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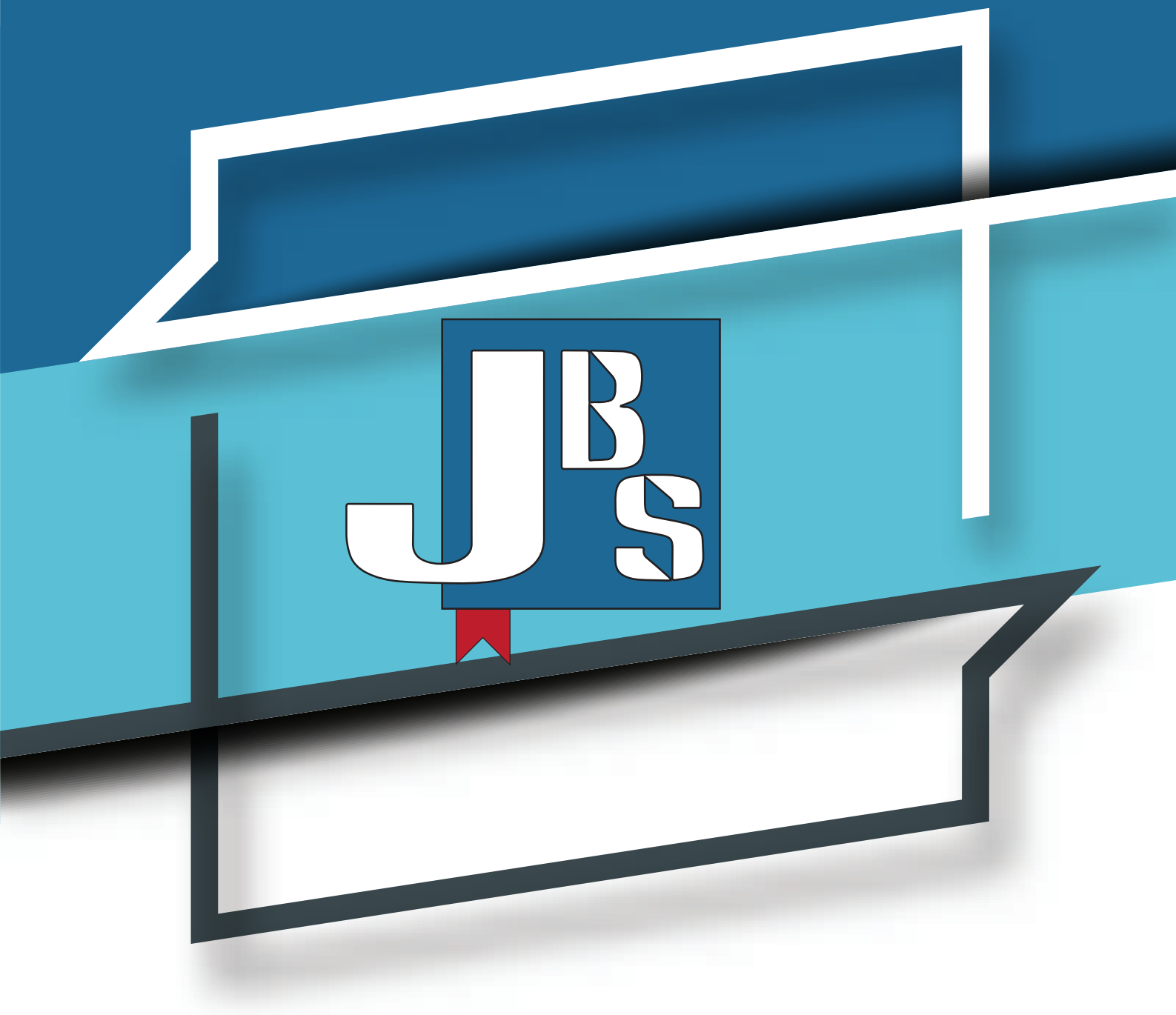

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CONTENTS

RESEARCH ARTICLE
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Causal relationships among the body-related and egg-related traits in crayfish: A case study on Turkish freshwater crayfish *Pontastacus leptodactylus* (Astacidae: Decapoda)

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Abstract

In this study, the direct and indirect causal relationships among the length, weight, egg diameter, egg weight, and egg quantity of female *Pontastacus leptodactylus* were analyzed using a path analysis. A total of 79 egg-bearing female crayfish with a total weight (WT; 39.1 ± 16 g) and total length (TL; 109.6 ± 18.1 mm), were sampled from Eğirdir Lake, Türkiye, in 2022 and 2023. Significant direct effects were observed several traits, such as crayfish length and weight, weight and egg diameter, length and egg quantity, egg diameter and egg quantity, weight and egg quantity, length and egg weight, and egg quantity and egg weight (p<0.05). These relationships indicate that larger females tend to produce a higher number of eggs and have greater overall reproductive potential. In contrast, no significant relationships were found between length and egg diameter, weight and egg weight, and egg diameter and egg weight (p>0.05), suggesting that body size may not directly influence egg size in this species. Regarding indirect effects, crayfish length was found to significantly influence egg weight through egg quantity (p<0.05). This suggests that larger females indirectly affect egg weight by producing more eggs. Although the direct effect of egg diameter on egg weight was not significant (p>0.05), its indirect effect of egg diameter on egg weight through egg quantity was significant (p<0.05), indicating that egg diameter plays a role in reproductive output through its influence on egg numbers. The findings of this study contribute to a better understanding of the direct and indirect causal relationships between growth-related traits and egg-related traits in freshwater crayfish. These insights can be utilized in sustainable fisheries management and aquaculture practices by informing selective breeding programs to enhance reproductive success or guiding conservation strategies that protect larger females in natural populations.

