da je dodavanje glistenjaka prilikom proizvodnje aljme imalo značajne pozitivne uticaje na visinu biljke, broj listova, broj izdanaka, masu cele biljke i prinos po parcelici. Konkretno, primena glistenjaka u količini od 40 t ha^{-1} pokazala je najviši učinak kod posmatranih parametara, povećavajući broj listova, broj izdanaka, masu cele biljke odnosno prinose po parcelici na 64,78, 21,18, 302,96 g po biljci, odnosno 4,86 kg m^{-2}. Prinosi po parcelici u tretmanima sa najvišom i najnižom primenom glistenjaka povećani su za 49,1% odnosno 3,9% u poređenju sa kontrolom. S tim u vezi, postojala je jaka pozitivna veza između količine primenjenog glistenjaka i prinosa po parcelici.

Ključne reči: *Allium fistulosum*, crni luk, organska đubriva, glistenjak, aljma.

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THE IMPACT OF NITROGEN FERTILIZATION ON THE ALKALOID CONTENT AND GROWTH TRAITS OF DATURA (*DATURA STRAMONIUM* L.)

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Abstract: *D. stramonium* is a wild plant species belonging to the nightshade family *Solanaceae* and grows on the edges of cotton fields, empty lands and roadsides. The plant is one of the most important economic weed plants that contain both toxic and medicinal properties. Studies have shown that the plant contains toxic tropane alkaloids such as atropine, hyoscyamine, and scopolamine. It is known that cultivation techniques have a great influence on the quality and quantity of secondary metabolites. For this reason, it is important to determine the optimal values of the agronomic factors that influence plant growth and production. This study was conducted in the experimental area of the Agricultural Faculty of Dicle University in the 2010 and 2011 growing seasons so as to evaluate the impact of nitrogen fertilizer rates (0, 50, 100 and 200 kg ha^{-1}) on some agronomical characteristics such as fresh and dry herb yield and total alkaloid content of Datura (*Datura stramonium* L.). The results revealed that significant ($P \leq 0.05$) differences were observed between nitrogen doses in the 2010 and 2011 growing seasons in terms of seed yield, fresh and dry herb yield and total alkaloid yield of seeds. However, seed yield changed between 545 kg ha^{-1} and 625 kg ha^{-1}, fresh herb yield between 8000 and 24483 kg ha^{-1}, dry herb yield between 2190 kg ha^{-1} and 5083 kg ha^{-1} and alkaloid content between 0.259% and 0.366%, respectively. The results showed that fresh and dry herb yields increased with increasing nitrogen doses.

Key words: Datura, seed yield, herb yield, total alkaloid content.

Introduction

The genus *Datura* belongs to the *Solanaceae* family, which is known for the synthesis of a number of tropane alkaloids. The main active substances are hyoscyamine and scopolamine, and this ratio for *D. stramonium* is about 2:1 (EFSA, 2008). Hyoscyamine (atropine) and scopolamine are the predominant

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tropane alkaloids in the *Datura* genus, occurring in all plant organs. These alkaloids have spasmolytic and anesthetic properties (Iranbakhsh et al., 2006; Alinejad et al., 2020). Its leaves contain about 0.2–0.6% of alkaloids. There are three species spreading in Turkey such as *D. metel* L, *D. innoxia* L. and *D. stramonium* L. *D. stramonium* is a plant distributed throughout most temperate regions of the world. *D. stramonium* has been traditionally used to treat asthma, coughs and cramps (Ceylan, 1994; Cespedes-Mendez et al., 2021). The plant is cultivated for medicinal purposes due to its alkaloids. *D. stramonium* grows in the wild in many zones in Turkey. In southeastern Anatolia of Turkey, *D. stramonium* is found in fields as a weed in many summer crops, particularly in maize, cotton and vegetable crops (Baytop, 1984). It is stated that the total alkaloid content of *D. stramonium* varies between 0.2 and 0.7% in plants grown in arid conditions and between 0.2 and 0.5% in plants grown in moist soils (Kulaksiz and Kan, 2002).

Nutritional elements such as nitrogen are important factors influencing alkaloid production. The excessive amount of nitrogen used in the plant causes it to get too much nitrogen and protein by blocking the conversion of certain factors, which also leads to an accumulation of nitrogen (NO_3, NO_2^{-}) (Maynard et al., 1976). Both the herbage yield and the biologically active substances are very important for the cultivation of medicinal plants. Nitrogen (N) is one of the major nutrients that support crop growth and is the most responsive nutrient required for herbage plants (Ozyigit et al., 2016; Izadi et al., 2021; Izadi et al., 2022; Alinejad et al., 2020). Therefore, it is very important to determine the suitable dosage. Up to date, there have been studies reporting on the effects of nitrogen doses on the secondary metabolites of many alkaloid bearing plants (Ruminska and Gamal, 1978; Ozguven et al., 1986; Cakmak and Ozguven, 1987; Gupta et al., 2011; Al-Humaid, 2005; Ciesiolka et al., 2005; Oster et al., 2014; Carrubba, 2015). Increasing nitrogen doses also causes an increase in the number of vegetative parts and total alkaloids (Nassar et al., 2015). In some agronomic studies, a nitrogen dose of 150 kg ha^{-1} has been reported to be suitable for a high proportion of vegetative parts and a nitrogen dose of 200 kg ha^{-1} for seed yield (Esendal et al., 2000). In another study, an increase in dry and fresh herb and seed yield was observed as nitrogen doses increased from 100 to 800 kg ha^{-1} (Al Humaid, 2005). The aim of this study was to investigate the effects of different nitrogen doses on *D. stramonium L.* regarding some agronomic characteristics and alkaloid content under semi-arid growing conditions in Diyarbakir, Turkey.

Material and Methods

Plant material and field experiment

Two-year field experiments were conducted in 2010 and 2011 at the experimental area of the Department of Field Crops of Dicle University (latitude

37°53´N and longitude 40°16´E, 680 m above sea level). The soil properties of the experimental plot were loamy-clayey with a pH of 7.46, 14.5 kg ha^{-1} P_2O_5, 0.48% organic matter and 11.02% lime. The soil was analyzed for total soil organic matter, total salts, lime, soil pH, and soil structure as described by Klute (1986).

D. stramonium seeds were collected from the Cinar district of Diyarbakir, Turkey. The experiments were laid out in a completely randomized block design with 3 replications. Four different nitrogen doses (0, 50, 100, 200 kg ha^{-1}) in the form of ammonium nitrate (33%) were used in the study. The seeds were scattered by hand on the prepared field at the first week of April in 2010 and 2011. Half of the nitrogen was applied at sowing and the remaining half of the nitrogen at the first irrigation. The *D. stramonium* seeds were sown into the previously prepared plots in rows 60 × 30 cm apart. The plot size was 7.2 m^2 (3 m × 2.4 m) at sowing and 2.4 m^2 (1.2 m x 2 m) at harvesting for both herbage and seed yields. Each plot consisted of four rows with a distance of 60 cm. Harvesting was done by hand when the seeds were ripe and the leaves were in full bloom.

Five plants randomly selected were taken from each plot to record growth and yield parameters. In the experiment, plant height (cm), plant stem diameter (mm), number of branches per plant, number of capsules per plant, capsule width (cm), capsule length (cm), number of seeds per capsule, thousand-seed weight (g), seed yield (kg ha^{-1}), fresh herbage yield (kg ha^{-1}), alkaloid content of seed (%) and alkaloid content of leaf (%) were investigated.

Climatic and weather conditions

The general climatic characteristics of the south-eastern Anatolia region are hot and dry in summer with temperatures above 30°C. Winter precipitation is erratic. Spring and fall are generally mild, but sudden hot and cold spells are common in both seasons in the region.

The experimental area has a long-term (70-year) average precipitation of 469.9 mm, an average temperature of 15.8 ^{0}C, and a relative humidity of 54.3%. The meteorological data for the 2010 and 2011 growing seasons showed an average temperature of 17.7 ^{0}C and 15.5 ^{0}C, and precipitation of 398 mm and 574 mm, respectively (State Meteorology Institute, Diyarbakir, Turkey).

Determination of the total alkaloid content

The determination of total alkaloids was performed according to the Harborne (1973) and EFSA (2008). According to this method, the dried plant material was crushed in a blender and soaked in 50 ml of 0.1 N HCL. Then the solution was kept at room temperature for one night and transferred to a percolation tube. After maceration, the mixture was transferred to a tube percolator and the percolation process was continued with distilled water. Then, a few drops of the percolation were dropped into Dragendorff reagent. The process was continued until the mixture was orange. The perchlorate was transferred to a 250-ml separating funnel and after decanting, the medium was made alkaline with 10% ammonia solution. Then the solution was dissolved with 4×25 ml of chloroform ($CHCl_3$) by shaking for 2 min each. The extracts were collected, and $CHCl_3$ was eluted completely from the media. The residue was dried at 105 ^{0}C for 30 min. The remaining material was dissolved in 25 ml of boiling water and 3 ml of ethanol of 95^0 was added, using methyl red as an indicator, and titrated with 0.01 N HCL. The titration, already showing a transparent yellowish color, turned pink again at the end. The calculation was made by counting 0.00289 alkaloid acid in each 1 ml of 0.01 N HCl. This value was multiplied by the acid used to determine the amount of alkaloids from 3-g samples. This value can then be converted into a percentage.

Statistical analysis

The obtained data was subjected to an analysis of variance (MSTAT-C, 1980) and the LSD test ($p<0.05$) was performed to compare the means.

Results and Discussion

The nitrogen treatments significantly affected plant height in the 2010 and 2011 growing seasons (Table 1). The highest plant height was 114.17 cm obtained with N_{100} doses, while the lowest plant height was 95.7 cm recorded in the N_0 control plot (Table 1). The plant height partially increased with an increase in applied N doses, but a decrease was observed in N_{200} doses. Al-Humaid (2005) reported that the highest dose of fertilizers resulted in a reduction in plant height, number of branches and leaves per plant, and fresh and dry weights of leaves, stems, crown flowers, and fruits of *D. stramonium* plants. Increasing nitrogen doses caused an increase in plant height in 2011. Namely, the highest value was 77.8 cm obtained with the N_{200} dose and the lowest value was 59.2 cm obtained with N_0 doses in both years (Table 1). The differences arising between the two years of the trial may be caused by changes in the amount of annual rainfall. Esendal et al. (2000) reported that nitrogen fertilization had an increasing effect on plant height, and plant height varied between 40.24 and 67.83 cm. Similar results were observed by these researchers in an experiment conducted in the second year. Kulaksiz and Kan (2002) verified that increasing N doses (ranging between 0 and 150 kg ha^{-1}) resulted in higher plant height.

Table 1 shows that the plant stem diameter was not affected significantly by the different fertilization applications. Plant stem diameter increased with increasing nitrogen dose so that the highest plant stem diameter was obtained at

N_{200} nitrogen doses with 1.87 cm, and the lowest plant stem diameter was produced at N_0 doses with 1.61 cm. The highest plant stem diameter (1.53 cm) was obtained at N_{200} nitrogen doses, while the lowest (1.19 cm) was obtained at N_0 nitrogen doses. As for plant height, the differences between the years may be due to the variations in annual rainfall. Our findings are higher than those of Esendal et al. (2000), who reported plant stem diameters between 0.72 and 0.86 cm.

The differences in the number of branches per plant between the N fertilization treatments were not significant for 2010 but were significant for 2011 (Table 1). The lowest number of branches (1.97 per plant) was obtained at N_{00} nitrogen doses, while the highest number of branches (2.3 per plant) was obtained at N_{200} nitrogen doses in 2011 (Table 1). The mean number of branches per plant was 3.84 and 2.18 in 2010 and 2011, respectively. However, the differences in the number of branches could be associated to the changes in the amount of annual rainfall between the experimental years. Our plant height results were higher than the results of Esendal et al. (2000), who reported the number of branches between 1.37 and 1.50 per plant. These variations may be due to differences in genotype, ecological conditions, and used agricultural techniques.

Table 1. The mean values of plant height, plant stem diameter, number of branches, number of capsules, capsule width, capsule length, and number of seeds per capsule of *D. stramonium* at different nitrogen doses in the 2010 and 2011 growing seasons.

2010							
Nitrogen doses (kg ha^{-1})	Plant height (cm)	Plant stem diameter (mm)	Number of branches per plant	Number of capsules per plant	Capsule width (cm)	Capsule length (cm)	Number of seeds per capsule
0	95.70c	1.61	3.63	1.53b	2.05b	2.07	293.9c
50	106.97ab	1.66	3.80	1.87a	2.18ab	2.69	314.1bc
100	114.17a	1.73	3.93	1.83a	2.36a	2.92	355.6a
200	102.45bc	1.87	4.00	1.67ab	2.13b	2.58	323.7b
Mean	104.83	1.72	3.84	1.73	2.18	2.56	321.8
LSD (0.05)	10.06	NS	NS	0.23	1.89	NS	21.48
2011							
0	59.20c	1.19	1.97b	1.20	1.88a	2.23b	239.3b
50	64.40bc	1.40	2.07b	1.53	1.89a	2.41ab	272.0a
100	73.93ab	1.48	2.33a	1.67	1.83b	2.63a	281.7a
200	77.80a	1.53	2.37a	1.80	1.91a	2.68a	281.3a
Mean	68.83	1.40	2.18	1.55	1.88	2.49	268.5
LSD (0.05)	12.19	NS	0.24	NS	0.58	2.69	31.02

*Means in the same column followed by the same letter are not significantly different according to LSD at 0.05 level, NS: Not significant.

According to the data from the 2010 growing season, the highest number of capsules per plant was 1.87 at N_{50} nitrogen doses, while the lowest was 1.53 per plant at N_0 nitrogen doses (Table 1). The increase in nitrogen doses in 2011 caused a slight increase in the number of capsules. The highest value of 1.80 per plant was achieved with the N_{200} nitrogen doses; the lowest value was 1.20 with the N_0 nitrogen doses (Table 1). Despite similar nitrogen doses and observed differences due to low rainfall during the growing season, the obtained results are in agreement with those of Esendal et al. (2000).

The width of the capsules has a great positive impact on the number of seeds they contain. The mean of the capsule width was determined to be 2.18 and 1.88 cm for the 2010 and 2011 growing seasons, respectively. In 2010, the highest capsule width (2.36 cm) was obtained with N_{100} nitrogen doses; the lowest capsule width (2.05 cm) was obtained with N_0 nitrogen doses (Table 1). In 2011, increasing nitrogen doses affected the capsule width positively. The highest value (1.91 cm) was determined for N_{200} nitrogen doses, and the lowest value (1.88 cm) was determined for the N_0 dose (Table 1). This situation might be due to precipitation differences between years. The capsule width increased with increasing nitrogen levels. Esendal et al. (2000) reported that the capsule width of the plants obtained from seeds collected from different places varied between 2.35 and 2.48, which is consistent with our results.

Theoretically, a high capsule density would produce more seeds per capsule and potentially generate a higher seed yield. The capsule length showed mean values of 2.56 cm and 2.49 cm in 2010 and 2011, respectively. The highest capsule length was 2.92 cm at N_{100}, and the lowest was 2.07 cm at N_0 nitrogen doses in 2010. The capsule length decreased with increasing nitrogen doses in the 2011 growing season. The highest capsule length was recorded at N_{200} nitrogen doses (2.68 cm) and the lowest was 2.23 cm at N_0 (Table 2). Esendal et al. (2000) reported that the nitrogen doses led to a linear increase in capsule length.

To obtain high seed yield, a high number of seeds per capsule is required. The number of capsules per plant is an important yield-contributing character for assessing the seed yield of *D. stramonium*. The maximum number of seeds per capsule was found to be 355.6 per capsule at doses of N_{100}, while the minimum value was 293.9 per capsule at N_0 nitrogen doses (Table 2). Increasing nitrogen doses enhanced the number of seeds per capsule, with the highest number of seeds per capsule at N_{100} doses (281.7 pcs), and the lowest number of seeds per capsule (239.3 pcs) at the N_0 treatment (Table 2). The determined differences between two years could be caused by the varying annual precipitation. Esendal et al. (2000) determined that the number of seeds per capsule varied in a range of 342–384 pcs. The number of seeds per capsule obtained in this study was lower than that of Esendal et al. (2000), which is probably due to the differences in the genotype, ecological conditions and agricultural practices applied.

Table 2. The effects of different nitrogen doses on thousand-seed weight, seed yield, fresh and dry herbage yield, and alkaloid content of seeds and leaves of *D. stramonium* in 2010 and 2011.

Nitrogen doses (kg ha^{-1})	Thousand-seed weight (g)	Seed yield (kg ha^{-1})	Fresh herbage yield (kg ha^{-1})	Dry herbage yield (kg ha^{-1})	Alkaloid content of seed (%)	Alkaloid content of leaf (%)
2010						
0	4.53b	546	13813b	3210b	0.312b	0.251c
50	5.09ab	657	20290ab	4317ab	0.352a	0.318a
100	5.41a	726	23356a	4500a	0.366a	0.323a
200	4.44b	569	24483a	5083a	0.323b	0.276b
Mean	4.87	625	20486	5178	0.338	0.292
LSD (0.05)	0.65	NS	741.8	124.2	0.019	0.019
2011						
0	5.55	412c	8000b	2190	0.259	0.195b
50	5.66	541b	8757b	2413	0.288	0.288a
100	5.54	675a	9713b	2522	0.320	0.304a
200	5.02	552b	12010a	2727	0.305	0.303a
Mean	5.44	545	9620	2463	0.293	0.272
LSD (0.05)	NS	10.50	191.8	NS	NS	0.063

*Means in the same column followed by the same letter are not significantly different according to LSD at 0.05 level, NS: Not significant.

The thousand-seed weight is one of the important yield components determining the quality of the seed. The maximum 1000-seed weight (5.41 g) was measured with the application of nitrogen doses at N_{100}, while the minimum 1000-seed weight (4.44 g) was recorded at N_{200} in 2010 (Table 2). The increase in nitrogen doses in 2011 caused greater seed weight. The maximum 1000-seed weight (5.66 g) was found in plants grown at N_{50} level, while the minimum thousand-seed weight (5.02 g) was measured at N_{200} level (Table 2). The 1000-seed weight results are lower than the findings of Losak and Palenicek (2005), Ceylan (1994) and Esendal et al., (2000). It is thought that these differences arise from differences in the studied material, the genotypes, and the ecological conditions.

The seed yield of *D. stramonium* is one of the latest products in the production of alkaloids. A high seed yield depends on many factors. Some of these factors are referred to as good agricultural practices, the number of plants per unit area and irrigation. In term of seed yield, the minimum seed yield (546 kg ha^{-1}) was recorded in the control, while the maximum seed yield (726 kg ha^{-1}) was obtained at the N_{100} level in 2010 (Table 2). Enhanced nitrogen doses increased seed yield in the 2011 growing season. The highest seed yield was obtained at the N_{100} level with 675 kg ha^{-1} and the lowest seed yield was obtained at the N_0 level with 412 kg ha^{-1} (Table 2). The results obtained in this study were lower than those of Esendal et al. (2000) and Kulaksiz and Kan (2002), but the findings of Losak and Palenicek (2005) and Ozguven et al. (1986) are similar to their lower values. These differences may be caused by the studied material, ecological conditions, and changes in agricultural practices. The results of this study in terms of seed yield are similar to those of Izadi et al. (2021), which were obtained by fertilizing with urea and were below the value of 180 kg ha^{-1}.