Keywords: Freshwater crayfish, Fecundity, Path analysis, Modelling, Morphometry

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1. Introduction

The number and size of eggs produced by crustaceans vary depending on factors such as reproductive period, temperature, photoperiod, age, nutrition, body size, body weight, molting, and claw loss (Pinheiro et al., 2003; Veríssimo et al., 2011; Mazlum & Eversole, 2005; Czerniejewski & de Giosa, 2013; Sheppard et al., 2024). Although egg productivity is generally proportional to body size (Hamasaki & Kawai, 2023), variations in egg size can also affect the number of eggs. For example, in crayfish, as in fish, larger females typically produce larger eggs (Mason, 1977). Penaeid shrimp can lay between 200,000 and 1,000,000 eggs, freshwater shrimp (*Macrobrachium rosenbergii*) can produce between 20,000 and 80,000 eggs, and lobsters (*Homarus* spp.) can produce between 5,000 and 80,000 eggs. Freshwater crayfish produce various numbers of eggs under natural and controlled conditions. For example, the cambarid crayfish species *Procambarus acutus acutus* produces 106-556 eggs (Eversole & Mazlum, 2002), *Procambarus zonangulus* produces 189-764 eggs (Eversole & Mazlum,

2002; Mazlum, 2003), *Procambarus clarkii* produces 100-700 eggs (Mazlum & Eversole, 2004), and *Orconectus* sp. produces approximately 500 eggs (Graczyk et al., 2019). Among astacid crayfish, *Austropotamobius pallipes* produces 20 eggs (Sáez-Royuela et al., 2006), while *Pontastacus leptodactylus* produces about 137-400 eggs (Köksal, 1988; Berber et al., 2006; Berber & Mazlum, 2009; Berber et al., 2011; Boyalik & Berber, 2020). Among parastacid crayfish, *Cherax quadricarinatus* can produce 150-600 eggs (Yeh & Rouse, 1994), *Cherax destructor* can produce 124-960 eggs, and *Cherax tenuimanus* can produce 200-600 eggs (Austin, 1998), reflecting the influence of reproductive season, temperature, age, and nutritional conditions.

The narrow-clawed crayfish, *Pontastacus leptodactylus*, is Türkiye's only native crayfish species, with increasing demand due to its high economic value and export potential (Mazlum et al., 2021; Alvanou et al., 2024). P. *leptodactylus*, whose reproductive cycle consists of specific stages (Mazlum & Yılmaz, 2006), mates in October-November when the water temperature is 7-12°C, and spawning occurs 4-6 weeks later when the temperature is 6-11°C. Therefore, the egg incubation period continues throughout winter and spring. *P. leptodactylus* can carry its eggs for 5-6 months in temperate climates and 6-7 months or more in colder climates. Under natural conditions, the development period of *P. leptodactylus* eggs can last 150-210 days or more (Aydın & Dilek, 2004; Köksal, 1988), and they reproduce only once a year (Köksal, 1988).

Egg productivity in crayfish is expressed in two ways: "Ovarian-eggs" represent the reproductive potential of crayfish, while "Pleopod-eggs" represent actual reproduction (Corey, 1987; Eversole & Mazlum, 2002; Eversole et al., 2002; Mazlum & Eversole, 2004; Berber & Mazlum, 2009). Pleopod-eggs, which indicate eggs laid by the crayfish, are influenced by many factors under natural conditions, resulting in significant variations in egg numbers among crayfish species (Corey, 1987; Eversole et al., 2002).

It is known that crayfish morphological characteristics are influenced by both genetic and environmental factors (Li et al., 2024). Therefore, individuals of the same species may exhibit phenotypic diversity depending on changing environmental conditions (Li et al., 2024). Morphometric relationships provide information on growth and reproductive potential characteristics of individuals in relation to environmental conditions (Hossain et al., 2019; Berber et al., 2020; Gören & Karayücel, 2022; Garabaghi et al., 2022; Benzer & Benzer, 2022; Gültepe et al., 2024; Boyalık et al., 2023; Alvanou et al., 2024; Roljić et al., 2024). For example, claws, an important morphometric structure used for survival, show variability rates of 38.67 and 66.74 in female and male individuals, respectively (Mazlum et al., 2007; Li et al., 2024). Additionally, larger claws in crayfish are advantageous during agonistic encounters, particularly for males during the mating season. In females, larger claws are beneficial for defending their eggs or rejecting unwanted male suitors during the breeding period (Bovbjerg, 1956; Buřič et al., 2010).

It is known that the relationship between morphometry and egg productivity varies depending on the species and habitats of crayfish (Hossain et al., 2019). This study aims to elucidate the causal relationships between body-related and egg-related traits of the *P. leptodactylus* population in Türkiye to support sustainable fishing policies, and aquaculture practices.

2. Material and Methods

2.1. Sampling

Seventy-nine egg-bearing female crayfish were collected from Eğirdir Lake, Türkiye, on May 9, 2022, and May 15, 2023, using fyke nets with a 16 mm mesh size. The crayfish had an average weight (WT) of 39.1 ± 16 g and a total length (TL) of 109.6 ± 18.1 mm. The crayfish were transported live and on ice in a styrofoam box by bus to the Faculty of Marine Sciences and Technology at Iskenderun Technical University. Measurements were conducted at the Marine Sciences Application and Research Unit.

2.2. Measurements and statistical analysis

It has been reported that crayfish length affects crayfish weight, egg diameter, egg number, and egg weight; crayfish weight affects egg diameter, egg weight, and egg number; egg diameter affects egg number and egg weight; and egg number affects egg weight (Berber, 2005; Hubenova et al., 2002; Eversole & Mazlum, 2002). Therefore, measurements of length, weight, egg diameter, egg weight, and egg number for the sampled female crayfish individuals were used in the study. The total length (TL) of female crayfish was measured to the nearest millimeter, and wet weight (WW) was measured to the nearest 0.01 g. Pleopod eggs were removed from each crayfish using watch markers forceps and counted. Egg subsamples (n=75) were removed from each ovigerous female, and egg diameters were measured with a dissection microscope equipped with a calibrated ocular micrometer. Egg weight was calculated from the mean weight of 75 eggs, weighed using a digital scale (0.0001 g) after wiping the eggs with a paper towel.

In the initial evaluation of these variables, significant correlations were found between the variables, supporting previous literature (Figure 1). Therefore, considering these relationships between variables, a path model was tested to evaluate both direct and indirect effects together (Figure 2).

Since the variables had different scales of measurement (Table 1), each variable was standardized using Z-transformation prior to performing the path analysis.