The data presented in Table 2 reveal that the different fertilization applications had a significant effect on the fresh herbage yield. The fresh herbage yield increased with an increase in nitrogen doses in the first experimental year, with the highest (24483 kg ha^{-1}) fresh herbage yield at N_{200} doses and the lowest fresh herbage yield of 13813 kg ha^{-1} at the N_0 nitrogen level. The second-year data showed that the highest fresh herbage yield was 12010 kg ha^{-1} obtained at the N_{200} level, and the lowest fresh herbage yield was 8000 kg ha^{-1} obtained at the N_0 level (Table 2). Ruminska and Gamal (1978) indicate in their study that the growth of *Datura innoxia* increased with increasing the amount of nitrogen fertilization. Ceylan (1994) also reported an increase in fresh herbage yield with an increase in nitrogen doses. However, compared with previous research data related to fresh herbage, our results showed a high value compared to the findings of Esendal et al. (2000), who reported 7038–9232 kg ha^{-1}. Similarly, it was observed by Oster et al. (2014) in *Duboisia*, another alkaloid plant, that mineral N fertilization significantly increased biomass.

The different nitrogen doses were statistically significant ($P \leq 0.05$) for dry herbage yield in 2010, but not statistically significant in the 2011 growing season. In 2010, the highest dry herbage yield (5083 kg ha^{-1}) was obtained at the N_{200} nitrogen dose, and the lowest dry yield (3210 kg ha^{-1}) was obtained at the N_0 level (Table 2). In 2011, the nitrogen doses enhanced the dry herbage yield, 2727 kg ha^{-1} was recorded as the highest value at the N_{200} nitrogen level, and 2190 kg ha^{-1} was recorded as the lowest value at the N_0 level. Our findings are higher than those of Ceylan (1994), who reported that increasing nitrogen doses increased dry herbage yield and the yield ranged from 100 to 300 kg ha^{-1}. Alaghemand et al. (2013) also reported that the dry weight of total foliage of henbane increased with increasing nitrogen fertilization.

Datura species are important for alkaloid production. Seeds, leaves, flowers and roots contain alkaloids. Leaves and seeds are used commercially for the production of alkaloids. High level of alkaloids depends on many factors, such as environmental conditions, nutrition level and different agronomical practices. The most important of these factors is fertilization. The highest alkaloid content was found to be 0.366% for N_{100} nitrogen doses, while the lowest alkaloid content was found to be 0.312% for N_0 nitrogen doses during the 2010 growing season (Table 2). The highest value (0.320%) was obtained for N_{100} nitrogen doses and the lowest value (0.259%) was obtained for N_{200} nitrogen doses in 2011. The increase in nitrogen doses in 2011 initially led to an increase in the alkaloid content of seeds, and then to a decrease. A similar tendency was observed in the study of Al-Humaid (2005). Gholamhosseinpour et al. (2011), working with periwinkle, have reported that nitrogen is a component of alkaloids that plays an important role in the synthesis of alkaloids. Therefore, increasing nitrogen nutrient leads to an increase in alkaloid content. Regarding the alkaloid content of seeds, our results are lower than those of Ceylan (1994) and Kulaksiz and Kan (2002), but higher than those of Akin and Ceylan (1986).

The effects of nitrogen doses on the alkaloid content of the leaves were significant in both years. The lowest alkaloid content of the leaf (0.251%) was obtained at the N_0 level, while the highest alkaloid content of the leaf (0.323%) was obtained at the N_{100} level in 2010. There was a trend towards an increase in the alkaloid content of the leaf with increasing nitrogen levels in the 2011 growing season. The highest value of 0.304% was obtained at N_{100} and the lowest value of 0.195% was obtained at the N_0 fertilization level. There were differences in the alkaloid content of the leaf between years, and the mean values of 2010 were higher than those of 2011. It is assumed that the higher precipitation in the second year was responsible for the high results in the first year of the experiment. As reported by Elzenga (1958), rainfall generally reduces the alkaloid content and the alkaloids are washed from the plant.

This situation shows that different ecological factors influence the secondary metabolites. Ceylan (1994) reported that the alkaloid content changed with increasing nitrogen doses, and the amount of alkaloid content increased with enhancing nitrogen doses. Al-Humaid (2005) showed that nitrogen fertilization in Datura plants caused an increase in alkaloid content at moderate fertilizer doses, while a decrease was observed at high doses. Our result is consistent with that of Ozguven et al. (1986), Tanker and Tanker (1990) and Kaya and Gurel (1997). There are also many studies showing that alkaloid content increases with increasing nitrogen doses in various alkaloid-producing plants (Ghorbanpour et al., 2014; Losak and Palenicek, 2005). Haadi-e-vincheh et al. (2013) describe this situation as follows: alkaloids contain nitrogen and are usually derived from amino acid precursors, since a higher availability of nitrogen doses would lead to higher levels of alkaloids in plants.

Conclusion

This study was carried out to determine the appropriate nitrogen dose to obtain high fresh and dry herb and seed yields. A high seed yield was obtained with 100 kg ha^{-1} nitrogen dose, and a significant decrease was observed with an application of 200 kg ha^{-1}. Fresh and dry herb yields were higher than 200 kg ha^{-1}, which corresponds to the highest nitrogen dose. The alkaloid contents of the seeds and leaves were found to be close to each other. It was determined that the alkaloid content in the seeds and dry leaves of the first year was higher than that of the second year. These results showed that *D. stramonium* can be cultivated to produce tropane alkaloids in semi-arid conditions with appropriate cultivation techniques.

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UTICAJ ĐUBRENJA AZOTOM NA SADRŽAJ ALKALOIDA I OSOBINE RASTA TATULE (*DATURA STRAMONIUM* L.)

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R e z i m e

D. stramonium je divlja biljna vrsta koja pripada porodici pomoćnica *Solanaceae* i raste na rubovima pamučnih polja, ruderalnih površina i pored puteva. Ova biljka je jedna od ekonomski najvažnijih korovskih biljaka koje sadrže i toksična i lekovita svojstva. Istraživanja su pokazala da ova biljka sadrži toksične tropanske alkaloide kao što su atropin, hiosciamin i skopolamin. Poznato je da način gajenja ima veliki uticaj na kvalitet i kvantitet sekundarnih metabolita. Iz tog razloga, važno je odrediti optimalne vrednosti agronomskih faktora koji utiču na rast i proizvodnju biljaka. Ovo istraživanje je sprovedeno u eksperimentalnoj oblasti Poljoprivrednog fakulteta Univerziteta Didžle u vegetacijskim sezonama 2010. i 2011. godine kako bi se procenio uticaj količine azotnog đubriva (0, 50, 100 i 200 kg ha^{-1}) na neke agronomske osobine kao što su kao prinos sveže i suve herbe i ukupan sadržaj alkaloida tatule (*Datura stramonium* L.). Rezultati su pokazali da su uočene značajne ($P \leq 0,05$) razlike između doza azota u vegetacijskim sezonama 2010. i 2011. godine u pogledu prinosa semena, prinosa sveže i suve herbe i ukupnom prinosu alkaloida semena. Međutim, prinos semena se menjao između 545 kg ha^{-1} i 625 kg ha^{-1}, prinos sveže herbe između 8000 i 24483 kg ha^{-1}, prinos suve herbe između 2190 kg ha^{-1} i 5083 kg ha^{-1} odnosno sadržaj alkaloida između 0,259% i 0,366%. Rezultati su pokazali da se prinosi sveže i suve herbe povećavaju sa povećanjem doza azota.

Ključne reči: tatula, prinos semena, prinos herbe, ukupan sadržaj alkaloida.

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EFFECTS OF THE APPLICATION OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ON THE CONSISTENCY LIMITS AND COMPACTION CHARACTERISTICS OF DIFFERENTLY TEXTURED SOILS

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Abstract: Notwithstanding their beneficial characteristics, the agricultural utilization of organic wastes may have an adverse effect on soil properties if used improperly. To evaluate proper use, a laboratory study was conducted to investigate the effects of different sewage sludge application doses (0, 2, 4, and 8% weight/weight) on the consistency limits and the compaction characteristics of three differently textured soils. The application of sewage sludge significantly improved the consistency limits and reduced the compactibility. The efficacy depended on the amount applied. The rates of increase in liquid limit (LL) values at 8% sewage sludge were 58.7% for sandy loam, 43.4% for loam, and 16.2% for clay soil. As the application dose increased, the optimum moisture content (OMC) values increased and the maximum dry bulk density (MBD) values decreased. The highest application dose decreased the MBD by 9.5% in sandy loam, by 6.5% in loam, and by 13.7% in clay-textured soils. The rates of increase in OMC values were 73.4%, 53.8%, and 27.1%, for sandy loam, loam, and clay, respectively. The results presented in this study clearly indicated that the application of sewage sludge made the soils more resistant to mechanical forces, since the increase in the proportion of OMC over LL and PL implied that the soil was easier to till at higher moisture contents without any deformation, which also resulted in a higher workable range.

Key words: Atterberg limits, compactibility, sewage sludge, soil degradation, soil friability.

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Introduction

Soil, one of nature's most complex and diverse ecosystems, is one of the most neglected, essential, and precious non-renewable natural resources. According to estimates, 36–75 billion tonnes of fertile soil is lost every year due to poor farming practices and degradation (Gobinath et al., 2021). To maintain soil sustainability and recover it to its former condition, simple, proper, and economic steps should be taken into account. The application of wastes (e.g., crop residues, biosolids, composts, etc.) to the soil is generally the most economical way of increasing organic matter content, thus maintaining its sustainability. The utilization of organic wastes as an organic matter not only provides an opportunity to recycle beneficial plant nutrients and increase crop productivity through the improvement of the physical (Lindsay and Logan, 1998; Asghari et al., 2009), chemical (Angin et al., 2012, 2017), and biological (Tejada et al., 2006; Adewole and Ilesanmi, 2012) properties of soils, but also decreases the environmental concerns regarding the disposal of these materials.

Sewage sludge is an inevitable waste byproduct of wastewater processes, which is a concentrated suspension of semi-solid materials, usually rich in mineral nutrients and organic matter. The utilization of sewage sludge as a source of organic matter is known to improve the soil properties such as water retention capacity, water availability, aggregate stability (Tsadilas et al., 2005), bulk density (Cogger et al., 2013), porosity (Tsadilas et al., 2005), hydraulic conductivity (Aggelides and Londra, 2000), compactibility (Stone and Ekwue, 1993), penetration resistance (Kumar et al., 1985), and crusting (Pagliai et al., 1983). In addition to the positive effects, the agricultural utilization of sewage sludge not only provides a cost-effective method of sludge disposal but is also essential to decreasing the environmental issues associated with the disposal of this material in landfills and the high volumes generated (Smith, 1995; Angin et al., 2012). The amount of sludge produced during wastewater treatment ranges from 35 to 85 g of dry solids per day for the equivalent of one person (Foladori et al., 2010). Notwithstanding its beneficial characteristics, sewage sludge may have an adverse effect on soil properties if used improperly. Therefore, greater attention is being paid to the management and proper use of sewage sludge for agricultural purposes.

Soil structure is a key indicator of soil quality because it influences processes critical to soil productivity, environmental and water quality, and agricultural resilience (Bronick and Lal, 2005). The risks of undesirable degradation effects such as erosion, flooding, and compaction can be reduced by the improvement of soil structure (Connolly, 1998; Six et al., 2004). Among these effects, compaction is perhaps the most encountered problem in agricultural areas due to conventional and intensive agricultural practices. The Proctor test has been employed to characterize the compaction resistance of agricultural soils and to evaluate the compaction status of soils (Wagner et al., 1994; Ekwue and Stone, 1995, Zhang et al., 1997; Hakansson and Lipiec, 2000). Parameters for comparing the compactibility of soils are the maximum dry bulk density (MBD) according to the Proctor test and the optimum moisture content (OMC), at which the maximum dry bulk density is reached. The maximum dry bulk density may be used as a reference value for evaluating the compaction status of soils (Hakansson and Lipiez, 2000). The corresponding water content indicates the moisture status for maximum compaction at a defined energy impact. The agronomic importance of these parameters is elucidated by Wagner et al. (1994). Wagner et al. (1992) have also found that the best soil fragmentation during tillage is obtained at the Proctor optimum moisture content. The sensitivity of the soil to compaction and the maximum dry soil bulk density in the case of maximum compaction are closely related to the consistency limits. Therefore, the consistency limits (Atterberg) are accepted as an important indicator of the mechanical behavior of the soil (Dexter and Bird, 2001; Hemmat et al., 2010; Sari et al., 2017). Consistency limits could be used to determine the ideal and practicable water content range for tillage operations with the least amount of effort and risk of structural degradation and deformation (Dexter and Bird, 2001). Therefore, studies aimed at improving the mechanical properties of agricultural soils should be given special attention. Several studies have found a significant and positive relationship between the optimum soil water content for tillage and the liquid and plastic liquid limits (Terzaghi et al., 1988; Mueller et al., 1990; Dexter and Bird, 2001; Mueller et al., 2003; Barzegar et al., 2004). However, there is little information about the effects of sewage sludge application on soil compaction, especially on the consistency limits.

To evaluate the effects of sewage sludge on sustainable soil management properly, it is important and necessary to determine the influences of sewage sludge on the consistency limits (Atterberg) and the parameters of the Proctor compaction test. Therefore, this study was carried out to investigate the influences of sewage sludge application on the consistency limits and compaction characteristics of three differently textured soils.

Material and Methods

A pot experiment with four sewage sludge application rates and three replicates was carried out under laboratory conditions with a relative humidity of 60±5% and an average temperature of 25±2ºC. The soil samples used in the experiment were under similar tillage and crop management practices and were collected from the 0-20-cm depth of widely distributed great soil groups (sandy loam [Ustorthent], loam [Fluvaquent] and clay [Haplustert]) (Soil Survey Staff, 2014) in Erzurum, Türkiye. The collected soils were air-dried under laboratory conditions, crumbled by hand, sieved through an 8-mm sieve, and homogenized by mixing thoroughly after sieving. Anaerobically stabilized sewage sludge obtained from the Ankara Metropolitan Municipality Treatment Plant, passed through a 2-mm sieve, was applied to the soils within the doses of 0, 2, 4, and 8% weight/weight basis (w/w) (dry matter). The prepared samples were thoroughly mixed (including the control) and placed into 36 plastic pots (0.25 m wide and 0.40 m long) to a depth of 0.15 m. The amount of soil in each pot was 17000 g for sandy loam, 16000 g for loam, and 14000 g for clay. To achieve microbial activity and organic matter mineralization, the experimental soils were incubated for 120 days at near field capacity moisture content.

The main descriptive properties of the soils and sewage sludge used in the study are presented in Table 1. A WDXRF spectrometer (Rigaku ZSX-100e) was used to determine the concentrations of O, Ca, Si, Mg, K, Al, P, S, Fe, Na, Mn, and Sr in sewage sludge and soils. The Bouyoucos hydrometer method was used to determine the particle size distribution (Gee and Or, 2002). The bulk density was determined using soil sampling rings (volume of 100 cm^3) (Grossman and Reinsch, 2002). Particle density was determined by the pycnometer method (Flint and Flint, 2002). The pH and electrical conductivity (EC) measurements of soils and sewage sludge were carried out in 1:2.5 (soil:water) and saturation extracts, respectively (Rhoades, 1996; Thomas, 1996). While the soil organic matter content was determined by using the Smith-Weldon method, the organic matter content of the sewage sludge was determined using the loss-on-ignition method described by Nelson and Sommers (Nelson and Sommers, 1996). A Schreiber calcimeter was used to determine the lime content of the soils and sewage sludge (Loeppert and Suarez, 1996). The cation exchange capacity (CEC) of the soils was determined with a flame photometer using 1-M neutral ammonium acetate (Sumner and Miller, 1996). The aggregate stability values of the soils were determined using the standard Yoder wet sieving method (Nimmo and Perkins, 2002).

The liquid limit (LL) values of the soils were determined by the standard drop-cone penetrometer (McBride, 2002). The plastic limit (PL) values of the soils were determined using the rod formation method (McBride, 2002). The “ASTM D427-04” standard method was used to determine the shrinkage limit (SL) of the soils (ASTM, 1992). The plasticity index (PI) was calculated by subtracting the PL from the LL. The difference in moisture content between SL and PL values was determined as the friability index (FI). The standard Proctor compaction test (ASTM, 2000) was used to determine the compaction test curves, optimum moisture content and maximum dry bulk density values of the soils.

The SPSS Statistical Package v.20.0 was used to conduct the statistical analysis (IBM, 2011). The data were subjected to a one-way ANOVA, followed by a comparison of the relevant means using the Tukey’s multiple comparison test at the significance level of $p<0.05$. Furthermore, correlation and regression analyses were carried out to assess the influences of sewage sludge application on the investigated parameters.

Table 1. Initial characteristics of the soils and sewage sludge used in the study.

Properties	Soil I	Soil II	Soil III	Sewage sludge (SS)		
Clay (%)	16.6±1.2	25.9±1.1	64.2±0.1	-	Particle size distribution (%) (dry sieving)	
Silt (%)	24.5±0.2	40.7±2.5	19.1±0.1	-	2000–1000 μ	24.8
Sand (%)	58.9±1.2	33.4±1.4	16.7±0.1	-	1000–500 μ	32.4
Textural class	Sandy loam	Loam	Clay	-	500–250 μ	21.9
Great soil group	Ustorthent	Fluvaquent	Haplustert	-	250–100 μ	9.8
CEC (cmol kg^{-1})	22.5±1.3	40.7±1.3	47.0±1.5	-	100–53 μ	7.7
$CaCO_3$ (%)	0.5±0.02	0.5±0.03	0.9±0.04	1.2±0.03	<53 μ	3.4
Organic matter (%)	1.9±0.07	1.2±0.09	1.1±0.03	45.5±1.02	(wet sieving)	
pH	6.6±0.08$^{\Psi}$	7.8±0.02$^{\Psi}$	7.3±0.04$^{\Psi}$	7.2±0.11$^{\Omega}$	2000–1000 μ	3.8
EC (mS cm^{-1})	0.5±0.1$^{\Psi}$	0.9±0.1$^{\Psi}$	1.1±0.1$^{\Psi}$	14.1±0.2$^{\Omega}$	1000–500 μ	16.3
Bulk density (g cm^{-3})	1.32±0.02	1.21±0.02	1.07±0.03	0.67±0.02	500–250 μ	18.7
Particle density (g cm^{-3})	2.66±0.02	2.63±0.02	2.67±0.02	1.93±0.03	250–100 μ	10.1
X-ray fluorescence spectrometer analysis (concentration, %): O	47.29	46.96	47.71	47.62	100–53 μ	15.0
Ca	3.85	4.88	2.10	13.18	<53 μ	36.1
Si	31.67	30.25	32.51	9.44		
Mg	1.44	1.51	1.77	1.25		
K	2.09	1.75	1.93	0.96		
Al	8.43	8.32	8.90	3.54		
P	0.17	0.16	0.05	2.27		
S	0.04	0.05	0.07	2.21		
Fe	1.82	2.42	2.18	1.81		
Na	1.75	1.42	0.48	0.23		
Mn	0.04	0.06	0.05	0.04		
Sr	0.01	0.01	0.01	0.02		

$^{\Psi}$ Determined in the 1:2.5 (soil: water) extract. $^{\Omega}$ Determined in the saturation extract.

Results and Discussion

Effects of sewage sludge on soil consistency limits

The application of sewage sludge had significant effects on the consistency limits of the soils studied (Table 2). The highest values were obtained from clay soil, which had the highest clay content. While the investigated parameters showed significant positive correlations with clay content, they showed a negative correlation with sand and silt. The amount of organic matter, sand, and clay, which affect the characteristics of the diffused double layer of the soil, has significant effects on the consistency limits (Canbolat and Öztaş, 1997; Canbolat et al., 1999; Hemmat et al., 2010). Clay, which reflects a high specific surface, has been reported to be the most important fraction of soil affecting consistency limits (De Jong et al., 1990; Stanchi et al., 2015). Keller and Dexter (2012) stated that soils containing negligible amount of organic matter, should have at least 10% of clay in the texture to exhibit plastic properties.

Table 2. Effects of sewage sludge on the organic matter content, aggregate stability, and consistency limits of soils.

Parameters	Application dose (w/w)	Soil I (sandy loam)	Soil II (loam)	Soil III (clay)
OM (%)	0%	1.9±0.4c*	1.2±0.1c	1.1±0.1c
	2%	3.4±0.3b	3.9±0.1b	2.0±0.2bc
	4%	3.6±0.1b	4.3±0.3ab	2.3±0.3b
	8%	4.4±0.3a	4.7±0.4a	3.3±0.4a
	Mean	3.3±0.9B	3.5±0.8A	2.2±0.7C
	p	.000	.000	.000
	R^2	.841	.825	.822
AS (%)	0%	32.7±0.8c	31.4±1.9b	31.4±2.1c
	2%	40.9±3.9b	33.1±2.0b	35.9±3.3bc
	4%	46.5±2.6b	39.1±0.4a	42.2±4.0ab
	8%	59.5±5.4a	41.6±2.0a	46.3±4.3a
	Mean	44.9±10.7A	36.3±4.6C	39.0±6.7B
	p	.000	.000	.004
	R^2	.880	.841	.773
LL (%)	0%	27.0±0.5d	34.7±0.7d	70.3±1.7d
	2%	32.8±0.9c	41.8±1.2c	74.7±1.9c
	4%	36.8±1.1b	46.2±1.6b	78.5±1.1b
	8%	42.9±1.5a	49.7±1.5a	81.7±1.7a
	Mean	34.9±6.1C	43.1±6.0B	76.3±4.6A
	p	.000	.000	.000
	R^2	.975	.893	.938
PL (%)	0%	20.6±1.7d	23.0±1.4d	44.2±1.9c
	2%	24.0±0.5c	29.1±0.8c	47.9±2.4b
	4%	28.7±0.7b	33.8±2.6b	51.9±0.4a
	8%	32.4±0.8a	38.8±1.1a	53.3±1.5a
	Mean	26.4±4.8C	31.2±6.3B	49.3±4.0A
	p	.000	.000	.001
	R^2	.960	.951	.655
PI (%)	0%	6.4±1.3c	11.7±1.2ns	26.2±0.6ns
	2%	8.9±0.7bc	12.7±1.0	26.9±1.9
	4%	8.1±0.7ab	12.4±1.0	26.6±0.8
	8%	10.5±0.8a	10.9±2.1	28.4±1.7
	Mean	8.5±1.7C	12.0±1.4B	27.0±1.5A
	p	.004	.464	.271
	R^2	.798	.347	.861
SL (%)	0%	7.1±1.3d	9.9±0.3d	16.8±0.8b
	2%	8.9±0.6c	13.5±1.0c	19.5±2.3a
	4%	11.8±0.6b	16.8±1.2b	21.7±0.8a
	8%	13.8±0.7a	20.5±1.0a	22.2±1.1a
	Mean	10.4±2.8C	15.1±4.2B	20.0±2.5A
	p	.000	.000	.005
	R^2	.917	.959	.700
FI (%)	0%	13.5±1.3c	13.1±1.1c	27.4±1.0c
	2%	15.0±0.2c	15.6±0.2b	28.4±0.9ab
	4%	16.9±1.2b	17.0±1.4ab	30.2±0.8ab
	8%	18.6±0.1a	18.3±0.4a	31.0±2.1a
	Mean	16.0±2.1B	16.0±2.2B	29.3±1.9A
	p	.001	.001	.031
	R^2	.864	.834	.603

OM: organic matter; AS: aggregate stability; LL: liquid limit; PL: plastic limit; PI: plasticity index; SL: shrinkage limit; FI: friability index. w/w: weight/weight *The letters in each column (capital letters) show the differences between the soils, whereas the letters in the columns (small letters) show the differences between the application doses (mean±std). ns: not significant.

The efficacy of the sewage sludge on the consistency limits depended on the amount of sewage sludge applied (Table 2). Sewage sludge played a significant role in increasing the LL, depending on the application dose. Among the application doses tested, the highest LL values were observed in the 8% treatment. When compared with the controls, the LL values of sandy loam, loam, and clay soil increased by 58.7%, 43.4%, and 16.2%, respectively. Similarly, the highest PL values were determined at an 8% sewage sludge application dose. As compared with the control, the rates of increase in PL values at the 8% sewage sludge application were found to be 57.0% for sandy loam, 68.9% for loam, and 20.6% for clay soil. The increase in LL and PL values could be related to an increase in the adsorption surface and water-holding capacity due to the addition of organic material. This statement is consistent with the findings of Rixon et al. (1991), Lindsay and Logan (1998), Bhushan and Sharma (2002). The organic matter content of sandy loam soil was 1.9% in the control and increased to 3.4%, 3.6%, and 4.4% after the application of 2, 4, and 8% sewage sludge, respectively. The organic matter content of loam soil increased from 1.2% to 3.9%, 4.3%, and 4.7% and that of clay soil from 1.1% to 2.0%, 2.3%, and 3.3% after 2, 4, and 8% sewage sludge application, respectively. The addition of sewage sludge to the soil not only augmented the aggregate stability of the soil, but also the absorptive capacity of the soil to absorb water, which therefore increased the consistency limits.

The effects of sewage sludge application on PL and LL values were more pronounced in soils with lower clay content and higher sand content. While the highest rates of increase in PL and LL values were determined in sandy loam soil, the lowest rates were determined in clay soil. The correlation coefficients between sewage sludge (SS) and LL were found to be 0.985^{**} for sandy loam, 0.969^{**} for loam, and 0.951^{**} for clay. The correlation coefficients between SS and PL were determined as 0.981^{**} for sandy loam, 0.974^{**} for loam, and 0.915^{**} for clay (Table 3). The organic matter content of soil, which is a great factor in increasing the specific surface area and consequently water retention, influences LL and PL of coarse-textured soils much more than other textured soils (Smith et al., 1985; Hemmat et al., 2010). The increase in LL and PL values of soils allows soils to be easily cultivated in wider and higher ranges of soil water contents (Lindsay and Logan, 1998). Although aggregate stability plays a major role in the soil swelling behavior, there is limited information on the relationship between AS and consistency limits. Significant positive correlations were observed between AS, –LL and –PL parameters. The correlation coefficients between AS–LL and AS–PL were 0.986^{**} and 0.954^{**} for sandy loam, 0.928^{**} and 0.911^{**} for loam, and 0.879^{**} and 0.835^{**} for clay, respectively (Table 3). The relationships between sewage sludge application –LL, –PL and –PI were presented in Table 2 and Figure 1. In general, irregular changes in PI values were obtained upon the application of sewage sludge to soils. The results showed that an increase in the LL value might not result in an increase in the PI value due to the simultaneous increase in the PL value. Regression analyses showed that the sewage sludge application had a high and positive linear relationship with LL (R^2=0.975), PL (R^2=0.960) and PI (R^2=0.798) for sandy loam soil. The same relationships were observed for loam and clay soils (Table 2, Figure 1).

Table 3. The Pearson's correlation coefficients between the parameters.

		SS	OM	AS	LL	PL	PI	SL	FI	OMC
Soil I (sandy loam)	OM	.925**	-							
	AS	.964**	.897**	-						
	LL	.985**	.922**	.986**	-					
	PL	.981**	.919**	.954**	.980**	-				
	PI	.783**	.731**	.862**	.838**	.714**	-			
	SL	.962**	.912**	.931**	.958**	.976**	.701*	-		
	FI	.936**	.863**	.915**	.939**	.959**	.680*	.875**	-	
	OMC	.967**	.925**	.937**	.960**	.970**	.725**	.929**	.952**	-
	MBD	-.966**	-.877**	-.920**	-.944**	-.964**	-.683*	-.952**	-.910**	-.955**
Soil II (loam)	OM	.917**	-							
	AS	.929**	.845**	-						
	LL	.969**	.947**	.928**	-					
	PL	.974**	.892**	.911**	.975**	-				
	PI	-.229	.042	-.123	-.104	-.322	-			
	SL	.981**	.895**	.919**	.970**	.993**	-.312	-		
	FI	.922**	.851**	.859**	.946**	.974**	-.329	.940**	-	
	OMC	.937**	.799**	.933**	.929**	.946**	-.278	.945**	.911**	-
	MBD	-.934**	-.887**	-.943**	-.944**	-.928**	.133	-.933**	-.881**	-.941**
Soil III (clay)	OM	.915**	-							
	AS	.853**	.763**	-						
	LL	.951**	.837**	.879**	-					
	PL	.915**	.748**	.835**	.953**	-				
	PI	.521	.612*	.510	.571	.297	-			
	SL	.853**	.711**	.758**	.849**	.932**	.151	-		
	FI	.799**	.635*	.756**	.886**	.873**	.428	.638*	-	
	OMC	.904**	.752**	.860**	.894**	.865**	.475	.834**	.719**	-
	MBD	-.953**	-.952**	-.838**	-.919**	-.843**	-.617*	-.737**	-.801**	-.781**
General correlation	OM	.678**	-							
	AS	.783**	.633**	-						
	LL	.377*	.367*	.336*	-					
	PL	.419*	.241	.238	.981**	-				
	PI	.370*	.315*	.204	.966**	.898**	-			
	SL	.412*	.281	.278	.877**	.937**	.745**	-		
	FI	.268	.429**	.196	.977**	.963**	.938**	.808**	-	
	OMC	.503**	.342*	.330*	.950**	.976**	.859**	.938**	.922**	-
	MBD	-.428**	-.459**	-.431**	-.974**	-.959**	-.936**	-.897**	-.924**	-.945**

SS: sewage sludge; OM (%): organic matter; AS (%): aggregate stability; LL (%): liquid limit; PL (%): plastic limit; PI (%): plasticity index; SL (%): shrinkage limit; FI (%): friability index; OMC (%): optimum moisture content; MBD (%): maximum dry bulk density. **The correlation is significant at the 0.01 level. *The correlation is significant at the 0.05 level.

Among the sewage sludge application doses tested, the highest PI values were obtained at 8% (10.5%) for sandy loam, 2% (12.7%) for loam, and 8% (28.4%) for clay soils. Stanchi et al. (2009) found a significant positive correlation between soil organic matter content and LL and PL, but no significant relationship between organic matter and PI. On the other hand, Ball et al. (1996), Blanco-Canqui et al. (2006), and Zentar et al. (2009) obtained a significant positive correlation between soil organic matter content and PI. As a result of a study conducted on 44 soil samples, McBride and Bober (1989) have stated that an increase in the soil organic matter content leads to a decrease in PI values. In this study, a significant positive correlation (0.315^{*}) was found between the soil organic matter content and the PI. When the correlation coefficients between organic matter content and PI were examined for each soil, correlation coefficients were found to be 0.731^{**} for sandy loam, 0.042 for loam, and 0.612^{*} for clay soil (Table 3). The reason for the inconsistencies could lie in the different inherent soil properties originating from the parent material, clay type, clay mineralogy, and clay content (De Jong et al., 1990; Hemmat et al., 2010). Lal and Shukla (2004) have reported that an increase in the organic matter content of mineral soil generally leads to an increase in both LL and PL values. Therefore, an increase in organic matter content is expected to have coherent effects on PI.

The application of sewage sludge significantly increased both the shrinkage limit (SL) and the friability index (FI) of soils studied (Table 2). The highest SL and FI values were obtained with the highest dose of sewage sludge application (8%). When compared with the controls, the SL values of sandy loam, loam, and clay soil increased by 94.2%, 106.7%, and 32.3%, respectively, at a dose of 8% sewage sludge application. The shrinkage limit of the soils increased with the increase in the sewage sludge application dose.

As compared with the control, the SL values of sandy loam increased by 25.4%, 66.0%, and 94.2% at 2, 4, and 8% sewage sludge applications, respectively. Similar increases were observed in the loam and clay soils. The highest SL values were obtained in clayey soil, which has the highest clay content. Our result suggested that an increase in clay content increased the magnitude of shrinkage due to the micropore volume, pore volume of clay-matrix organized in clay aggregates, and the interaggregate pore volume (Boivin et al., 2004). The increase in SL values with the application of sewage sludge may be attributed to an increase in soil organic matter, which increases the moisture content due to its high-water absorption capacity. The strong correlation between the SL and the soil organic matter content supports this argument. The correlation coefficients between OM and SL were found to be 0.912^{**} for sandy loam, 0.895^{**} for loam, and 0.711^{**} for clay soil (Table 3). An increase in FI may extend optimal tillage and the cultivation period, as the soil structure is only minimally disturbed (Munkholm, 2011; Obour et al., 2017; Seleiman et al., 2019). As observed for SL and PL values, the highest increase in FI values was found at an 8% sewage sludge application dose (Table 2). In general, a higher sewage sludge application dose increased the FI value.

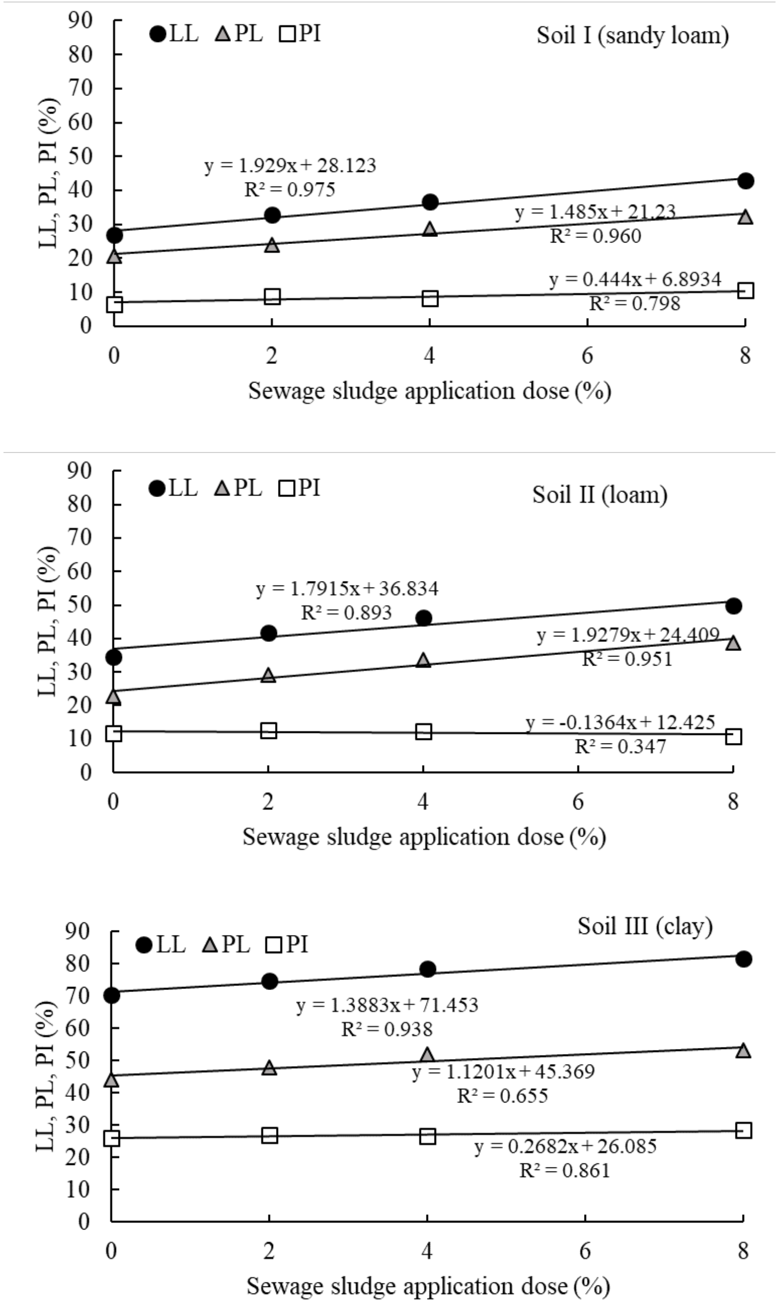

Figure 1. The relationship between the sewage sludge application dose and LL, PL, and PI.

These results showed that the application of sewage sludge made the soil friable at a higher water content compared to the control, making the soil more tillable and cultivable at a higher water content. The correlation coefficients between the soil OM content and the FI were 0.863^{**} for sandy loam, 0.851^{**} for loam, and 0.635^{*} for clay soil (Table 3). Similar results were reported by Macks et al. (1996), Watts and Dexter (1998), Munkholm (2011).