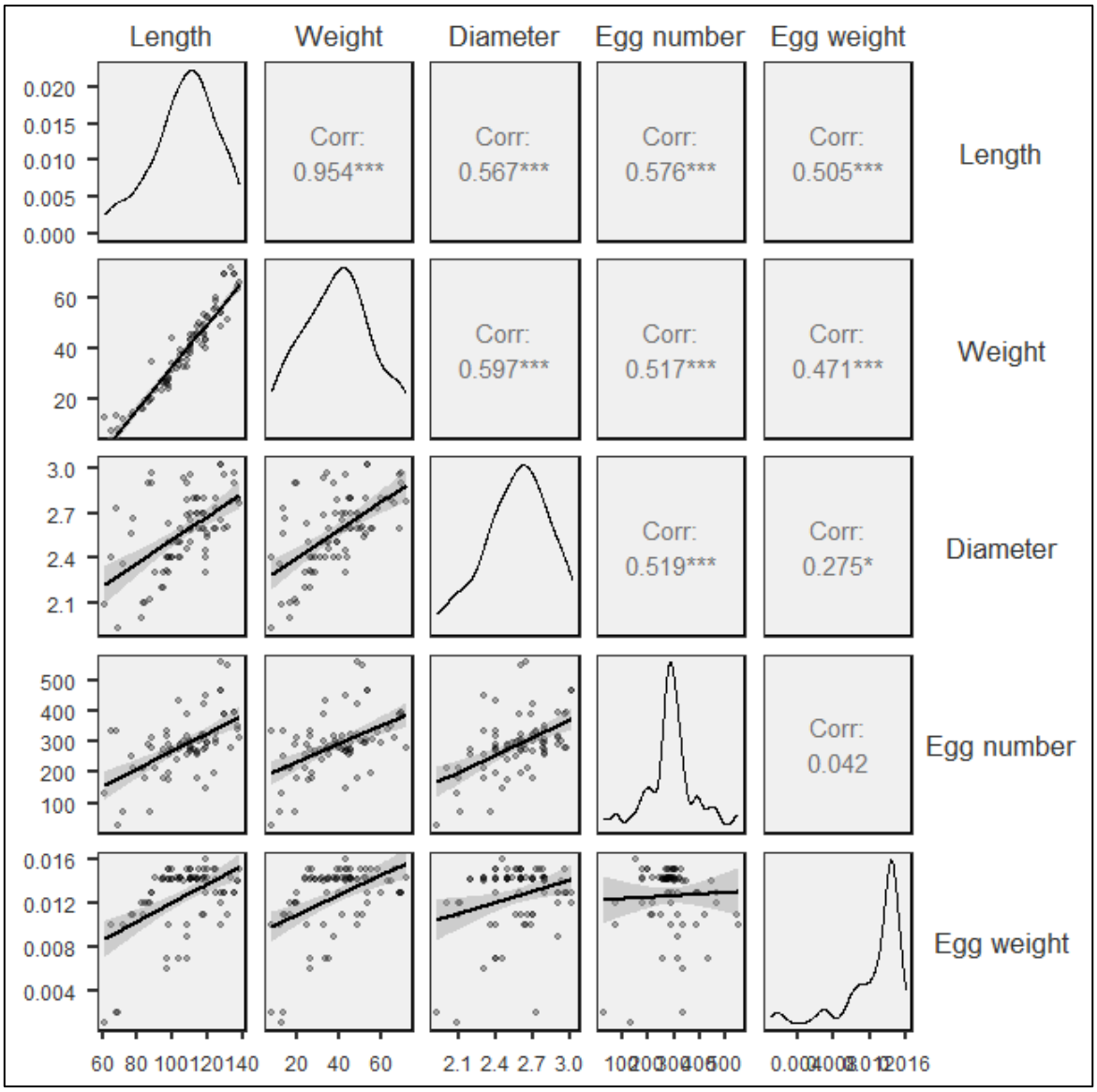

Figure 1. Linear relationships, correlations, and distributions of variables

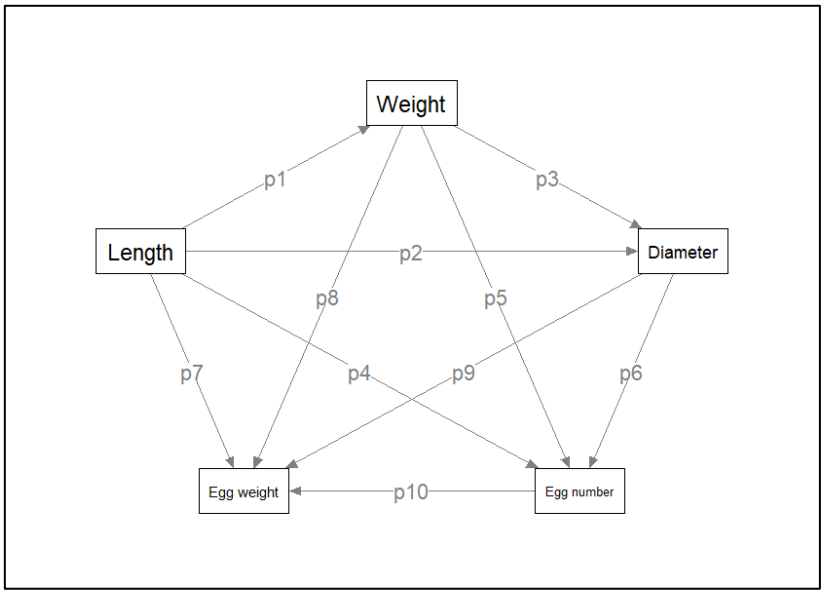

Figure 2. Path coefficients (parameters) in the tested conceptual model

Table 1. Descriptive statistics for the sampled 79 individuals

	Length (mm)	Weight (g)	Diameter (mm)	Egg number	Egg weight (mg)
N	79	79	79	79	79
Mean	107	39.1	2.58	290	0.0127
Median	110	39.7	2.60	289	0.0142
Standard deviation	18.1	16.0	0.250	90.8	0.00309
Minimum	61	7.70	1.93	27.0	0.00100
Maximum	138	71.9	3.02	556	0.0160

Path analysis provides an estimate of the significance and importance levels of hypothetical cause-effect relationships among a set of variables. Therefore, this analysis method is preferred in determining the direct, indirect, and total effects in causality among variables, especially when indirect relationships are significant (Rutherford & Choe, 1993; Webley, 1997; Sarwono, 2007).

In this study, five estimation methods were considered: "Maximum Likelihood," "Generalized Least Squares (GLS)," "Weighted Least Squares (WLS)," "Diagonally Weighted Least Squares (DWLS)," and "Unweighted Least Squares (ULS)." Among these, the Weighted Least Squares (WLS) method provided the best results in estimating the path analysis parameters.

As seen in the conceptual model (Figure 2), the hypotheses to be tested in the study in terms of direct and indirect effects are summarized as follows.

Direct Effects:
• The direct effect of body length on body weight (p1), egg diameter (p2), egg number (p4), and egg weight (p7).
• The direct effect of body weight on egg diameter (p3), egg number (p5), and egg weight (p8).
• The direct effect of egg diameter on egg number (p6) and egg weight (p9).
• The direct effect of egg number on egg weight (p10).

Indirect Effects:
The 11 hypotheses to be tested in terms of indirect effects are given in Table 2.

Statistical analyses were performed using the JAMOVI program, which utilizes the R ecosystem (R Core Team, 2022; The Jamovi Project, 2023).

Table 2. Hypotheses tested for indirect effects using path analysis

Hypothesis	Path	Parameter
Body length has an indirect effect on egg weight through body weight, egg diameter, and egg number.	Length ⇒ Weight ⇒ Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p1*p3*p6*p10
Body length has an indirect effect on egg weight through body weight and egg diameter.	Length ⇒ Weight ⇒ Egg Diameter ⇒ Egg Weight	p1*p3*p9
Body length has an indirect effect on egg weight through body weight and egg number.	Length ⇒ Weight ⇒ Egg Number ⇒ Egg Weight	p1*p5*p10
Body length has an indirect effect on egg weight through body weight.	Length ⇒ Weight ⇒ Egg Weight	p1*p8
Body length has an indirect effect on egg weight through egg diameter and egg number.	Length ⇒ Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p2*p6*p10
Body length has an indirect effect on egg weight through egg diameter.	Length ⇒ Egg Diameter ⇒ Egg Weight	p2*p9
Body length has an indirect effect on egg weight through egg number.	Length ⇒ Egg Number ⇒ Egg Weight	p4*p10
Body weight has an indirect effect on egg weight through egg diameter and egg number.	Weight ⇒ Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p3*p6*p10
Body weight has an indirect effect on egg weight through egg diameter.	Weight ⇒ Egg Diameter ⇒ Egg Weight	p3*p9
Body weight has an indirect effect on egg weight through egg number.	Weight ⇒ Egg Number ⇒ Egg Weight	p5*p10
Egg diameter has an indirect effect on egg weight through egg number.	Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p6*p10

3. Results

It was determined that the conceptual model tested was significant as a whole (Chi-square = 303, df = 10, $p<0.001$) and that all endogenous variables in the model were also significant for the model ($p<0.05$) (Table 3). Accordingly, the most important exogenous variables in the model were, in order, weight, number of eggs, egg weight, and egg diameter. Results for direct and indirect effects are provided in Figure 3, Table 4, and Table 5.

Table 4 shows that the relationships between length-weight (β=0.95), weight-egg diameter (β=0.63), length-number of eggs (β=0.94), egg diameter-number of eggs (β=0.33), weight-number of eggs (β=-0.57), length-egg weight (β=1.04), and number of eggs-egg weight (β=-0.42) are significant ($p<0.05$), while the relationships between length-egg diameter (β=-0.04), weight-egg weight (β=-0.38), and egg diameter-egg weight (β=0.14) were not significant ($p>0.05$).

From the tested path model, it is observed that the dependent variable for all variables was egg weight. Among the independent variables with respect to egg weight, it is seen that the variable with the greatest impact on egg weight was the length of the organism. An increase of one unit in the organism's length increases the egg weight by 1.0378 units, while a one-unit increase in the number of eggs decreases the egg weight by -0.4228 units (Table 4). It was interesting that the effects of organism weight and egg diameter on egg weight were not significant ($p>0.05$).

Regarding indirect effects (Table 5), the effect of crayfish length on egg weight through the number of eggs was significant (p4*p10, $p<0.05$). Although the effect of egg diameter on egg weight was not significant ($p>0.05$, Table 4), the effect of egg diameter on egg weight through the number of eggs was significant (p6*p10, $p<0.05$).

Table 3. Some statistics and significance levels for the dependent variables

Variable	R^2	95% Confidence Intervals		Wald X^2	df	p
		Lower	Upper			
Weight	0.910	0.862	0.942	797.6	1	< 0.001
Egg Diameter	0.353	0.184	0.519	43.1	2	< 0.001
Egg Number	0.414	0.242	0.573	55.8	3	< 0.001
Egg Weight	0.368	0.198	0.532	46.0	4	< 0.001