Effects of sewage sludge on Proctor compaction test parameters

As the sewage sludge application dose increased, the optimum moisture content (OMC) values increased and the maximum dry bulk density (MBD) values decreased (Table 4, Figures 2 and 3). A higher amount of sewage sludge generally resulted in a higher OMC and a lower MBD. For all soils studied, the highest OMC and the lowest MBD values were obtained at the highest sewage sludge application doses. While the regression analysis between the sewage sludge application dose and OMC had a high and positive relationship (R^2=0.949), the regression analysis between the sewage sludge application dose and MBD generally had a high and negative relationship (R^2=0.981) (Table 4). The lowest OMC and the highest MBD values were obtained in sandy loam soil, which has the highest sand content and the lowest clay content. The results obtained show that the OMC values increased and the MBD values decreased with the associated decrease in sand content and the increase in clay content. Similar results regarding the effects of soil texture on OMC and MBD were reported by Smith et al. (1997), Nhantumbo and Cambule (2006), Sari et al. (2017). While the mean OMC value of the non-amended (control) soils was 21.0%, the OMC values at 2, 4 and 8% sewage sludge application doses were found to be 24.9%, 28.0%, and 30.6%, respectively. These values were 1.68, 1.64, 1.58, and 1.52 g cm^{-3} for the MBD of the control, 2, 4, and 8% application doses, respectively (Table 4). The lossiest compaction state was achieved with the application of 8% sewage sludge. These findings clearly show that the sewage sludge application made the soil more resistant to compaction and extended the range of workability in the field without deforming it. Extending the workability range makes soil more easily tilled without any mechanical compactions or deformations. The sewage sludge application showed a significant positive correlation with the OMC and a significant negative correlation with the MBD. The correlation coefficients between SS and OMC were found to be 0.967^{**} for sandy loam, 0.937^{**} for loam, and 0.904^{**} for clay soil. Moreover, the coefficients between SS and MBD were determined to be -0.966^{**}, -0.934^{**}, and -0.953^{**} for sandy loam, loam, and clay, respectively. On average for all soils, the correlation coefficient between SS–OMC was significant (0.503^{**}) compared to a significant correlation coefficient of -0.428^{**} between SS–MBD (Table 3). General correlation coefficients between SS–OMC and –MBD were determined to be 0.503^{**} and -0.428^{**}, respectively (Table 3). Many studies have shown that improving the structural stability of soil significantly decreases the compactness of the soil (Baumgartl and Horn, 1991; Ball et al., 1996; Buck et al., 2000; Batey, 2009; Sari et al., 2017). Agricultural management practices that provide organic matter to the soil are known to increase structural stability and AS. The organic matter addition to soil, which is known to increase the cohesive forces between mineral particles and organic components, improves structural stability and AS (Chenu et al., 2000; Yazdanpanah et al., 2016). Organic matter decreases the impact of compaction force on the soil through its elastic properties and by increasing the amount of stable aggregates (Soane, 1990; Nawaz et al., 2013; Holthusen et al., 2020). A significant positive effect was found between sewage sludge application and AS in the present study. The AS of the sandy loam soil was 32.7% in the control and increased to 40.9%, 46.5%, and 59.5% after 2, 4, and 8% sewage sludge applications, respectively. The AS of the loam soil increased from 31.4% to 33.1%, 39.1%, and 41.6%, and that of the clay soil from 31.4% to 35.9%, 42.2%, and 46.3% after 2, 4, and 8% sewage sludge applications, respectively. It is apparent that the increase in AS significantly increased OMC and significantly decreased MBD, as revealed by the significant correlations between the variables. The correlation coefficients between AS and OMC were 0.937^{**} for sandy loam, 0.933^{**} for loam, and 0.860^{**} for clay soil. The coefficients between AS and MBD were -0.920^{**}, -0.943^{**}, and -0.838^{**} for sandy loam, loam, and clay, respectively.

Table 4. Effects of sewage sludge on the Proctor compaction test parameters of soils.

Parameters	Application dose (w/w)	Soil I (sandy loam)	Soil II (loam)	Soil III (clay)
OMC (%)	0%	14.6±1.2d	18.9±2.0c	29.6±2.2c
	2%	18.5±0.9c	22.0±1.8c	34.2±1.0b
	4%	22.6±1.7b	25.4±1.8b	36.1±0.6ab
	8%	25.3±1.0a	29.0±1.3a	37.6±0.6a
	Mean	20.2±4.4C	23.8±4.2B	34.4±3.4A
	p	.000	.000	.000
	R^2	.928	.977	.818
MBD (g cm^{-3})	0%	1.89±0.03a	1.70±0.03a	1.46±0.03a
	2%	1.84±0.01b	1.66±0.01ab	1.41±0.02a
	4%	1.75±0.02c	1.63±0.01b	1.35±0.03b
	8%	1.71±0.02d	1.59±0.02c	1.26±0.02c
	Mean	1.80±0.08A	1.64±0.05B	1.37±0.08C
	p	.000	.001	.000
	R^2	.906	.988	.997

OMC: optimum moisture content; MBD: maximum dry bulk density; w/w: weight/weight. *The letters in each column (capital letters) show differences between the soils, whereas the letters in the columns (small letters) show the differences between the application doses.

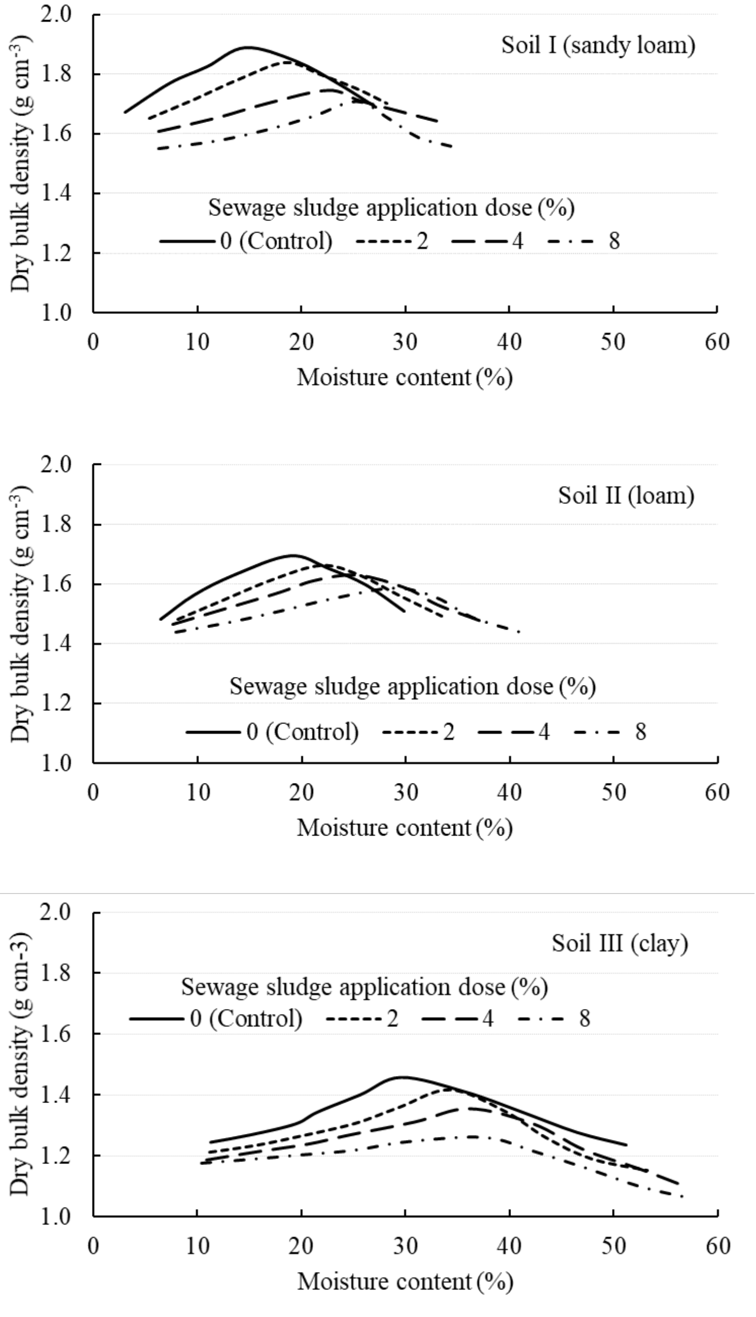

Figure 2. Proctor compaction test curves of soils studied.

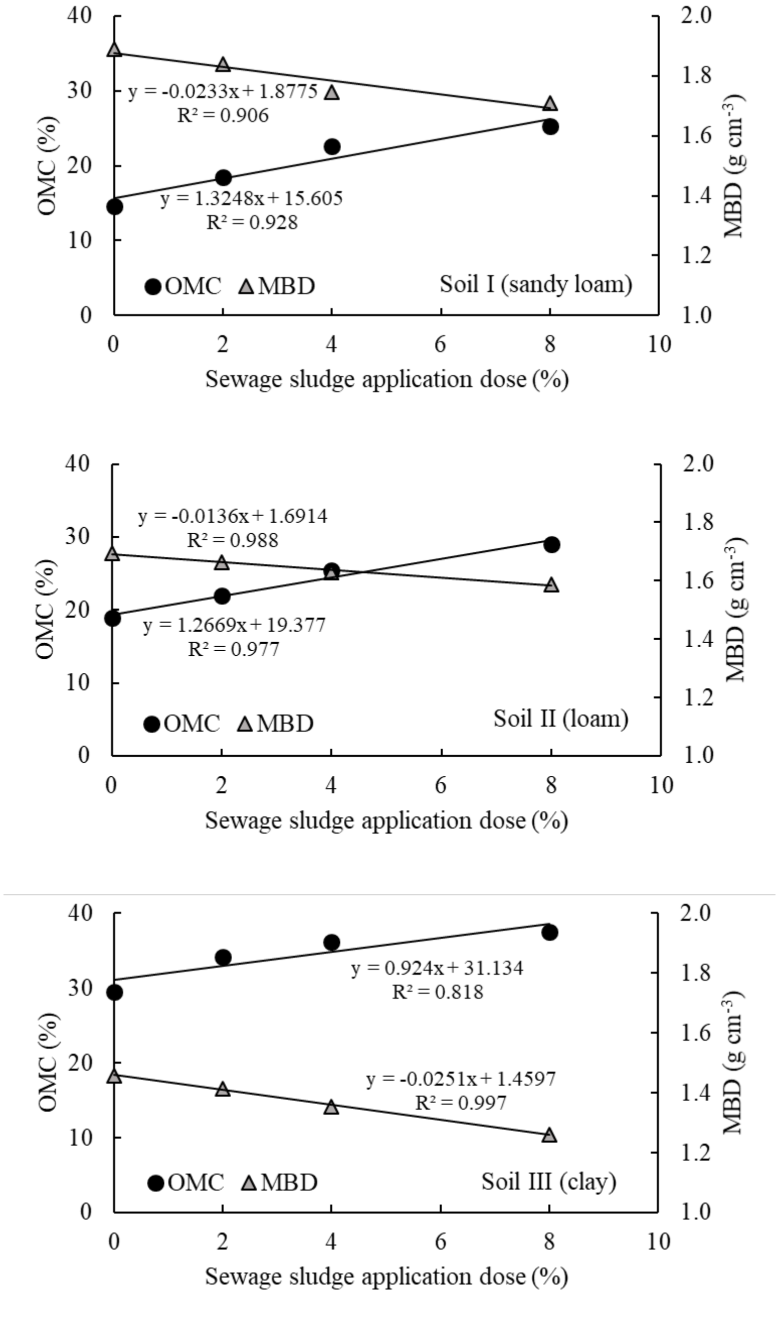

Figure 3. The relationship between the sewage sludge application dose and OMC and MBD.

Soil LL and PL values were evaluated as useful indicators of soil physical and mechanical properties, such as compressibility and strength, providing strategic indicators for management actions. The application of sewage sludge increased the water content at LL and PL of OMC where the maximum soil compaction occurred. Increasing the sewage sludge application dose increased the OMC of the control of the sandy loam soil, for which the initial LL and PL values were 54.0% and 70.7%, respectively. These values were reached at 56.3%, 61.4%, 59.0% for LL, and 77.1%, 78.7%, 78.1% for PL in response to 2, 4, and 8% sewage sludge application doses, respectively. Similar increases were observed in loam and clay soils. A significant and positive correlation between OMC–LL and –PL was found in several studies (Mueller et al., 2003; Aksakal et al., 2013; Sari et al., 2017). Overall, the correlation coefficients between OMC and LL, OMC and PL were found to be 0.950^{**} and 0.976^{**}, respectively.

Conclusion

The application of sewage sludge was found to improve the consistency limits and Proctor compaction parameters of soils. The response was not only dependent on the amount of sewage sludge applied, but also on the initial soil properties, mainly the soil texture. The application of sewage sludge at a dose of 2% of the soil was sufficient to significantly improve the soil mechanical properties, due to the development and stabilization of soil aggregates. The results obtained from this research have shown that soils increase their ability to withstand mechanical forces or withstand greater mechanical forces when properly mixed with sewage sludge. The increase in the optimum moisture contents of the liquid limit, plastic limit, and friability index indicated that soils amended with sewage sludge became more friable than the control soils at a relatively higher moisture content. Increasing the moisture content extends the range of workability in the field and allows the soil to be workable without degradation (aggregate breakdown and compaction) at higher moisture content. The application of sewage sludge has shown that it is possible to improve both soil tillage and workability simultaneously. The quality assessment of sewage sludge intended for agricultural use requires careful consideration of parameters such as nutrient content, organic matter, and potential contaminants. This evaluation is crucial to ensure that the sewage sludge, when utilized as soil amendment agent, has positive effects on soil properties and complies with environmental standards. The electrical conductivity of the sewage sludge used in this study was high, therefore, it requires careful and controlled use in terms of soil salinity when applied in high doses.

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UTICAJI PRIMENE ANAEROBNO RAZGRAĐENOG KANALIZACIONOG MULJA NA GRANICE KONZISTENCIJE I ZBIJENOST ZEMLJIŠTA RAZLIČITE TEKSTURE

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R e z i m e

Bez obzira na njihove korisne karakteristike korišćenje organskog otpada u poljoprivredi može imati negativan uticaj na svojstva zemljišta ukoliko se nepravilno koristi. Radi procene pravilne upotrebe kanalizacionog mulja sprovedena je laboratorijska studija kako bi se istražili efekti primene različitih doza (0, 2, 4 i 8% mas.) na granice konzistencije i zbijenost tri zemljišta različite teksture. Primena kanalizacionog mulja značajno je poboljšala granice konzistencije i smanjila zbijenost. Efikasnost je zavisila od količine primenjenog materijala. Stepeni povećanja vrednosti gornje granice plastičnosti (engl. *liquid limit* – LL) pri primeni 8% kanalizacionog mulja bile su 58,7% za peskovitu ilovaču, 43,4% za ilovaču, i 16,2% za glinovito zamljište. Kako se doza primene povećavala, vrednosti optimalnog sadržaja vlage (engl. *optimum moisture content* – OMC) su se povećavale, a vrednosti maksimalne suve gustine zemljišta (engl. *maximum dry bulk density* – MBD) su se smanjivale. Najviša doza mulja je smanjila MBD za 9,5% kod peskovite ilovače, za 6,5% kod ilovače, i za 13,7% kod glinovitog zemljišta. Stepeni povećanja vrednosti optimalnog sadržaja vlage bili su 73,4, 53,8, odnosno 27,1% za peskovitu ilovaču, ilovaču i glinu. Rezultati prikazani u ovoj studiji jasno su pokazali da je primena kanalizacionog mulja učinila zemljište otpornijim na delovanje mehaničke sile, s obzirom na to da je povećanje optimalnog sadržaja vlage u odnosu na LL i PL ukazalo na lakšu obradu zemljišta pri većim sadržajima vlage bez negativnih deformacija, što je dovelo i do povećanja intervala vlage pri kojem se zemljište može obrađivati.

Ključne reči: Aterbergove granice, zbijenost, kanalizacioni mulj, degradacija zemljišta, drobljivost zemljišta.

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EFFECTS OF COVID-19 ON THE FOOD SECURITY STATUS OF RURAL FARMING HOUSEHOLDS. EVIDENCE FROM NIGERIA

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Abstract: For the past couple of decades, food insecurity has become a major global phenomenon, which makes zero hunger the second Sustainable Development Goal. Nevertheless, COVID-19 has set in and posed a serious threat to the food system. Thus, there is a need to assess its effect on food security. This study, therefore, examined the effects of COVID-19 on the food security of rural farming households in Enugu State, Nigeria. Data collected from 120 households were analysed using descriptive statistics, the food security index, and logistic regression. The results revealed that the majority (64.5%) of the households with a shortfall index of 0.313 were food insecure, while only 35.5% were food secure with a surplus index of 0.109. The average daily equivalent calorie consumption of adults in food insecure and food secure households was 1552.52 and 2506.88 kcal, respectively. Low food availability (p<0.01), an increase in food prices (p<0.01), and the inability to harvest crops (p<0.1) increased the probability of food insecurity. Thus, the COVID-19 pandemic, due to the imposed lockdown has affected household food security. In contrast, access to credit (p<0.01), education (p<0.1), cooperative memberships (p<0.01), and income (p<0.05) positively influenced food security status. Reducing rational consumption, eating less expensive food, skipping meals, borrowing money to buy food, allowing children to eat first, and engaging in additional small-scale productivity activities were the major food insecurity coping strategies adopted by households during COVID-19. The study recommends the provision of farm inputs and financial support to farmers by governments and NGOs to curb the adverse effects of COVID-19 on food security.

Key words: COVID-19, food insecurity, rural households, smallholder farmers, coping strategies.

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Introduction

For the past couple of decades, food insecurity has been one of the major global phenomena. It is highly concentrated in developing nations, particularly in sub-Saharan Africa (SSA). The problem of malnutrition keeps increasing in SSA, as the number of undernourished people was reported to have increased by 32 million people between 2015 and 2019, making the number of undernourished people in SSA 239.1 million (FAO, IFAD, UNICEF, WFP, and WHO, 2020). Rural dwellers, who are mostly farmers, were the hardest hit in terms of malnutrition and poverty (Global Hunger Index, 2019; World Bank, 2019). In Nigeria, food insecurity was reported to be more concentrated among the rural population (Nigeria Millennium Development Goals End-Point Report, 2015).

Currently, about 690 million people, or 8.9% of the world's population, are undernourished globally (FAO, IFAD, UNICEF, WFP, and WHO, 2020). World food insecurity increased by 60 million (8.7%) between 2014 and 2019 (FAO, IFAD, UNICEF, WFP, and WHO, 2020). This shows that food insecurity is on the rise globally. In Africa, the number of undernourished people keeps increasing. Between 2014 and 2019, food insecurity in Africa increased by 17.6 per cent (FAO, IFAD, UNICEF, WFP, and WHO, 2020). Currently, 19.1% of the population (over 250 million people) is undernourished.

Globally, several programmes have been targeted at alleviating the high rate of food insecurity. For instance, the member states of the United Nations agreed to work towards halving the number of people suffering from hunger by 2015 and achieving seven Millennium Development Goals (MDGs). However, this was not met, although tremendous progress was recorded as the world's percentage of hungry people declined from 23.3% in 1990–1992 to 12.9% in 2014–2016 (Millennium and Goals, 2015). Nigeria is part of this phenomenon, as the country was unable to reduce the number of hungry people by half in 2015. This implies that food insecurity remains a major global concern and that a lot of effort has to be put in place to reduce the level of food insecurity (Mukaila et al., 2020; Falola et al., 2023).