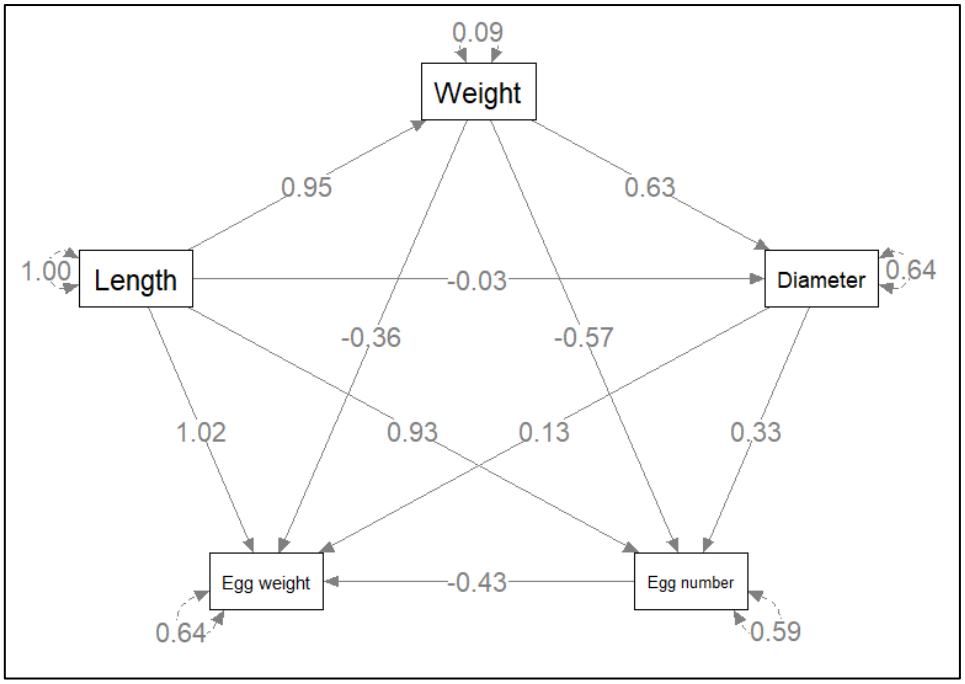

Figure 3. Regression coefficients (β) for causal relationships between variables (direct effects) and variance estimates for the variables

Table 4. Path analysis results for direct effects

Dependent	Independent	Estimate	SE	Z	*p*
Weight	Length	0.9539	0.0338	28.242	< 0.001
Egg Diameter	Length	-0.0358	0.3015	-0.119	0.906
Egg Diameter	Weight	0.6280	0.3015	2.083	0.037
Egg Number	Length	0.9364	0.2869	3.263	0.001
Egg Number	Weight	-0.5713	0.2947	-1.939	0.053
Egg Number	Egg Diameter	0.3283	0.1071	3.066	0.002
Egg Weight	Length	1.0378	0.3175	3.269	0.001
Egg Weight	Weight	-0.3812	0.3133	-1.217	0.224
Egg Weight	Egg Diameter	0.1432	0.1176	1.218	0.223
Egg Weight	Egg Number	-0.4228	0.1169	-3.618	< 0.001

Table 5. Path analysis results for indirect effects

Path	Parameter	Estimate	SE	Z	*p*
Length ⇒ Weight ⇒ Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p1*p3*p6*p10	-0.083	0.054	-1.553	0.120
Length ⇒ Weight ⇒ Egg Diameter ⇒ Egg Weight	p1*p3*p9	0.086	0.082	1.050	0.294
Length ⇒ Weight ⇒ Egg Number ⇒ Egg Weight	p1*p5*p10	0.230	0.135	1.706	0.088
Length ⇒ Weight ⇒ Egg Weight	p1*p8	-0.364	0.299	-1.216	0.224
Length ⇒ Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p2*p6*p10	0.005	0.042	0.118	0.906
Length ⇒ Egg Diameter ⇒ Egg Weight	p2*p9	-0.005	0.043	-0.118	0.906
Length ⇒ Egg Number ⇒ Egg Weight	p4*p10	-0.396	0.163	-2.423	0.015
Weight ⇒ Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p3*p6*p10	-0.087	0.056	-1.556	0.120
Weight ⇒ Egg Diameter ⇒ Egg Weight	p3*p9	0.090	0.086	1.051	0.293
Weight ⇒ Egg Number ⇒ Egg Weight	p5*p10	0.242	0.141	1.709	0.087
Egg Diameter ⇒ Egg Number ⇒ Egg Weight	p6*p10	-0.139	0.059	-2.339	0.019

4. Discussion

Despite the negative correlations predicted between live weight and both egg weight and egg number, these relationships were not statistically significant (p>0.05). Similar results have been reported in previous studies, where a negative correlation between total egg number and average egg weight was observed (Berber & Mazlum, 2009; Gören et al., 2019; Cilbiz, 2020). It has been reported that females of the same size might show differences in developmental stages due to slowing or stopping growth rate at later ontogenetic stages, which could affect egg number (Longshaw & Stebbing, 2016; Hamasaki et al., 2022). However, a significant positive correlation was found between live weight and egg diameter (p<0.05), highlighting that egg diameter (size) affects both the condition and survival rates of the offspring (Huner, & Lindqvist, 2020). The relationship between egg size and female weight reflects the effects of energy allocation (Eversole & Mazlum, 2002; Bernardo, 1996). Larger eggs contain more yolk and have longer incubation period, as larger females tend to invest more energy into reproduction rather than growth (Mazlum & Eversole, 2004; Mazlum & Eversole, 2005; Rey et al., 2017). Conversely, no significant relationship was found between body size and egg diameter (p>0.05). A study by Mazlum & Eversole (2004) on *Procambarus clarkii* reported that smaller females had lower fecundity (200 eggs/female) compared to larger females (700 eggs/female) with a positive linear correlation between egg number and total length. This differs from our findings, as we observed significant positive relationships between size and both egg number and egg weight (p<0.05). As body size increases, both individual egg weight and total egg number rise. However, despite a positive relationship between the body size and weight, the relationships between egg diameter, egg number, and egg weight differ (Table 4). This likely reflects significant variability in live weight among organisms of the same age (Berber & Mazlum, 2009), suggesting that body size is a more

reliable criterion than weight in breeding, cultivation, and stock management studies. Genetic structure and biotic/abiotic factors also play a role (Skurdal et al., 2011), as crayfish in warmer climates reach sexual maturity earlier than those in colder climates. Genetic structure leads to greater egg production in larger and older crayfish compared to younger ones. A positive relationship between crayfish size and egg number has been reported in many crayfish species (Yeh & Rouse, 1994; Reynolds, 2002; Cilbiz, 2020). In our study, we found a significant positive correlation between egg diameter and egg number (p<0.05); however, egg diameter had no significant effect on egg weight(p>0.05). Notably, as egg number increased, individual egg weight decreased significantly (p<0.05), suggesting a trade-off between the number of eggs and the resources allocated to each egg (Table 4). This negative relationship likely reflects energy limitations, where a greater number of eggs results in smaller energy reserves for each individual egg.

In terms of indirect effects, the effect of crayfish size on egg weight through egg number (p4*p10: -0.396) was found to be significant (p<0.05) (Table 5). The direct effect of crayfish size on egg weight was also significant and positive (1.0378), while the direct effect of egg number on egg weight was significant but negative (-0.4228). Considering the coefficients of direct effects, a one-unit increase in crayfish size leads to a 1.0378-unit increase in egg weight, while a one-unit increase in egg number reduces egg weight by 0.4228 units. When considering the coefficient of the indirect effect (p4*p10: -0.396), it is observed that egg number has a greater impact on egg weight than crayfish size itself. This suggests that an increase in the number of eggs leads to smaller individual egg weights due to energy trade-offs. It is known that genetic structure and biotic and abiotic factors in the ecosystem also affect these relationships (Cilbiz, 2021; Skurdal et al., 2011). A positive relationship between crayfish size and egg number has been reported in many crayfish species (Reynolds, 2002; Cilbiz, 2020).

Regarding egg productivity, previous studies have shown that *P. leptodactylus* produces different numbers of eggs across different populations. For example, the average egg production per female is reported as 211 eggs in Lake Eğirdir (Köksal, 1988), 240 eggs in Lake İznik (Aydın et al., 2015), and 276 eggs on the northern coast of the Caspian Sea (Kolmykov, 2001). In the Divzak Lake population in Poland, egg production ranges from 210 to 410 eggs, while in the Mazurian Lake population, the average is 374 eggs (Stypinskaya, 1978). Similarly, females in lakes in Norway, Finland, and Lithuania produce 204, 210, and 139 eggs, respectively (Karimpour & Taghavi, 2002). These differences in egg productivity can be attributed to variations in genotypes, environmental conditions, and food availability across the different various *P. leptodactylus* populations (Mirheydari et al., 2013; Gitau et al., 2024). Genetic factors can influence reproductive traits, with larger and older crayfish typically producing more eggs. Environmental conditions, such as water temperature and food availability, also play a key role, with warmer climates and richer food sources potentially leading to higher egg production.

The effect of egg number also shows itself in the indirect effect, where the direct effect of crayfish egg diameter on egg weight (0.1432) is not significant (p>0.05), but the effect of egg diameter on egg weight through egg number (p6*p10) is negative and significant (p<0.05). It is thought that as egg number increases, egg diameters decrease and smaller females mating with large males produce more eggs. Similar results have been found in (*Astacus astacus*) (Abrahamsson, 1971), *A. leptodactylus* (Berber & Mazlum, 2009), and signal crayfish (*Pacifastacus leniusculus*) (Math et al., 2004). Additionally, besides crayfish size, food availability in the environment affects crayfish productivity. A similar explanation was proposed by Matthews & Reynolds (1992) for *Austropotamobius pallipes*. Crayfish require food and optimal temperatures for rapid growth of offspring. Increased fecundity also depends on food and temperature parameters (Momot, 1984). For long-lived species with low fecundity (*Pacifastacus, Austropotamobius*, and *Astacus* species) living in low-energy and low-nutrient environments, it is suggested to form larger adult populations and to eutrophize the environment to increase fecundity (Eversole & Mazlum, 2002). Short-lived species or those with short hatching times (*Cherax*) show high fecundity (Morrissy, 1975), while some species reproduce almost continuously (*Procambarus*) (Huner, 1978). *Cherax* and *Procambarus* exhibit the highest numbers in terms of production, biomass, and yield per unit area (Morrissy, 1980; Huner & Romaire, 1981; Huner, 1978). Variations in average yields of crayfish in high-nutrient, high-energy environments show about five times the fluctuation compared to the approximately two-fold variation observed for *Orconectes virilis* in low-nutrient, low-energy environments (Momot, 1984).

With increasing egg number, egg diameters decrease. In crayfish, eggs are attached to the mother's pleopods throughout the development period and hatching occurs there. Mazlum & Eversole (2004) noted that some crayfish (*Procambarus acutus acutus*) do not release all their eggs but keep them in the ovary within the body. Some crayfish have also been found to have eggs in their stomachs. Previous studies have shown that smaller-sized females mating with large males produce more eggs (Galeotti et al., 2006). This situation suggests that larger crayfish will emerge from larger eggs and will provide an advantage in the population. It has been reported that the adaptation period and natural habitat of crayfish can affect egg productivity (Gören et al., 2019).

5. Conclusion

In conclusion, for the species *P. leptodactylus*, size of the crayfish appears to be the most important factor affecting

both directly and indirectly egg number and egg weight for aquaculture, breeding, and sustainable fishing management.

Conflict of interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Ethical Approval

The paper is not currently being considered for publication elsewhere, and it reflects the author's' own research and analysis in a truthful and complete manner.

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Effect of peat-based feed additive on performance of laying hens

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Abstract

A strategy for improving the efficiency of the poultry industry is balanced nutrition for poultry, which plays a key role in achieving maximum productivity while maintaining health and reducing production costs through the use of feed additives or unconventional feed ingredients. Feed additives are mainly used to meet the needs of birds, improve their health, stimulate digestion, increase feeding efficiency, and enhance disease resistance. They positively affect the gastrointestinal tract, metabolism, immune system, suppress pathogens, and improve intestinal integrity.

For this purpose, the research aimed to determine the impact of using a peat-based bioregulatory feed additive on the egg productivity of laying hens of the same-age industrial flock of the "Hy-Line Brown W-36" cross and to conduct a qualitative assessment of the eggs. A total of 480 laying hens (day-old), divided into five groups, were raised for 240 days. The feeding of hens in the groups consisted of five experimental diets: a basal diet and the basal diet mixed with a peat-based feed additive at levels of 0.5, 0.75, 1.0, and 1.25 kg/t.

Experimental data showed a positive effect of using a peat-based feed additive in the composition of compound feeds for laying hens on their growth, overall productivity and product quality. The feed intake, calculated at a gram/hen/day rate, was high in Groups 1 and 2; however, the control group exhibited an even greater overall feed intake. A larger egg weight was noted for Groups 3 and 4. Based on these results, this study found that certain supplements did successfully improve egg shell integrity in older laying hens compared to a control. Thus, the use of peat-based feed additive for laying hens has a greater effect on egg-laying intensity (85.38%), average egg weight (63.24g), egg mass yield (664.1kg). The results obtained emphasize the necessity of including organic bioregulators in the diets of laying hens to achieve optimal productivity.