In 2015, following the MDGs, the United Nations Development Programme (UNDP) committed to 17 Sustainable Development Goals (SDGs), including the issue of food insecurity. Zero hunger by 2030 was the second SDG; this shows how serious the challenge of food insecurity is for world leaders. The target of the second SDG was to improve nutrition, achieve food security, and end hunger. Efforts have been made by governments, UNDP, and other agencies to achieve zero hunger by 2030. Nevertheless, COVID-19 has set in and posed a serious threat to the food system. This could affect achieving SDG 2 by 2030.

The immediate preventive measures such as lockdown, border closure, and movement restrictions established globally to contain the spread of COVID-19 created serious hindrances to agri-food systems, economic activity, and, consequently, nutrition, food security, and people’s livelihoods (FAO, 2021). Furthermore, farmers were negatively affected as they were unable to buy inputs and sell their products, disrupting the national and international food supply chain and the entire food system (Andam et al., 2020; Amare et al., 2020; Chiemela et al., 2021; NAERLS and FMARD, 2020). The pandemic results in reductions in labour availability and an increase in food prices due to its effects on the food supply chain (Egwue et al., 2020; Swinnen, 2020). The pandemic also resulted in a decline in agricultural productivity and consequently a reduction in farmers’ earnings, which could affect the food security of their households.

Most studies on the recent COVID-19 in Nigeria have tended to focus on the economy, education, health, and industrial sectors (Abdullahi et al., 2020; Ajibo, 2020; Gabriel et al., 2020; Hassan et al., 2020; Jacob et al., 2020; Nnabuife et al., 2020; Obayori et al., 2020; Ololo et al., 2020; Onyekwena and Ekeruche, 2020). However, there is little research on how COVID-19 affects household food security, especially in rural farming households, and how they cope with the situation of food insecurity during the pandemic. This raises the need to assess the effect of COVID-19 on household food security. This study, therefore, investigated the food security status of rural farming households and the effects of COVID-19 on their food security status. It also analysed the coping strategies of farming households in relation to food insecurity during the COVID-19 crisis. This was to find out how they survived the crisis and whether or not they adopted a healthy coping strategy. This would enable appropriate policy interventions to curb the effects of COVID-19 on the food security of farming households.

Material and Methods

The study area

The study area was Enugu State, one of the thirty-six states of Nigeria. It is bordered by Ebonyi State to the east, Benue State to the northeast, Abia and Imo States to the south, Anambra State to the west, and Kogi State to the northwest. The state has a population of 3,257,298 people (National Population Commission, 2006), with an annual population growth rate of 2.3%, and occupies an area of 71,161 square kilometres. About eighty-five per cent of the population resides in rural areas, and seventy-five per cent of the rural dwellers are engaged in agriculture and allied activities (Ezike, 1998; Obetta et al., 2020). The state is located at latitudes $5^0$55’N and $7^0$08’N of the equator and longitudes $6^0$55’ E and $7^0$08’ E of the Greenwich meridian (Mukaila et al., 2022). It has seventeen local government areas (LGAs), which were grouped into six agricultural zones based on agroecology.

Sampling technique and data collection

Sampling of the farming households was done using a multistage sampling technique. A random selection method was used because the majority of the rural population in Enugu State are farmers. Thus, three LGAs were randomly selected in the first stage. The second stage involved the random selection of two rural communities, making a total of six rural communities. The third stage of the sampling techniques involved a random selection of twenty households from each community. This resulted in a total number of 120 farming households that served as respondents in the study.

The population for this study consisted of rural farming households. Primary data collected through the use of structured questionnaires was used in this study. The data collected contained information on the socioeconomic characteristics of the farming households, the food consumed by the households in the last twenty-four hours, the impact of the COVID-19 pandemic on their food security, and how they coped with the situation. The data were collected after the ease of lockdown in Nigeria. The researchers observed COVID-19 preventive measures such as social distancing, and the use of nose masks, hand gloves, and hand sanitisers to ensure the safety of researchers and respondents.

Data analysis

Descriptive statistics (such as mean, percentage, and frequency), the food security index, and logistic regression were used to analyse the data collected. Descriptive statistics were used to describe the socioeconomic characteristics of rural household heads.

This study used the food security index to measure the food security of the farming households following Babatunde et al. (2007), Mukaila et al. (2020), Omotesho et al. (2006), and Yusuf et al. (2015). The recommended calorie intake of 2260 kilocalories (kcal) per adult equivalent per day by the FAO was used as the food security line. The daily per capita calorie intake was determined by dividing the estimated daily calorie consumption of the household by the household size measured in adult equivalent with the use of male adult scale weights. The calories available in food items were estimated using food nutrient composition. A household with a daily per capita calorie consumption of up to 2260 kcal was considered food secure. Those whose household members consumed less than 2260 kcal per capita per day were considered food insecure.

The food security index is expressed as follows:

$$Z = \frac{I}{R} \quad (1)$$

where Z is the food security index, I is the daily per capita calorie intake of the household and R is the daily per capita calorie requirement of the household.

The headcount ratio (HR) is a measure of food security status and it is defined as

$$HR = \frac{M}{N} \quad (2)$$

where M is the total number of the food-secure and N is the sample population.

The food insecurity gap (FIG_i) was used to measure the depth of food insecurity among rural households. It is expressed as:

$$FIG_i = \frac{TR_i - TC_i}{TR_i} \quad (3)$$

The total food insecurity gap or shortfall index is expressed as:

$$TFIG_i = \frac{\sum(TR_i - TC_i)}{TR_i} \quad (4)$$

The squared food insecurity gap was used to examine the severity of food insecurity among food-insecure households. It is expressed as:

$$SFIG = \frac{\sum(FIG_i)^2}{M} \quad (5)$$

where TC_i is the total calorie consumed by the i^{th} food-insecure household, TR_i is the total calorie required for the i^{th} food-insecure household and $\sum$ is the summation.

Logistic regression was used to investigate the factors that affected farming household food security, and some variables were incorporated into the model to measure the effects of COVID-19 on farming household food security. Logistic regression is a predictive model that can perfectly account for dichotomous dependent variables. Therefore, it has been widely used in food security studies (Babatunde et al., 2007; Mukaila et al., 2020; Omotesho et al., 2006; Salau et al., 2019). It is explicitly represented as:

$$Y = \beta_0 + \beta_1 LFA + \beta_2 IFP + \beta_3 IHC + \beta_4 LO + \beta_5 ED + \beta_6 MO + \beta_7 FE + \beta_8 HS + \beta_9 CM + \beta_{10} IN + \beta_{11} EXT + \beta_{12} AC + \varepsilon \quad (6)$$

where Y is the food security status, LFA is the low food availability, IFP is the increase in food prices, IHC is the inability to harvest the crop, LO is the low output, ED is the educational level, MO is the major occupation, FE is farming experience, HS is the household size, CM is cooperative membership, IN is he income, EXT is the access to extension, AC is the access to credit, β_{1-12} are the coefficients of the regressors and ε is the error term.

Table 1. Description of the variables.

Variable name	Description	Expected sign	Unit of measurement
Food security status	A household with 2260 kcal per capita consumption was considered food secure and coded 1, 0 if otherwise		2260 kcal per capita (adult equivalent) consumption per day
Low food availability	This was measured in terms of the low availability of foodstuff in the household as farmers were unable to engage in their normal activities. 1 if low food availability affects their food consumption, 0 if otherwise	-	Dummy
Increase in food prices	This was measured in terms of high food prices during the pandemic. 1 if an increase in food prices affects the household food consumption, 0 if otherwise	-	Dummy
Inability to harvest crop	Measured in the form of movement restrictions imposed by COVID-19 which affects farmers' ability to harvest their products. 1 if farmers' inability to harvest their crops affects their food consumption, and 0 if otherwise	-	Dummy
Low output	Measured in the form of the effects of COVID-19 on farmers' output. 1 if low output affects their food consumption, 0 if otherwise	-	Dummy
Education	The educational level of the household head	+	Years
Major occupation	1 if farming is the major occupation, 0 if otherwise	+ / -	Dummy
Farming experience	Years of farming experience of the household heads	+	Years
Household size	The number of persons living in the same household and eating together.	+/-	Adult equivalent
Cooperative membership	Membership of the household head in a cooperative. 1 if the household head belongs to a cooperative, 0 if otherwise	+	Dummy
Income	Monthly income of the household head	+	Naira
Access to extension services	Access to agricultural extension services by farmers in the previous farming season	+	Number of contacts
Access to credit	Access to credit facilities from formal and informal sources. 1 if a household head has access to credit, 0 if otherwise	+	Dummy

Source: Authors' computation.

Gujarati (2004) and Greene (2005) suggested the derivation of the marginal effects of the explanatory variables in the logistics regression model. This should enable a comprehensive interpretation of the coefficient of the logistic regression model. Therefore, the marginal values of the explanatory variables were estimated to show their predictive power.

A three-point Likert rating scale was employed to examine the food insecurity coping strategies adopted by rural households during the COVID-19 crisis. The three-point Likert scale ranged from always (3), occasionally (2), to never (1). The mean value ($\bar{x}$ = 2) of the three values was used as the cut-point. The mean value of the smallholder households was calculated for each of the coping strategies listed. All the mean scores equal to or greater than 2 were regarded as widely adopted coping strategies during the pandemic, and all scores less than 2 were considered less adopted.

Results and Discussion

Socioeconomic characteristics of the farmers

The socioeconomic characteristics of the rural farmers are presented in Table 2. The results reveal that the majority of the farming household heads were male (90.8%). This implies that the males were likely to be responsible for the needs and wellbeing of the household and had the responsibility of providing food for the household. The majority were married (86.7%) and the average household size was six persons. However, rural households prefer a large household size, which could serve as a family labour force for their farming activities (Mukaila et al., 2021). The rural farming household heads had an average age of 52 years. This shows that although the household heads were elderly, they were still economically active enough to carry out farming activities effectively. Forty per cent of the farmers had no formal education. However, the majority possessed some level of education, though not advanced, which could help them in the decision-making process. This is because the level of farmers' education can enhance their ability to make the right decision on the use of inputs, which in turn increases their productivity (Akanbi et al., 2022; Falola et al., 2022). A larger percentage (68.3%) of the rural household heads did not belong to a cooperative society where they could benefit from economies of scale and have access to relevant agricultural information. This could affect their access to credit, as one of the major roles of a cooperative society is the provision of financial support to its members.

Farming is the major occupation of 85 per cent of rural household heads. This implies that agriculture serves as a means of livelihood and a source of income for the rural population. Thus, any disruption to agricultural activities would affect the livelihoods of the rural population. The rural household heads had an average farming experience of 21 years. This implies that they were experienced farmers who had knowledge of farming activities.

Table 2. Socioeconomic characteristics of the smallholder farmers.

Characteristics	Categories	Frequency	Percentage	Mean
Gender	Male	109	90.8	
	Female	11	9.2	
Age	Less than 40	11	9.2	52
	41 to 50	32	26.6	
	51 to 60	65	54.2	
	Above 60	12	10	
Marital status	Married	104	86.7	
	Single	5	4.2	
	Widow(er)	11	9.2	
Household size	Less than 4	23	19.2	6
	5 to 8	86	71.7	
	Above 8	11	6.7	
Educational status	No formal education	48	40	
	Primary education	40	33.3	
	Secondary education	26	21.7	
	Tertiary education	6	5	
Cooperative association	Non-member	82	68.3	
	Member	38	31.6	
Major occupation	Farming	102	85	
	Artisan	8	6.7	
	Business	6	5	
	Civil servant	4	3.3	
Farming experience (years)	Less than 10	28	23.3	21
	11 to 20	34	28.3	
	21 to 30	27	22.5	
	Above 30	31	25.8	
Access to extension services	Yes	39	32.5	
	No	81	67.5	
Farm size (hectares)	Less than 2	103	85.8	1.8
	2 to 3	14	11.7	
	Above 3	3	2.5	
Access to credit	Yes	43	35.8	
	No	77	64.2	
Monthly income (₦)	< 20,000	45	17.5	26,333.3
	20,001 to 40,000	56	55	
	40,001 to 60,000	15	20.8	
	> 60,000	4	6.7	

Source: Field survey, 2020.

They had an average farm size of 1.8 hectares, which implies that they were smallholder farmers. Access to agricultural extension services (32.5%) was very low among the smallholder farmers. The low access to extension services could negatively affect their productivity as extension agents disseminate useful information to farmers. In the same vein, only 35.8% had access to credit. This could affect the level of their agricultural investments and could be the reason why they operate on a small scale. The smallholder farmers had an average monthly income of ₦26,333.3 (USD 63.99). However, this was low for a household with an average of six people. The low income was due to the impact of the COVID-19 pandemic on their production activities, as farmers stated during the field survey that the COVID-19 crisis negatively affected their income. A study conducted by UNDP (2020a) also reported that the income-generating capacity of farmers and food system agents was adversely affected due to the pandemic.

Food security status of rural farming households during the pandemic

Table 3 presents the results of the food security indices. The results show that the majority (64.5%) of the rural farming households were food insecure, while only 35.5% were food secure. The headcount ratio for food-insecure households was 0.645 and 0.355 for food-secure households, which implies that about two-thirds of the sampled population was food insecure. The daily per capita calorie intake of food-insecure and food-secure farming households was 1552.52 kcal and 2506.88 kcal, respectively. Thus, food-insecure households fell short of calorie requirements by 31.3 per cent while food-secure households had a surplus of calorie requirements by 10.9 per cent. The severity of food insecurity among food-insecure households was 0.098. These results imply that food insecurity is a serious challenge for rural households during the COVID-19 pandemic. This suggests that the COVID-19 pandemic has disrupted rural households' food consumption and increased rural farming households' food insecurity. This supports the opinion of FAO (2020a) that COVID-19 has led to an increase in hunger globally. It is worth noting that the pandemic, as a result of lockdown and movement restrictions, led to low household food availability, an increase in food prices, the inability of farmers to harvest their crops, and low crop output as their agricultural activities were disrupted. These consequently affected the food consumption of rural households.

Table 3. Results of rural farming household food security indices.

Food security indices	Food insecure	Food secure
Percentage of rural households	64.5	35.5
Headcount ratio	0.645	0.355
Per capita calories available per day	1552.52	2506.88
Squared food-insecure gap	0.098	
Shortfall/surplus index	0.313	0.109

Source: Field survey, 2020.

Effects of COVID-19 on the food security status of rural farming households

Table 4 presents the result of the logistic regression used to examine the effects of COVID-19 on food security measured by low household food availability, an increase in food prices, farmers' inability to harvest their crops, and low output due to the lockdown imposed by the government to contain the spread of COVID-19. The effects of some socioeconomic characteristics on farming household food security were also presented in Table 4.

Table 4. Effects of COVID-19 on the food security of rural farming households.

Variables	Coefficient	Std. Err.	Z	P>z	Marginal effects
Low food availability	-0.702352***	0.219017	-3.21	0.001	-0.1553
Increase in food price	-1.733186***	0.663286	-2.61	0.009	-0.4102
Inability to harvest crop	-0.929071*	0.541315	-1.72	0.086	-0.1998
Low output	-0.735977	0.508014	-1.45	0.147	-0.1627
Educational level	0.157944*	0.086652	1.82	0.068	0.0349
Major occupation	0.476884	0.759786	0.63	0.530	0.1054
Farming experience	-0.012473	0.022348	-0.56	0.577	-0.0028
Household size	-0.246732	0.363295	-0.68	0.497	-0.0545
Cooperative memberships	1.859568***	0.604476	3.08	0.002	0.4328
Income	0.094915**	0.039221	2.42	0.015	0.0214
Access to extension	-0.780762	0.554817	-1.41	0.159	-0.1726
Access to credit	1.382905***	0.506836	2.73	0.006	0.3057
Constant	-2.231246	1.715658	-1.30	0.193	
Pseudo R^2	0.2518				
LR chi^2	39.98				
Prob > chi^2	0.0000				
Log-likelihood	-59.39673				

Source: Field survey, 2020. *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$.

The coefficient of low household food availability had negative and significant ($p<0.01$) effects on the food security of rural farming households. A percentage decrease in food availability increases the likelihood of being food insecure by 15.53%. This implies that the households experiencing low food availability due to the pandemic were affected by food insecurity. This is because household food security depends on the availability of food. The pandemic led to a decrease in food availability in rural farming households, as farmers were unable to engage in their normal farming activities during the lockdown, especially in the first phase of the pandemic. This result corroborates the study by UNDP (2020b) that COVID-19 containment measures increased the number of people who experienced a deterioration in food security.

The coefficient of the increase in food prices was negative and significant ($p < 0.01$) in relation to the food security of farming households. A percentage increase in food prices decreases the likelihood of being food secure by 41.02%. Thus, high food prices due to the pandemic increased the likelihood of being food insecure among rural farming households. This supports the view of Devereux et al. (2020) and Egwue et al. (2020) that COVID-19 has increased food prices, which could consequently affect household food consumption. As food prices increased and household income decreased due to the pandemic, household purchasing power for food would decrease. This would reduce household food consumption and food security.

The movement restriction imposed by COVID-19, which prevents the farmers from harvesting their crops, had a negative and significant effect on the food security status of the farming households ($p < 0.1$). The result suggests that a percentage increase in farmers' inability to harvest their crops decreased the probability of being food secure by 19.98%. This implies that the imposed lockdown and movement restrictions, which inhibit farmers from accessing their farms to harvest their products, increased the likelihood of being food insecure. This is because rural smallholder households depend on their farm output to survive. Thus, any disruption to their farming activities would have a severe impact on their income, food security, and well-being. Therefore, the COVID-19 pandemic, as a result of the lockdown measures, has negatively affected the availability of food and food security of rural farming households. This is in line with the FAO (2021) statement that the pandemic has affected the agri-food systems, people's livelihoods, food security, and nutrition.

The level of education had a positive and significant effect on the food security status of the farming households ($p<0.1$). A percentage increase in the educational status of the household heads increased the probability of being food secure by 3.49%. This implies that households whose heads are educated are more likely to be food secure than households whose heads are not educated. This could be because education enhances the decision-making process and provides access to relevant information, which could likely increase the chance of being food secure. This corroborates the findings of Egwue et al. (2020) and Oyebanjo et al. (2013) that the level of education enhanced household food security.

The coefficient of cooperative memberships had a positive and significant effect on the food security status of farming households ($p<0.01$). A percentage increase in cooperative membership will increase the likelihood of being food secure by 43.28%. This implies that cooperative membership is an enhancer of rural food security. Thus, a farming household whose head is a member of a cooperative association is likely to be food secure, while those that do not belong to the cooperative society are likely to be food insecure. This could be a result of the benefits such as economies of scale, access to agricultural information, and credit derived from the society by its members. Mukaila et al. (2020) and Oyebanjo et al. (2013) also reported that members of cooperatives had a high probability of being food secure.