Keywords: Feed additives, Laying hens, Performance, Egg production

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1. Introduction

In poultry farming, feed constitutes one of the primary expenses, accounting for approximately 65-70 percent of the total production cost. Modern poultry feeding strategies must include not only protein and energy sources but also other essential elements such as limiting amino acids, vitamins, antioxidants, enzyme preparations, and various biologically active substances. Deficiencies or imbalances in these critical components can result in metabolic disorders, stunted growth, decreased productivity, and poor product quality. Therefore, incorporating specialized feed additives and biologically active substances into poultry diets is crucial for enhancing productivity and maintaining health. Given the challenges of feed shortages and rising costs, it is important to explore innovative ways to improve the biological value of feed rations. Optimizing compound feed formulations to meet the nutritional needs of poultry is essential. This requires a comprehensive approach that integrates both scientific research and the practical implementation of innovative

feeding solutions to address the limitations of traditional feed components and ensure that poultry receive all the essential elements for optimal growth and development (Papunidi et al., 2018; Ponomarenko et al., 2020; Tarasova, 2020).

The components of feed rations, including the addition of necessary biologically active additives, not only help maintain poultry health but also enhance egg production parameters, such as egg size and quality.

In modern poultry farming, the effective use of feed additives is a key aspect of optimizing feeding, as they enhance the nutritional completeness of feeds and their bioavailability to poultry. The efficiency of nutrient absorption directly influences the reduction of feed costs per unit of production, which, in turn, is crucial for economic viability.

The rapid development of the feed additives market and the continuous emergence of new formulations underscore the need for their testing under various conditions. Scientific research and practical experience demonstrate that the proper use of feed additives is essential for achieving high egg production. Investments in scientifically validated feed additives are justified by the overall improvement in efficiency and profitability of poultry farming (Kryukov et al., 2020).

The effectiveness of using available feed complexes in poultry farming is determined by several key aspects, including quantitative, qualitative, and economic indicators. These aspects are interrelated, and their combined improvement creates optimal conditions for enhancing poultry productivity. The overall enhancement of these parameters enables the maximization of agricultural poultry productivity. Qualitative and quantitative improvements in poultry nutrition can lead to increased productivity, better bird health, and reduced production costs, ultimately boosting the overall efficiency and profitability of poultry farming (Danilenko et al., 2022).

The use of high quality and safe feed additives, as well as regular monitoring of gut health, are important measures to maintain poultry health and productivity (Belkaid & Hand, 2014).

The aim of the present study was to determine the effect of a natural biologically active feed additive (peat-based additive) on the performance of laying hens, including egg productivity, egg weight, growth performance, daily feed intake, and morphometric parameters of eggs.

2. Material and Methods

2.1. Experimental site, design, animals and management

The experiment was conducted at the poultry farm AO "Floreni" and in the laboratory of the Department of Animal Science and Quality Control of the Technical University of Moldova. A total of 480 laying hens of the cross "High Line Brown W-36" aged from 17-34 weeks were individually weighed and randomly assigned to four experimental treatments. Each treatment consisted of 5 groups and 96 hens in each replicate. A completely randomized design (CRD) was used in this experiment (Fisinin, 2004).

Before feeding the experimental diets to laying hens, the feed ingredients were analysed at the Department of Animal Resources and Product Quality Control of the Technical University of Moldova. The experiment lasted 17 weeks (17-34 weeks of hens' age), during which five groups received different diets (Table 1): basic ration (CG), basic ration supplemented with 0.5 kg/tone of peat-based feed additive (EG1), basic ration supplemented with 0.75 kg/tone of peat-based feed additive (EG2), basic ration supplemented with 1.0 kg/tone of peat-based feed additive (EG3) and basic ration supplemented with 1.25 kg/tone of peat-based feed additive (EG4). The experimental diets were prepared using local ingredients and formulated to maintain a constant energy to protein ratio to meet the minimum requirements for laying hens (Fisinin, 2009).

All diets were isocaloric and isonitrogenous. The composition, calculated nutrient content, and feed cost of the different dietary treatments are shown in Table 2.

Table 1. Scheme of the experiment

Group	Number of laying hens	Features of Feeding
Control group (CG)	96	Basic compound feed (BCF)
Experimental group 1 (EG1)	96	BCF + PFA* 0.5 kg/ton
Experimental group 2 (EG2)	96	BCF + PFA* 0.75 kg/ton
Experimental group 3 (EG3)	96	BCF + PFA* 1.0 kg/ton
Experimental group 4 (EG4)	96	BCF + PFA* 1.25 kg/ton

**PFA: peat feed additive*

Table 2. Composition of the basal diet (%)

Ingredients	Content	Nutrients	Content
Corn	63.15	Metabolic energy (MJ/kg)	11.67
Wheat bran	4.74	Crude protein	16.00
Soybean meal	21.24	Calcium	3.23
Limestone	7.76	Total phosphorus	0.62
Dicalcium phosphate	0.40	Available phosphorus	0.32
Soybean oil	1.53	Methionine	0.38
Choline chloride	0.09	Lysine	0.85
$NaHCO_3$	0.09	Methionine + Cysteine	0.72
Mineral premix	0.90	Isoleucine	0.52
Methionine	0.05	Threonine	0.54
Phytase	0.03	Tryptophan	0.17
Vitamin premix 1	0.03	Valine	0.68

[1]The premix for per kilogram diet included: vitamin A, 12,000 IU; vitamin D3, 1500 IU; vitamin E, 25 IU; vitamin K3, 1.0 mg; vitamin B1, 1.6 mg; riboflavin, 5.0 mg; pantothenic acid, 15 mg; nicotinic acid, 20 mg; vitamin B6, 6.0 mg; biotin, 0.2 mg; folic acid, 0.5 mg; vitamin B12, 0.01 mg; choline, 500 mg; copper, 20 mg; iron, 90 mg; zinc, 80 mg; manganese, 80 mg; iodine, 0.45 mg; selenium, 0.2 mg. [2]Metabolic energies are calculated value and others are measured values.