The income of smallholder farmers had a positive effect on the food security status of the farming household ($p< 0.05$). A percentage increase in the income of smallholder farmers increased the probability of their households being food secure by 2.14%. This implies that the income of household heads is a significant enhancing factor in household food security. A household whose head has a high income is likely to be food secure, while a household with a low income has a high chance of being food insecure. In the era of the pandemic, when food prices rise, higher-income households have enough money to buy food, while low-income households have little money to buy food items. The decline in income and the increase in food prices due to the pandemic make food insecurity a major concern among low-income earners (Aromolaran et al., 2020; FAO, 2020b). This supports Falola et al. (2023), Salau et al. (2019), and Omotesho et al. (2006), who found that income enhanced the food security of farming households.

Access to credit positively influenced the food security of farming households ($p< 0.01$). A percentage increase in access to credit increased the likelihood of being food secure by 30.57%. This implies that households whose heads can access credit are likely to be food secure, while those whose heads are unable to access credit are likely to be food insecure. This is because of the financial hardship and inability to get food items that are not produced by the farming households as a result of the COVID-19 lockdown which has lowered food availability in their households during the period, so borrowing money became one of the ways out. Therefore, farmers who could access credit used the money for household consumption to curb the effect of COVID-19 on their food security status. A similar result was reported by Frimpong and Asuming-Brempong (2013) and Ibrahim et al. (2016), indicating that access to credit positively affects food security.

Farming household food insecurity coping strategies during COVID-19

The food insecurity coping strategies adopted by farming households during the COVID-19 pandemic are shown in Table 5. The farming households were able to cope with the food insecurity situation during the pandemic by adopting a less expensive diet ($\bar{x}$ = 2.69). This strategy was ranked first among the coping strategies. It enabled them to procure more food to feed their households with the available money. However, this method could restrict them to consuming a particular food, which might not give them the required nutrients for a healthy life. The FAO (2020c) also reported that the COVID-19 crisis has led to the adoption of coping strategies such as eating cheap and less preferred foods by people. To cope with food insecurity during COVID-19, farming households reduced their rational consumption ($\bar{x}$ = 2.65). This was the second most important coping strategy adopted and used to enable them to manage the food available in their households. This is in line with the FAO (2020c) report that households reduced the quantity of food to cope with the crisis. Borrowing money to buy food when there is no food in the household was widely adopted by rural farming households ($\bar{x}$ = 2.58). Household heads took out loans for consumption purposes during the pandemic. Some household heads even diverted credit meant for production activities to feed their households, which could affect the next planting season.

Table 5. Farming household food insecurity coping strategies during COVID-19.

Coping strategies	Always Freq (%)	Occasionally Freq (%)	Never Freq (%)	Likert Mean	Rank
Eating less expensive food	86 (71.7)	31 (25.8)	3 (2.5)	2.69	1
Reducing rational consumption	89 (74.2)	20 (16.7)	11 (9.2)	2.65	2
Borrowing money to buy food	75 (62.5)	40 (33.3)	5 (4.2)	2.58	3
Engaging in additional small-scale productivity activities	55 (45.8)	45 (37.5)	20 (16.5)	2.29	4
Buying food on credit	60 (50)	32 (26.7)	28 (23.3)	2.27	6
Skipping meals within a day	53 (44.2)	33 (27.5)	34 (28.3)	2.16	5
Backyard livestock production	42 (35)	48 (40)	30 (25)	2.10	7
Allowing children to eat first	40 (33.3)	41 (34.2)	39 (32.5)	2.01	8
Mortgaging and selling domestic assets	26 (21.7)	30 (25)	64 (53.3)	1.68	9
Eating wild fruits	11 (9.2)	42 (35)	67 (55.8)	1.53	10

Source: Field survey, 2020.

Engaging in additional small-scale productivity activities ($\bar{x}$ = 2.29) by the households was also adopted as a coping strategy to curb the effect of COVID-19 on the food security of the farming households. Some of the households processed palm fruits into palm oil in their land to earn money. The money they earned was used for household consumption. In the middle of the lockdown, when the farmers were unable to visit their farms and their savings were exhausted, they switched to buying food on credit ($\bar{x}$ = 2.27). They purchased food items in their neighbourhood that were sold at a higher price. Some rural households were able to cope with the crisis using these strategies. Skipping meals within a day was also adopted by farming households to cope with the situation ($\bar{x}$ = 2.16). This was common among the adults in the households when they had little food in their households. FAO (2020c) also reported that households reduced the frequency of meals to cope with the COVID-19 crisis. Smallholder farmers who kept some livestock such as goats and poultry in their backyards sold them to cope with the food insecurity situation during the pandemic ($\bar{x}$ = 2.10). This helped them get some money to purchase food items for their household consumption. Some of the household heads and adults in the households always allowed the children to eat first to ensure that the children did not starve ($\bar{x}$ = 2.01). This was done to lower the chances of severe malnutrition among the children in the household. Mortgaging and selling domestic assets ($\bar{x}$ = 1.68) and eating wild fruits ($\bar{x}$ = 1.53) were considered less adopted coping strategies as their mean scores were below the Likert mean score of 2. Using all these coping strategies during the pandemic suggests that the COVID-19 crisis has severely affected rural food security. Meanwhile, some of the coping strategies are detrimental to their nutrition and health, which could result in food and nutrition deficiency diseases in rural households.

Conclusion

This study examined the food security status of rural farming households, the effects of COVID-19 on the food security of rural farming households, and how they coped with the menace of food insecurity during the pandemic. The study revealed that the majority of the farming households were affected by food insecurity and fell short of their calorie intake by 31.3% during the COVID-19 crisis. The pandemic, as a result of lockdown and movement restrictions, led to low availability of food in the households, an increase in food prices, low crop output, and the inability to harvest the crop, which consequently increased the likelihood of being food insecure in the farming households. Educational qualifications, cooperative memberships, income, and access to credit enhanced the probability of being food secure in rural households. The coping strategies adopted by the farming households during the food insecurity situation were reducing rational consumption, eating less expensive and less preferred food, borrowing money to buy food, allowing children to eat first, engaging in additional small-scale productivity activities, and buying food on credit. It can, therefore, be inferred from this study that the COVID-19 crisis has disrupted the food security of rural farming households.

To mitigate the adverse effects of COVID-19 on household food security, the study recommends that governments and non-governmental organisations provide support to farming households. This could take the form of palliatives for rural households that can be distributed by cooperative societies for effective distribution. Financial assistance in the form of grants or loans at a low and affordable interest rate spread over reasonable periods to ease repayment is also important to mitigate the effect of the pandemic on food availability and consumption. This would also help the farmers to have enough capital to boost their food production and, consequently, improve their food security status. Since the agricultural activities of farmers have been disrupted, it is necessary for governments and agencies to provide free or subsidised agricultural inputs to farmers. This would enhance their planting activities for the next season, which in turn would result in food availability in the country and increase farmers' earnings.

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UTICAJI KOVIDA-19 NA PREHRAMBENU SIGURNOST RURALNIH POLJOPRIVREDNIH DOMAĆINSTVA. ISKUSTVA IZ NIGERIJE

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R e z i m e

U poslednjih nekoliko decenija, prehrambena nesigurnost je postala glavni globalni fenomen, zbog čega je iskorenjivanje gladi uvršteno na drugo mesto Ciljeva održivog razvoja. Ipak, pojava COVID-19 predstavila je ozbiljnu pretnju prehrambenom sistemu. Stoga postoji potreba da se proceni njegov uticaj na prehrambenu sigurnost. S tim u vezi, ova studija je ispitala uticaje COVID-19 na prehrambenu sigurnost ruralnih poljoprivrednih domaćinstava u državi Enugu u Nigeriji. Podaci prikupljeni od 120 domaćinstava analizirani su korišćenjem deskriptivne statistike, indeksa prehrambene sigurnosti i logističke regresije. Rezultati su pokazali da je u većini domaćinstava (64,5%) prehrambena sigurnost ugrožena (indeks 0,313), dok se samo 35,5% može smatrati prehrambeno sigurnim (indeks 0,109). Prosečna dnevna potrošnja kalorija odraslih osoba u domaćinstvima koja su bila prehrambeno nesigurna odnosno prehrambeno sigurna iznosi 1552,52 odnosno 2506,88 kalorija, redom. Niska dostupnost hrane ($p<0{,}01$), povećanje cena hrane ($p<0{,}01$) i nemogućnost žetve ($p<0{,}1$) povećali su verovatnoću prehrambene nesigurnosti. Tako je pandemija COVID-19, zbog nametnutog karantina, uticala na prehrambenu sigurnost domaćinstava. Nasuprot tome, pristup kreditu ($p<0{,}01$), obrazovanje ($p<0{,}1$), članstvo u zadrugama ($p<0{,}01$) i prihod ($p<0{,}05$) pozitivno su uticali na status prehrambene sigurnosti. Smanjenje veličine obroka, konzumiranje jeftinije hrane, preskakanje obroka, pozajmljivanje novca za kupovinu hrane, omogućavanje deci da jedu prva i uključivanje u dodatne nisko produktivne aktivnosti bile su glavne strategije suočavanja sa prehrambenom nesigurnošću koje su domaćinstva primenjivala tokom pandemije COVID-19. Rezultati istraživanja ukazuju da je potrebno da vlada i nevladine ogranizacije obezbede poljoprivredne inpute i finansijsku podršku poljoprivrednicima kako bi se suzbili štetni uticaji COVID-19 na prehrambenu sigurnost.

Ključne reči: COVID-19, prehrambena nesigurnost, ruralna domaćinstva, mali poljoprivrednici, strategije suočavanja.

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VARIABILITY OF AGRONOMIC TRAITS IN VEGETABLE PEA (*PISUM SATIVUM* L.) GENOTYPES

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Abstract: In this study, a total of 12 vegetable pea genotypes of different growing seasons were subjected to phenotypic characterization. The vegetable pea genotypes are a part of the collection maintained at the Institute of Field and Vegetable Crops Novi Sad. The plant material included 10 promising lines and two released cultivars, Tamiš and Dunav. The trial was carried out in 2022. It was set up at the Rimski Šančevi site, as a randomized block design in five replications. A total of 14 agronomic traits were analyzed. The obtained research results revealed divergence in the investigated plant material. The statistical significance of all sources of variation was determined by the LSD test. The height of the first fertile node was found to be the most variable feature, with a coefficient of variation of 40.54%. The tested genotypes were clustered into two groups and two subgroups within the second group. The correlation analysis of the examined quantitative traits revealed the presence of several statistically significant positive and negative correlations. Some of the most significant positive correlations were established between the grain weight per plant and the number of grains per plant and the yield of technologically mature grain, while the pod width and the number of fertile nodes per plant had the most negative correlations with the other tested traits.

Key words: vegetable pea, phenotypic characterization, genotype, correlations, divergence.

Introduction

Vegetable pea (*Pisum sativum* L.) is an annual plant from the legume family (Dozet et al., 2018). Peas used in human nutrition are mainly grown for their grain, less often for their pods. In addition to the seasonal use of fresh grains, large quantities of grain are preserved by sterilization and freezing. The importance and the quantity of the processed pea products placed peas among the major vegetable crops in the food canning industry (Đorđević et al., 2021). Peas have long been

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consumed because of their nutritional value, namely their high content of protein, starch, fibre, minerals and vitamins (Castaldo et al., 2021). As a rich and affordable source of protein for human and livestock nutrition, peas are a strategically important commodity for global food security. In addition, when included in crop rotation, peas enable the fixation of atmospheric nitrogen and have a beneficial effect on soil physical properties (Pavan et al., 2022). One of the most important tasks in pea breeding is the development of high-yielding and stable pea varieties (Kumar et al., 2022). Different plant traits affect pea yield, which depends on both the specific genotype and environmental conditions (Đinović, 1986). When a new cultivar is developed, knowledge of effective yield traits saves time and labor, and improves the chances of success (Ceyhan and Avci, 2015). To this end, researchers have already studied various traits that affect the yield of a pea. Đinović (1986) lists the following as the most important traits that affect the yield of pea grains: number of pods per plant, number of grains per plant, number of grains per pod, absolute weight and grain yield. Timmerman-Vaughan et al. (2005) describe pea grain yield as the result of four components: number of plants per unit area, number of pods per plant, number of grains per pod and grain weight. Greveniotis et al. (2021) measure the following traits in their studies: grain yield (kg/ha), 1000-grain weight (g), number of pods per plant, number of grains per pod, pod length (cm), pod width (mm), number of branches per plant, and plant height (cm). These traits vary depending on the genotype and the agroecological conditions.

During the breeding process, breeders use diverse material (wild relatives, populations, lines and cultivars) that are expected to contain variability in different traits. However, for some of these genotypes to be used, they should be well described to give the researcher an insight into their breeding value. The description and knowledge of the genotypes is a prerequisite for their use (Kumar et al., 2018). According to Ton et al. (2022), the description of some traits of local genotypes is very important for pea breeding. The first step in the description and classification of the germplasm is morphological characterization (Smith and Smith, 1989). The aim of the research was to determine the correlation between the tested traits, to assess the relative contribution of the variability of the tested traits to the total variability of the studied pea genotypes, and ultimately to group the divergent genotypes and thereby facilitate breeding work.

Material and Methods

Field trial and plant material

The field trial was set up at the Rimski Šančevi site in 2022, on a chernozem-type soil in an irrigation system at the Department of Vegetable and Alternative Crops of the Institute of Field and Vegetable Crops Novi Sad (45°19'55.7"N

19°50'14.9" E and 86 m above sea level). The experiment was set up as a randomized block design with five replications. The main plot consisted of two rows of plants with a distance of 20 cm between the rows, 5 cm in a row and a length of 3 m. The distance between the two plots was 80 cm for easier manipulation and tillage between the rows during the growing season.

The sample for the analysis consisted of 10 plants per replication, i.e., a total of 50 plants per one genotype. The examined material is part of the collection of the species *Pisum sativum* L. of the Institute of Field and Vegetable Crops Novi Sad. The research included 12 genotypes consisting of 10 prospective lines and 2 domestic varieties (Tamiš and Dunav) of vegetable peas. The lines were named S-1 to S-10, and the Tamiš and Dunav lines were designated S-T and S-D. Spring vegetable pea lines, which differ significantly in terms of several morphological and quantitative characteristics, were selected based on earlier observation and the determination of certain parameters important in pea breeding.

The vegetation period length of the studied vegetable pea genotypes was: S1 – 65 days, S2 – 61 days, S3 – 60 days, S4 – 66 days, S5 – 65 days, S6 and S7 – 70 days, S8 – 72 days, S9 and S10 – 76 days, ST – 63 days and SD – 64 days.

The tested genotypes were harvested manually at the time of technological maturity. The degree of maturity was determined with a tenderometer, where the degree of grain hardness is expressed in tenderometric values (TV) (Jovićević et al., 2009; Červenski et al., 2021). According to the tests, a grain hardness of 100 to 180 TV was deemed acceptable (Đinović et al., 1984). The tenderometric values at harvest in the tested genotypes ranged from 100 to 135.

The traits were examined based on morphological descriptors for peas in the protocol for distinctness, uniformity and stability tests UPOV. Quantitative traits were not assessed according to the UPOV protocol, but were measured and expressed according to the International System of Units (SI). The studied agronomic traits included: stem length (cm) and height of the first fertile node (cm) – measured using a meter; number of nodes per plant, number of fertile nodes per plant, number of pods per plant, number of pods per stem; pod length (mm) and pod width (mm) – measured with a ruler; number of grains per pod, number of grains per plant; grain weight per pod (g) and grain weight per plant (g) – measured using a precise analytical scale; yield of technologically mature grain (kg/ha) – determined based on the total grain weight obtained from each plot for each genotype; vegetation period from sprouting to technologically mature grain (expressed as number of days).

During the trial, cultivation practices were carried out to control weeds, diseases and harmful insects. Sprinkler irrigation (a system of sprinklers) was used three times during the growing season with a watering rate of 35 mm. Watering peas is necessary at localities with poor distribution of precipitation, as well as in extremely dry years. The time and rate of watering are determined as needed. At low soil moisture, crops are irrigated at germination and during the initial stages of plant development with a smaller amount of water. The irrigation norm of 10 mm is sufficient for wetting the top soil layer, connecting with winter moisture reserves and preventing the formation of soil crust. On average, 1–2 irrigations during flowering, fertilization and pod formation, with a watering rate of 30–40 mm can significantly increase the yield (the number of pods and grains), as well as improve the quality of pea seeds (Červenski et al., 2021).

Agrometeorological conditions

The meteorological conditions at the trial locality and during the trial period were represented by the following meteorological parameters: minimum and maximum monthly air temperature (°C), average monthly air temperature (°C) and ten-year average precipitation sum (mm). The values of the analyzed parameters were compared with the corresponding values of the multi-year average for the reference period from 1964 to 2014. The number of days during the growing season with maximum temperatures over 25°C stands out as an important parameter in vegetable pea production. The meteorological parameters for the examined locality were provided by the Republic Hydrometeorological Service of Serbia.

Tables 1, 2 and 3 show the precipitation deficit compared to the multi-year average during the entire vegetation period. Average temperatures during March were lower compared to the multi-year average, but slightly higher in mid-May and June with maximum temperatures above 25°C, which can have a negative effect on plant development during and shortly after flowering.

Table 1. Mean values of maximum, minimum and average air temperatures (°C) per 10-day periods at the investigated location during the growing season.

	Maximum (°C)			Minimum (°C)			Mean (°C)			Multiannual average (°C)		
Month	Period											
	I	II	III	I	II	III	I	II	III	I	II	III
February	18.0	18.4	18.1	-3.3	-3.8	-1.7	5.8	7.7	6.2	1.3	1.6	2.6
March	9.2	16.6	23.3	-4.5	-9.4	-6.3	2.5	4.3	10.3	4.3	6.2	8.8
April	22.3	24.8	23.2	-2.5	-1.4	1.6	10.4	9.3	13.1	10.8	10.8	13.5
May	26.0	31.4	32.3	8.5	6.0	11.7	17.2	19.6	19.7	15.8	17.2	17.9
June	33.0	33.7	36.2	12.8	12.4	15.3	23.2	22.0	24.9	19.2	20.0	20.9

Table 2. Precipitation sum (mm) per 10-day period at the investigated location during the growing season.

Month	Period			Sum	Multiannual sum
	I	II	III		
February	8	5	16	29	34.2
March	1	0	0	1	38.8
April	18	2	17	37	47.5
May	0	3	17	20	64.6
June	8	23	12	43	87.7

Table 3. Number of days with maximum temperatures above 25 °C.

	Period		
Month	I	II	III
February	0	0	0
March	0	0	0
April	0	0	0
May	1	9	6
June	10	10	8

Statistical data analysis

In view of the main statistical indicators for the analyzed traits, the following values were calculated: minimum and maximum values, mean value, standard error of the arithmetic mean, and coefficient of variation (%).

The least significant difference test (LSD) was conducted for all traits, at the significance threshold of 0.05 and 0.01.

The Pearson's correlation coefficient was calculated to determine the mutual dependence of the examined quantitative traits. The correlations between traits based on the intensity of the Pearson's coefficient values were divided according to Evans (1996):

r = 0.00–0.19 (very low);
r = 0.20–0.39 (low);
r = 0.40–0.59 (medium);
r = 0.60–0.79 (high);
r = 0.80–1.00 (very high).

Cluster analysis was conducted to group the genotypes based on similarities and differences in the tested traits. Cluster analysis is a multivariate method that allows the determination of clustering in a data set.

The obtained research results were statistically processed using the Statistica program version 14.0.1.25.

Results and Discussion

The basic statistical indicators and mean values of the examined traits are shown in Tables 4 and 5. The statistical significance of all sources of variation at both levels of significance (0.05 and 0.01) was determined by the LSD test for the examined agronomic traits of the studied genotypes.

Table 4. Main statistical indicators of the examined agronomic traits of the studied vegetable pea genotypes.

Trait	Minimum	Maximum	Mean ± Se	CV (%)
SL	51.42	84.42	60.4±2.92	16.76
HFFN	19.24	65.24	33.38±3.91	40.54
NNPP	9.66	17.1	13.09±0.67	17.79
NFNPP	4.94	6.7	5.81±0.17	10.4
NPPP	5.94	10.66	8.84±0.4	15.65
NPPS	1.19	1.89	1.54±0.07	14.89
PL	58.23	82.46	67.48±2.13	10.95
PW	11.7	14.13	12.7±0.18	4.87
NGPPo	5.82	8.3	7.05±0.19	9.34
GWPPo	1.6	3.34	2.55±0.15	20.62
NGPP	36.04	63.22	51.75±2.51	16.79
GWPP	9.3	21.8	17.63±1.28	25.09
YTM	3627.78	8502.78	6848.4±501.8	25.38
LVP	60	76	67.33±1.56	8.04

SL – stem length (cm); HFFN – height of the first fertile node (cm); NNPP – number of nodes per plant; NFNPP – number of fertile nodes per plant; NPPP – number of pods per plant; NPPS – number of pods per stem; PL – pod length (mm); PW – pod width (mm); NGPPo – number of grains per pod; GWPPo – grain weight per pod (g); NGPP – number of grains per plant; GWPP – grain weight per plant (g); YTM – yield of technologically mature grain (kg/ha); LVP – vegetation period from sprouting to technologically mature grain.

The height of the first fertile node (HFFN) was found to be the most variable trait with a coefficient of variation of 40.54%, ranging from 19.24 cm in genotype S-T to 65.24 cm in genotype S-7. According to the LSD test, no mutually significant difference was found between genotype S-T and genotypes S-1 and S-2, while being significantly different compared to the other examined genotypes. Significant variability for this trait was also noted by Ton et al. (2022). Literature sources state that the average values in vegetable peas for the HFFN range from 9.22 to 74.5 cm (Singh and Dhall, 2018; Kalapchieva and Yankova, 2019; Ton et al., 2022). The yield of technologically mature grain (YTM) varied significantly, with a coefficient of variation of 25.38% and an interval of variation from 3627.78 kg/ha (S-T) to 8502.78 kg/ha (S-9). Compared to the other tested genotypes, a significantly higher YTM was found in genotypes S-1, S-5, S-6, S-7, S-8, S-9 and S-10, while a significantly lower yield was achieved by genotypes S-3 and S-T. On average, the YTM was 6848.4 kg/ha. In the studies of other authors, the reported average values of technologically mature vegetable pea grain yield ranged from 1100 to 17900 kg/ha (Đorđević et al., 2001; Uher et al., 2009; Stanimirović et al., 2011; Červenski et al., 2016; Kanižai Šarić et al., 2016; Kumar et al., 2017; Dozet et al., 2018; Arunadevi et al., 2022). Plant productivity is determined by multiple factors that directly affect each other, so yield variability can be high and difficult to predict (Woźniak, 2013). A high coefficient of variation (25.09%) was determined for grain weight per plant (GWPP), with a variation interval of 9.3 g (S-T) to 21.8 g (S-9).

Table 5. Mean and LSD values of the examined agronomic traits of the studied pea genotypes.

Genotype	SL	HFFN	NNPP	NFNPP	NPPP	NPPS	PL	PW	NGPPo	GWPPo	NGPP	GWPP	YTM
S-1	54.12	21.26	10.6	5.94	7.9	1.31	71.56	13.32	6.88	3.34	43.6	20.50	7850.7
S-2	51.42	20.26	11.72	6.7	8.06	1.20	58.23	11.70	5.82	1.60	46.24	12.53	4814.94
S-3	54.98	26.98	9.66	5.02	5.94	1.19	64.03	14.13	6.07	1.79	36.04	10.40	3978.78
S-4	63.98	31.08	13.6	6.2	10.66	1.75	59.4	12.26	6.74	2.46	59.04	18.18	7024.68
S-5	61.16	26.02	11.94	5.78	9.74	1.7	67.94	13.14	7.04	2.72	54.42	19.59	7582.38
S-6	73.34	51	14.66	5.4	9.08	1.79	66.48	12.42	7.4	2.88	55.42	20.07	7947.42
S-7	84.42	65.24	17.1	4.94	9.2	1.89	62.88	12.6	7.2	2.83	54.64	21.23	8257.86
S-8	56.82	33.3	15.26	6.46	10.58	1.65	63.1	12.68	7.26	2.56	63.22	21.55	8402.94
S-9	66.06	40.24	16.06	6.7	10.16	1.53	79.8	12.94	7.58	2.86	60.58	21.80	8502.78
S-10	55.02	36.26	13.7	5.4	9.02	1.66	82.46	12.36	8.3	2.87	59.86	19.41	7568.34
S-T	51.44	19.24	11.42	5.7	7.54	1.33	65.72	12.46	7.42	1.89	41.9	9.30	3627.78
S-D	52.22	29.64	11.36	5.48	8.24	1.51	68.18	12.42	6.94	2.82	46.08	16.98	6622.2
Mean	60.42	33.38	13.09	5.81	8.84	1.54	67.48	12.70	7.05	2.55	51.75	17.63	6848.4
$LSD_{0,01}$	3.295	3.175	0.782	0.754	1.301	0.130	3.177	0.520	0.551	0.375	8.248	2.720	1061.06
$LSD_{0,05}$	2.504	2.413	0.594	0.573	0.989	0.099	2.415	0.395	0.419	0.285	6.268	2.067	806.40

SL – stem length (cm); HFFN – height of the first fertile node (cm); NNPP – number of nodes per plant; NFNPP – number of fertile nodes per plant; NPPP – number of pods per plant; NPPS – number of pods per stem; PL – pod length (mm); PW – pod width (mm); NGPPo – number of grains per pod; GWPPo – grain weight per pod (g); NGPP – number of grains per plant; GWPP – grain weight per plant (g); YTM – yield of technologically mature grain (kg/ha).

On the other hand, S-1, S-6, S-7, S-8 and S-9 had significantly higher GWPP compared to the other genotypes, but not compared to each other, while S-T and S-3 had significantly lower GWPP. The average GWPP was 17.63 g. According to the sources, the average grain weight per plant ranges from 1.52 to 118.4 grams (Ghafoor et al., 2005; Ceyhan et al., 2008; Siddika et al., 2013; Kumari et al., 2015; Barcchiya et al., 2018; Singh and Dhall, 2018; Kalapchieva and Yankova, 2019; Kumar et al., 2022; Singh and Prakash, 2022; Ton et al., 2022). The lowest grain weight per pod (GWPPo) of 1.6 g was measured in genotype S-2, while the highest

GWPPo of 3.34 g was found in genotype S-1. According to the LSD test, S-2, S-3 and S-T had significantly lower GWPPo compared to the other genotypes, while the S-1 genotype showed significantly higher GWPPo compared to the other genotypes. The mean value for this trait was 2.55 g, with a coefficient of variation of 20.62%. According to the literature, the average value of GWPPo ranged from 1.52 to 48.22 grams (Ghafoor et al., 2005; Ceyhan et al., 2008; Siddika et al., 2013; Kumari et al., 2015; Kalapchieva and Yankova, 2019; Kumar et al., 2022; Ton et al., 2022; Singh and Prakash, 2022).

The trait with the lowest value of the coefficient of variation (4.87%) was the pod width (PW), considering the narrow range from 11.7 mm in genotype S-2 to 14.13 mm in genotype S-3. A similar coefficient of variation for PW was also obtained by Singh and Dhall (2018). The PW in different studies ranged from 9.3 to 23.7 mm (El-Hak et al., 2012; Siddika et al., 2013; Afreen et al., 2017; Singh et al., 2017; Kumar et al., 2017; Singh and Dhall, 2018; Bardisi and Zyada, 2021).

The variability of different agronomic traits in plant material is an important source and factor in breeding work.

From the aspect of the indirect selection of traits correlated with the main breeding objectives and for a simultaneous selection of multiple traits, the assessment of correlation coefficients is important for plant breeders (Radinović et al., 2022). Correlation coefficients shown in Table 6 make it possible to identify direct connections between the examined agronomic traits of vegetable pea genotypes.

Table 6. The Pearson's correlation coefficients of the examined agronomic traits of the studied vegetable pea genotypes.

	NFFN	NNPP	NFNPP	NPPP	NPPS	PL	PW	NGPP	GWPP	NGPP	GWPP	YTM
SL	0.79**	0.68**	0.07	0.35**	0.46**	0.02	0.03	0.15**	0.21**	0.37**	0.47**	0.47**
HFFN		0.66**	-0.26**	0.07**	0.46**	0.07	-0.08	0.20**	0.24**	0.15**	0.30**	0.30**
NNPP			0.27**	0.46**	0.40**	0.10*	-0.13**	0.28**	0.16**	0.50**	0.50**	0.50**
NFNPP				0.73**	-0.12**	-0.01	-0.04	-0.01	-0.06	0.63**	0.46**	0.46**
NPPP					0.56**	0.02	-0.08*	0.12**	0.07	0.85**	0.71**	0.71**
NPPS						0.02	-0.11**	0.20**	0.17**	0.48**	0.49**	0.49**
PL							0.30**	0.52**	0.41**	0.15**	0.26**	0.26**
PW								0.03	0.09*	-0.05	0.03	0.03
NGPP									0.41**	0.32**	0.32**	0.32**
GWPP										0.16**	0.44**	0.44**
NGPP											0.83**	0.83**
GWPP												1.00**

SL – stem length (cm); HFFN – height of the first fertile node (cm); NNPP – number of nodes per plant; NFNPP – number of fertile nodes per plant; NPPP – number of pods per plant; NPPS – number of pods per stem; PL – pod length (mm); PW – pod width (mm); NGPPo – number of grains per pod; GWPPo – grain weight per pod (g); NGPP – number of grains per plant; GWPP – grain weight per plant (g); YTM – yield of technologically mature grain (kg/ha).

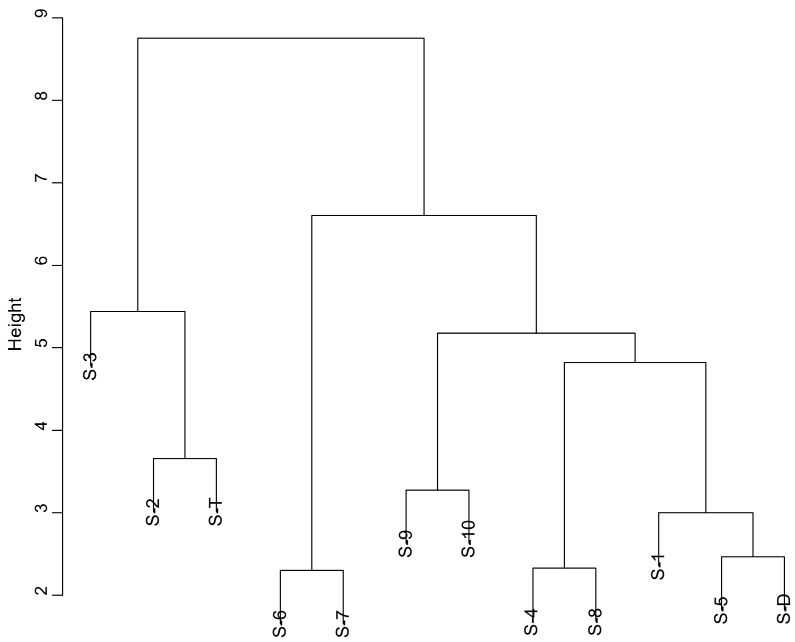
Based on the intensity of the obtained Pearson's coefficient values, at both levels of significance (0.05 and 0.01), a very strong positive correlation was established between the following traits: GWPP and YTM (1.00), number of pods per plant (NPPP) and the number of grains per plant (NGPP) (0.85), the NGPP and the GWPP (0.83), the NGPP and the YTM (0.83). Khan et al. (2017) also noted a high positive correlation between the NGPP and the GWPP. Contrary to our results, Khan et al. (2017) found that the stem length (SL) had a highly significant negative correlation with pod length (PL) and a negative correlation with the NGPPo. This research suggests that grain yield could be improved through the selection of genotypes with high GWPP and high NGPP.

A strong positive correlation was established between: SL and the HFFN (0.79), the number of fertile nodes per plant (NFNPP) and the NPPP (0.73), the NPPP and the GWPP (0.71), the NPPP and the YTM (0.71), the SL and the number of nodes per plant (NNPP) (0.68), the HFFN and the NNPP (0.66), the NFNPP and the NGPP (0.63). Similar to the results of our research, Dozet et al. (2011) found that the SL had a highly significant positive correlation with the HFFN. In the research conducted by Arunadevi et al. (2022), the NPPP showed a highly significant positive correlation with the YTM, which is in agreement with the results of this research. However, Arunadevi et al. (2022) also reported a highly significant positive correlation between the NPPP and the number of grains per pod (NGPPo), and a significant positive correlation between the NPPP and the PL, which is not in accordance with our results.

Panwar et al. (2018) stated that the NPPP had a negative correlation with the NGPPo. The most significant negative correlations in our research (with the height of the reciprocal ratio >-0.3) at both levels of significance (0.05 and 0.01) were found between the following traits: the HFFN and the NFNPP (-0.26), the NNPP and the PW (-0.13), the NFNPP and the number of pods per stem (NPPS) (-0.12), the NPPS and the PW (-0.11). The negative correlation between the NPPP and the PW (-0.08) was only significant at the 0.05% significance level. Both PW and the NFNPP exhibited the most negative correlations with the tested vegetable pea traits.

The distribution of genotypes by clusters and the mean values of the investigated agronomic traits for each cluster are presented in Graph 1. Using the complete linkage method, the vegetable pea genotypes were clustered into two groups and two subgroups, while the similarity between genotypes was determined based on Euclidean distances.

The first group (I), which consisted of genotypes S-2, S-3 and S-T, was characterized by the smallest NGPPo with 6.44 grains, the smallest GWPPo with 1.76 g, the smallest NGPP which was 41.39 on average, the smallest GWPP with 10.74 g, and the lowest YTM, which was 4140.5 kg/ha in group I. This group was also characterized by the lowest average SL of 52.61 cm, HFFN of 22.16 cm, NNPP of 10.93 nodes, NPPS of 1.24 pods, and PL of 62.66 mm. All three genotypes in group I were characterized by the shortest vegetation period (LVP) compared to the other examined genotypes, with an average vegetation period of 63.33 days.



Graph 1. Dendrogram of the tested vegetable pea genotypes for the testedagronomic traits.

In the second group (II), two genotypes were distinguished (S-6 and S-7), which separately formed subgroup IIa. What distinguishes these two genotypes from the others at first glance is precisely their "main" characteristic of the SL, which averaged 78.88 cm in this subgroup. The genotypes of this subgroup were characterized by the highest values for the HFFN, which was 58.12 cm, the NNPP with 15.88 nodes, the NPPS of 1.8 pods, the NGPPo with 7.3 grains, the GWPPo of 2.86 g, and the GWPP which was 20.65 g.

Subgroup IIb was characterized by the highest NFNPP with 5.99 fertile nodes, the highest NPPP with 9.47 pods, the greatest PL of 70.35 mm, and the highest NGPP with 55.26 grains. Although the average YTM in subgroup IIb was lower compared to subgroup IIa, genotypes S-8 and S-9 from subgroup IIb achieved the highest average YTM in the trial with 8402.94 kg/ha and 8502.78 kg/ha, respectively.

Grouping peas using multivariate techniques can provide breeders with considerable advantages, as many researchers have applied these techniques to group vegetable pea genotypes based on different traits (Singh et al., 2017; Hanci, 2019; Mohamed et al., 2019). Clustering genotypes into the groups based on similarities and differences between the examined traits will help breeders to select parental pairs for future crosses to breed for different traits.

Conclusion

The obtained research results indicate the presence of divergence with a medium level of phenotypic variability in the investigated plant material. The statistical significance of all sources of variation was determined by the LSD test for the examined agronomic traits. The Pearson's correlation matrix indicates that traits such as the grain weight per plant and the number of grains per plant can be used as selection criteria for the development of new high-yielding pea varieties. On the other hand, pod width and the sum of fertile nodes per plant had the most negative correlations with the tested pea traits, which directly indicates of the possibility to obtain weaker or lower values in the selection based on these traits. The examined genotypes were clustered into two groups based on similarities and differences between the examined traits, with the second group divided into two subgroups. The formation of homogeneous groups based on a combination of the examined agronomic traits will help breeders to select parental pairs for future crosses to breed for different traits.

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VARIJABILNOST AGRONOMSKIH SVOJSTAVA KOD GENOTIPOVA POVRTARSKOG GRAŠKA (*PISUM SATIVUM* L.)

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R e z i m e

U toku ovog istraživanja izvršena je fenotipska karakterizacija 12 genotipova povrtarskog graška, različite dužine vegetacije, koji pripadaju kolekciji Instituta za ratarstvo i povrtarstvo u Novom Sadu. Biljni materijal obuhvatao je 10 perspektivnih selekcionih linija i dve priznate sorte *tamiš* i *dunav*. Ogled je izveden tokom 2022. godine, postavljen je po slučajnom blok sistemu, u pet ponavljanja na lokalitetu Rimski Šančevi. Analizirano je 14 agronomskih osobina. Dobijeni rezultati istraživanja ukazuju na postojanje divergentnosti u ispitivanom biljnom materijalu. Analizom varijanse ispitivanih agronomskih osobina utvrđena je statistička značajnost svih izvora varijacije. Kao najvarijabilnija osobina istakla se visina prvog rodnog nodusa sa koeficijentom varijacije od 40,54%. Ispitivani genotipovi su primenom klaster analize grupisani u dve grupe sa dve podgrupe u okviru druge grupe. Korelacionom analizom ispitivanih kvantitativnih svojstava utvrđeno je postojanje nekoliko statistički značajnih pozitivnih i negativnih međuzavisnosti. Neke od najznačajnijih pozitivnih korelacija utvrđene su između osobina: masa zrna po biljci i prinos tehnološki zrelog zrna, kao i broja zrna po biljci i prinosa tehnološki zrelog zrna, dok svojstva širina mahune i suma rodnih nodusa po biljci imaju najviše negativnih korelacija sa ostalim ispitivanim osobinama.

Ključne reči: povrtarski grašak, fenotipizacija, genotip, korelacije, divergentnost.

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AN UPDATE ON APPLE CHLOROTIC LEAF SPOT VIRUS STATUS OF SWEET CHERRY IN BULGARIA

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Abstract: The sweet cherry (*Prunus avium* L.) is one of the most important stone fruit species in Bulgaria. The cherry is susceptible to many viruses. To gain a better insight into the phyto-virologic status of sweet cherry, a survey for the presence of apple chlorotic leaf spot virus (ACLSV), cherry leafroll virus (CLRVR) and raspberry ringspot virus (RpRSV), in addition to ilarviruses, was carried out in 32 commercial and 6 collection orchards in ten regions of Bulgaria between 2017 and 2022. A total of 1503 sweet cherry samples were collected from symptomatic and symptomless trees. These samples were tested by cocktail ELISA for ACLSV and DAS-ELISA for CLRVR, RpRSV and additionally for prune dwarf virus (PDV) and prunus necrotic ringspot virus (PNRSV) to detect mixed infections. Of the three viruses studied, only ACLSV was detected in commercial and collection sweet cherry orchards in all regions with intensive cherry cultivation in Bulgaria, in addition to ilarviruses. The extent of ACLSV virus infection was 8.8%. Single ACLSV infection was the most common, affecting 79.6% of infected sweet cherry trees, followed by ACLSV and PDV (14.4%) and least frequently by ACLSV and PNRSV (6.0%). The presence of ACLSV in orchards of different ages, including 'young' orchards, indicates that virus-free propagation material must be used when establishing new orchards to prevent the spread of pathogens with the planting material.

Key words: sweet cherry, ELISA, detection, viruses, ACLSV.

Introduction

The sweet cherry (*Prunus avium* L.) is one of the main fruit crops in Bulgaria in terms of cultivated area (12 649 ha in 2021) and production (52 615 t in 2021) (MA, 2022). The sweet cherry is mainly cultivated in southern Bulgaria.

The cherry is susceptible to many virus-associated diseases. More than 30 viruses have been reported, most of which belong to the *Ilarvirus, Potyvirus,* and *Trichovirus* genera (Myrta and Savino, 2008).

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Apple chlorotic leaf spot virus (ACLSV) of the genus *Trichovirus*, together with similar viruses – prune dwarf virus (PDV) and prunus necrotic ringspot virus (PNRSV), is one of the most common viruses in cherries (Mandic et al., 2007; Sánchez et al., 2015; Deltedesco et al., 2022). Generally, ACLSV-infected trees are symptomless, but incompatibility of scion-rootstock combinations in nurseries, deformation and discoloration, and necrosis of fruit in susceptible sweet cherry cultivars have been reported (Desvignes and Boye, 1989; Rana et al., 2011; Liu et al., 2014; Sánchez et al., 2015).

Cherries can also be infected by viruses belonging to the genus *Nepovirus.* Cherry leafroll virus (CLRV) and raspberry ringspot virus (RpRSV) are some of these nematode-borne viruses (Martelli and Uyemoto, 2011).

CLRV alone causes a slow decline in sweet cherries, but in mixed infections with either PDV or PNRSV, symptoms become much more severe and tree decline is accelerated (Eastwell and Howell, 2010; Lutes and Pscheidt, 2018). The symptoms induced by RpRSV-ch on sweet cherries can also be very severe, even leading to the death of the tree (Wetzel and Krczal, 2007).

In recent years, extensive studies have been conducted on the distribution of ilarviruses on sweet and sour cherries in Bulgaria (Kamenova et al., 2020; Borisova et al., 2021), while data on the spread of other viruses on cherries are insufficient and only available for individual regions and cultivars.

To gain a better insight into the sanitary status of sweet cherries, outside the ilarviruses, a survey was carried out for the presence of ACLSV, CLRVR, and RpRSV in commercial and collection orchards in different regions of Bulgaria.

Material and Methods

Sampling procedure and visual evaluation of virus symptoms

The surveys were carried out from April and May 2017 to May and June 2022 in several provinces, mainly in southern Bulgaria. Field inspections were conducted and samples were collected from thirty-two commercial and six collection sweet cherry orchards (four at the Institute of Agriculture (IA), Kyustendil, one at the Agriculture school, Kyustendil, and one at the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA), Troyan) (Table 1). Twelve individual home-growing sweet cherry trees from the Kyustendil region were also included. A total of 1503 sweet cherry samples were collected from symptomatic and symptomless trees. Each tree in the field and each sample in the laboratory were examined for symptoms. All samples (petals and leaves) were taken randomly from different branches at different heights around each sampled tree.

Serological tests – enzyme-linked immunosorbent assay (ELISA)

The samples were labeled and placed in isolated transparent bags, transported to the virology laboratory, and maintained at 4°C. All samples were tested for the presence of five viruses (ACLSV, CLRVR, RpRSV, PNRSV, and PDV) within a few days after sampling using ELISA. Two ELISA variants were performed – for the identification of CLRV and RpRSV, the double antibody sandwich variant (Clark and Adams, 1977) was applied and for the detection of ACLSV, the test was carried out according to a modified procedure named cocktail ELISA developed by Flegg and Clark (1979). At the same time, the samples were also analyzed by DAS-ELISA for the presence of PNRSV and PDV viruses to detect mixed infections. The commercial diagnostics sets manufactured by Loewe Phytodiagnostica GmbH were applied, using the protocol recommended by the producer. Samples showing an optical density (OD) three times the average of the negative controls were considered as positive.

Results and Discussion

Surveys and symptoms in the field

The orchards for field observation and sample collection were selected in some of the regions with the most intensive sweet cherry cultivation in Bulgaria, such as Kyustendil, Sliven, Plovdiv, Stara Zagora, and Burgas. To represent the different types of orchards, old (over 50 years old) and abandoned plantations (Kyustendil and Kazanlak), newly established ones (1 to 5 years old in Blagoevdrad, Burgas and Sliven), and middle-aged (10–15 years old in Plovdiv, Burgas, Pazardzhik, Sliven, and Kyustendil) were included.

The sweet cherry trees in each orchard were initially monitored by visual observation for the development of virus-like symptoms. Most of the trees in the surveyed orchards did not show any symptoms that could suggest virus infections and samples were collected randomly. However, there were some cases in which typical symptoms of stone fruit viruses, such as chlorotic diffuse rings or spots, interveinal chlorosis, deformations, and wrinkling on the leaves were sporadically observed in some sweet cherry orchards surveyed. Chlorotic and necrotic spots and lines on the leaves of trees of the "Bing" cultivar were noted in an orchard in Kazanlak. Chlorosis, necrotic spots, deformation, and wrinkling on the leaves of trees of the "Kozerska", "Lambert", "Van" cultivars were seen in orchards in the Aytos, Kyustendil, and Kazanlak regions. These symptoms were found in a serologically proven mix of ACLSV + PDV or ACLSV+ PNRSV infected trees (Figure 1 B, C). Most of the single-infected ACLSV trees were symptomless. These results confirm reports of natural infections of stone fruit trees by ACLSV as a latent virus (Nemeth, 1986; Sutic et al., 1999). Only in one case, ACLSV-infected trees showed symptoms in the form of rings and spots without necrosis around them on individual leaves of trees of the cultivar “Bigarreau Burlat” in the orchard of Gabarevo/ Kazanlak (Figure 1 A).

A B C

Figure 1. Symptoms on the leaves of sweet cherry trees. A/rings and spots on the leaf of the tree cultivar “Bigarreau Burlat” infected with ACLSV; B/chlorotic and necrotic spots and lines on the leaf of the tree cultivar “Bing” infected with ACLSV + PDV; C/chlorosis, deformation and wrinkling on the leaf of the tree cultivar “Kozerska” infected with ACLSV + PNRSV.

Most of the visual monitoring was carried out in April and May of 2017–2022, which did not allow the observation of symptoms on ripe cherry fruit. In our previous work, necrosis was found on sweet cherry fruit on trees infected with ACLSV (Borisova and Christov, 2014).

Serological detection

A total of 1503 samples of sweet cherry trees from ten different districts in Bulgaria were serologically analyzed for the presence of ACLSV, CLRV, and RpRSV, except for the presence of ilarviruses. The results of the ELISA tests showed that the samples tested were infected only with ACLSV in single or in mixed infection with ilarviruses. The presence of CLRV and RpRSV was not detected in any of the samples tested (Table 1).

The ELISA analysis revealed that 132 of 1503 tested samples were infected. The total infection rate was 8.8%, that with ACLSV was 7.0% and that with ACLSV+PDV/or PNRSV was 1.8% (Table 1).

Table 1. Extent of single and mixed infections with ACLSV in sweet cherry in the surveyed regions of Bulgaria.

Region/District	Orchard location	Samples		Positive for:	
		Number tested	Number infected (%)	ACLSV	ACLSV + PNRSV/or PDV
Southeastern/ Sliven	5 commercial orchards	166	17 (10.2)	17	0
Southeastern/ Burgas	4 commercial orchards (town Aytos)	160	9 (5.6)	3	5 (with PNRSV) 1 (with PDV)
Southeastern/ Burgas	4 commercial orchards (town Pomorie)	96	2 (2.1)	2	0
South-central/ Plovdiv	4 commercial orchards	132	12 (9.1)	11	1 (with PDV)
South-central/ Pazardzhik	1 commercial orchard (town Septemvri)	46	8 (17.4)	8	0
South-central/ Stara Zagora	2 commercial orchards (town Maglish)	81	1(1.2)	1	0
South-central/ Stara Zagora	3 commercial orchards (town Kazanlak)	135	34 (25.2)	30	3 (with PNRSV) 1 (with PDV)
Western/ Kyustendil	4 collection orchards, IA-Kyustendil	340	14 (4.1)	13	1 (with PDV)
Western/ Kyustendil	1 collection orchard, Agriculture School	35	2 (5.7)	2	0
Western/ Kyustendil	4 commercial orchards, single trees	103	14 (13.6)	9	5 (with PDV)
Western/ Dupnica	1 commercial orchard (town Bobov dol)	48	0	0	0
Western/ Pernik	1 commercial orchard (town Radomir)	47	17 (36.2)	7	10 (with PDV)
Southwestern/ Blagoevgrad	3 commercial orchards	103	2 (1.9)	2	0
North-central/ Lovech	1 collection orchard, RIMSA-Troyan	11	0	0	0
Total		1503	132 (8.8)	105 (7.0)	27 (1.8)

The difference in ACLSV incidence among the orchards ranged from 1.2% to 36.2%. The highest infection rate of 36.2% (14.9% of single and 21.3% of mixed infection with PDV) was recorded in the “old” sweet cherry orchard located in Radomir, Pernik district, followed by the infection rate of 25.2% (22.2% of single infection, 2.2% of mixed infection with PNRSV, and 0.8% with PDV) found in the orchards in Kazanlak, Stara Zagora district. The lowest infection in sweet cherry trees (1.2% and 1.9%) was noted in “young” orchards in Maglish, Stara Zagora, and in orchards of different ages in Blagoevgrad, respectively. All tested sweet cherry trees from the collection orchards at RIMSA-Troyan and the commercial orchard in Bobov dol, Dupnica were free from the tested viruses. No differences in virus prevalence were found for the different age groups of the investigated orchards.

According to the preliminary data, 13.3% of the sweet cherry in the Kyustendil region of Bulgaria was infected with ACLSV, and 33.4% of the tested trees were found to be infected with ACLSV and PDV or PNRSV (Borisova, 2005). In sweet cherry orchards in Spain, the infection with this virus was 16.0% (Sánchez et al., 2015) and 14.0% in the Mediterranean region (Myrta and Savino, 2008). A lower level of infection (only 4.0%) with this pathogen was found in Serbia (Mandic et al., 2007). Similar results were obtained in the study of sweet cherry cultivars in Ukraine – 3.6% (Pavlikuk et al., 2021).

ACLSV was very often detected in mixed infections with ilarviruses, PDV or PNRSV, in sweet cherry (Ulubaş, 2008; Sánchez et al., 2015; Deltedesco et al., 2022). ACLSV and PDV were the viruses that occurred most frequently in combination, accounting for 14.4% of infected sweet cherry trees, followed by ACLSV and PNRSV (6.0%) in our study. A low infection rate for the PNRSV-ACLSV combination in cherry trees was also reported by Mandic et al. (2007) and Çevik et al. (2011) in Serbia and Turkey, respectively.

None of the tested samples were positive for CLRV and RpRSV and these results are in agreement with the reported absence of these viruses in stone fruit species in the Kyustendil region in Bulgaria (Borisova et al., 2013). However, in a study on sap-transmissible viruses in sweet cherries, Milusheva and Zivondov (2009) reported that CLRV was the most prevalent virus, identified in 31.9% of the trees tested, followed by RpRSV – 26.0% in the Plovdiv region. In another study, the most widespread virus was also CLRV, identified in single or mixed infection in 56.4% of the cherry samples tested in the same region (Milusheva et al., 2014). In our study, no trees infected with CLRV or RpRSV were found, although the Plovdiv region was included. Different rates of CLRV infection have been reported in different regions and countries. According to Tryapitsyna and Vasiuta (2010), 21.63% of sweet cherry orchards in Ukraine were infected with CLRV, while Pavlikuk et al. (2021) reported the presence of the pathogen in 0.7% of sweet cherries. In Romania, CLRSV was not detected in the investigated sweet cherry orchards (Zagrai et al., 2022).

Conclusion

In the present study, of the viruses tested in all regions with intensive cherry cultivation in Bulgaria, only ACLSV was detected in commercial and collection sweet cherry orchards in addition to ilarviruses. The extent of ACLSV virus infection in 1503 sweet cherry trees tested was 8.8%. Single infection with ACLSV was most common, accounting for 79.6% of infected sweet cherry trees, followed by ACLSV and PDV (14.4%) and least by ACLSV and PNRSV (6.0%). The presence of ACLSV in orchards of different ages, including "young" orchards, indicates that virus-free propagation material must be used when establishing new orchards to prevent the spread of pathogens with planting material.

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AŽURIRANJE STATUSA VIRUSA HLOROTIČNE LISNE PEGAVOSTI JABUKE NA TREŠNJI U BUGARSKOJ

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R e z i m e

Trešnja (*Prunus avium* L.) je jedna od najvažnijih vrsta koštičavog voća u Bugarskoj. Trešnja je domaćin brojnih virusa. U cilju dobijanja boljeg uvida u fitovirusni status trešnje, u periodu 2017.-2022. godina sprovedeno je istraživanje prisustva virusa hlorotične lisne pegavosti jabuke (engl. *apple chlorotic leaf spot virus* – ACLSV), virusa uvijanja lista trešnje (engl. *cherry leafroll virus* – CLRVR) i virusa prstenaste pegavosti maline (engl. *raspberry ringspot virus* – RpRSV), pored ilarvirusa, u 32 komercijalna voćnjaka i 6 kolekcionih zasada trešnje u deset regiona Bugarske. Prikupljeno je ukupno 1503 uzorka trešnje sa stabala sa i bez simptoma. Uzorci su testirani primenom koktel ELISA za ACLSV i DAS-ELISA metode za CLRVR, RpRSV, kao i za virus kržljavosti šljive (engl. *prune dwarf virus* – PDV) i virus nekrotične prstenaste pegavosti koštičavih voćaka (engl. *prunus necrotic ringspot virus* – PNRSV) u cilju detekcije mešanih infekcija. Pored ilarvirusa, od druga tri proučavana virusa, samo je ACLSV uočen u komercijalnim voćnjacima i kolekcionim ili matičnim zasadima trešnje u svim regionima sa intenzivnim uzgojem trešanja u Bugarskoj. Zastupljenost virusa hlorotične lisne pegavosti jabuke bila je 8,8%. Pojedinačna infekcija virusom hlorotične lisne pegavosti jabuke bila je najzastupljenija, detektovana u 79,6% zaraženih stabala trešnje, a zatim slede ACLSV i PDV (14,4%) i ACLSV i PNRSV (6,0%). Prisustvo virusa hlorotične lisne pegavosti jabuke u voćnjacima različite starosti, uključujući i „mlade” zasade, ukazuje na to da se mora koristiti bezvirusni sadni materijal prilikom zasnivanja novih voćnjaka, kako bi se sprečilo širenje patogena sadnim materijalom.

Ključne reči: trešnja, ELISA, detekcija, virusi, ACLSV.

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The full name and address of the institution where the author is employed should be provided. It should be centred and written immediately after the author's name. If authors belong to different institutions, the numerals should be placed as the superscript before the name of institution to provide information on the institution where each of the stated authors is employed.

Abstract
The abstract is a short informative review of the content of the paper, which should enable the reader to estimate its relevance easily and accurately. It is in the interests of the author that the abstract contains terms used for indexing and browsing purposes. The references should not be given in the abstract. The abstract should include the aim of research, the methods, the results and the conclusion. It should contain between 200 and 250 words and be placed between the name of the authors' affiliations and key words. The title of the abstract should be bolded and indented pressing the tab key. The colon should be used after the title of the abstract, and then the text of the abstract should follow without any indentation.

Key words
Key words are terms or phrases which describe best the content of the article for the needs of indexing and browsing purposes. The number of key words should be 3 to 10. They should appear below the abstract. The title of key words should be bolded and indented by pressing the tab key. The colon should be used after the title, and then the list of key words in lower-case letters should be given with the full stop at the end. Key words should be provided in Serbian and English after abstract on both languages.

Introduction
The introduction should contain all the relevant information on past researches according to the stated problem and what can be achieved by further research. Reviewing the references, the author and the year should be provided, and the mentioned author should be cited in References. The title of the introduction should be centred and bolded, written in lower-case letters, below which using one line spacing, the text of the introduction should follow, justified. Each new paragraph should be indented pressing the tab key. These rules should be applied to all parts of the paper.

Material and Methods
The material and methods should be clearly outlined explaining all applied procedures in the paper. Generally known methods should be presented briefly, and a detailed explanation should be given if there is a deviation from previously published procedures. Papers, which have an experimental character, should provide the way of statistical data processing. This part, as well as the part Results and Discussion, if needed, may comprise certain subparts, too.

Results and Discussion
In the part Results and Discussion data obtained on the basis of observation and conducted experiments should be interpreted. In the comment of the results, references should be quoted at the end of the paper, providing the comparison between the obtained results and previous knowledge of the certain area.

Conclusion
All relevant items achieved in the researched area should be mentioned in the conclusion. Listing of all results with repetition of numbers previously specified in Results and Discussion should be avoided. Conclusion should not contain references.

Acknowledgements
Acknowledgements should contain the title and the number of the project that is the title of the program within which the paper was written, as well as the name of the institution which financed the project or program. It should be placed between the conclusion and references.

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The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

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The examples of listing references are the following:

Periodicals

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.

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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.

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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

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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.

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

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

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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.

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

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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.

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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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Primeri navođenja referenci su sledeći:

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

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

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

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

Izveštaj

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

Veb sajt

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

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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 uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

Nomenklatura

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Formule

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