2.2. Measurements

Feed consumption

Prior to feeding each morning, the required amount of feed for each group was weighed. The residual feed was collected and weighed again the following morning. The feed given and the feed residue were measured in grams using an electronic digital scale, and the averages were calculated. Daily feed consumption was recorded, and the average feed intake per bird per day was determined.

Body weight

The hens' body weights were measured in grams using an electronic digital balance. The initial body weight of each hen was recorded and measurements were repeated monthly. Body weight of birds was measured in grams using electronic digital scales.

Egg production

Egg production (EP) was recorded daily, and egg weight (EW) was measured biweekly. Before determining EW, a sample of 12 eggs from each experimental group was stored for 24 hours at room temperature. The intensity of egg production, egg weight, feed costs per 10 eggs and per kilogram of egg mass, egg mass output per laying hen, and the organoleptic qualities of the eggs were evaluated. Samples of eggs were randomly collected from each experimental group every month to assess egg quality parameters (Ergun et al., 1987).

Egg quality parameters were *shape index*, *shell strength*, *shell thickness*, *albumen index*, *yolk index*, *yolk color*, and *Haugh unit*. Shape index (%) = [(egg width (cm)/egg length (cm)] × 100; shell strength (kg/cm × cm) was determined by using a machine with spiral pressure system; shell thickness (mm) was determined in three different parts (upper and lower ends and middle) by using a micrometer; albumen index (%) = [(albumen height (mm)/average of albumen length (mm) and albumen width (mm)] × 100; yolk index (%) = [(yolk height (mm)/yolk diameter (mm)] × 100; yolk color was determined by using commercially available yolk color fan according to the CIE standard colorimetric system; Haugh unit = 100 × log (H + 7.57 − 1.7 × W × 0.37), where H = albumen height (mm) and W = egg weight (g)].

Feed conversion ratio

Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. Feed conversion ratio for all treatments was calculated.

Poultry's safety

Monitoring of the bird's safety in all groups was carried out throughout the experiment, which is an important aspect for determining the health and well-being of hens.

2.3. Statistical analysis

The obtained egg mass data were processed using the biometric method of variation statistics. The significance level of the obtained data was determined by Student's t-test as $^{*}p \geq 0.95$, $^{**}p \geq 0.99$, $^{***}p \geq 0.999$. Graphs were generated using Microsoft Excel 2016 applications. Data from the results of organoleptic evaluation of eggs were analyzed using one-way analysis of variance (ANOVA). Differences between means for each experimental group were assessed using Tukey's post hoc test (HSD), grouping using the Tukey method, and 95% confidence. Calculations were performed using the program Minitab 17.

3. Results and Discussion

The experimental introduction of a peat-based feed additive into the diet of laying hens is associated with a significant increase in egg production throughout the

laying period (Table 3). The maximum effect is observed with the optimal dosage of the additive – 1.0 kg/ton of feed. These results suggest a direct correlation between the dose of the feed additive and the level of egg production of poultry. Thus, the best results are achieved by incorporating a peat-based feed additive at a dosage of 1.0 kg/ton of feed into the diet of laying hens. It is important to note that a lower concentration of this additive does not significantly affect hen productivity. These findings provide valuable practical recommendations for optimizing egg production and effectively using organic bioregulators in poultry production.

Under current conditions, the main problem of poultry farms using highly productive crosses of foreign breeding is to ensure optimal feeding and care conditions for full development of genetic potential.

Practice shows that modern crosses, especially of foreign origin, are extremely sensitive to the conditions of keeping at domestic poultry farms due to fluctuations in temperature and humidity at different times of the year, changes in feed rations and their quality, as well as changes in light regime. All these factors have a negative impact on egg production and health of the flock. Additionally, the process of adaptation of imported laying hens to the infectious environment of domestic poultry farms does not always lead to successful results.

One of the key indicators determining the successful productivity of laying hens is the intensity of egg production, which requires an integrated approach to the creation of optimal housing conditions and management of influencing factors on the genetic potential of poultry.

Hens from the experimental groups were more resilient to negative environmental impacts, particularly the third experimental group. This indicates that environmental factors had minimal effect on the egg production of the third group, which received the peat-based feed additive at a dosage of 1.0 kg/ton of feed. The age at which peak egg production was reached was approximately the same for all groups, ranging from 30 to 34 weeks, which is consistent with the standards for this breed. However, the intensity of egg production varied significantly. The highest values for this indicator were recorded in the second and third experimental groups – 88.00% to 88.25%, which is 1.65% to 1.96% higher than the control group.

During the four-month period of comparative analysis relative to the control group, an increase in the average egg production intensity was observed in the different experimental groups. In the first experimental group this increase was 1.09%, in the second - 1.56%, and in the third - 1.92%, indicating increased egg production activity compared to the control group. In the fourth experimental group, the increase was 1.20%.

The laying hens of the third experimental group had the highest gross egg collection, which reached 10502 eggs. This exceeds the number of eggs in the control group by 3266 pieces, which is an increase of 45.13%. Compared with the first experimental group, the difference was 1765 pieces or 16.81%, and with the second 879 pieces or 8.37%. Compared to the fourth experimental group, the third group showed a superiority of 164 pieces, which is an increase of 1.57%.

It is important to note that in the experimental groups, better safety and high egg production was also noticed in both initial and middle laying hens. These values were higher by 18.18% and 20.74% in the first experimental group, 27.45% and 32.98% in the second, 39.08% and 45.18% in the third, and 38.36% and 42.86% in the fourth.

For tasting evaluation of poultry products, the method of organoleptic evaluation was used, which allows a more accurate assessment of the general response of the poultry organism to the impact of both internal and external factors affecting the physiological state and quality of the produced products.

Tasting evaluation of eggs was carried out using a five-point scale. The results demonstrate the positive effect of peat feed additive on the quality of egg products. The third (4.27 points), fourth (4.06 points) and second (4.05 points) experimental groups, in which increased doses of peat feed additive were used, received the highest scores in the tasting analysis (compared to the control group). Eggs from the first experimental group received a slightly lower number of points, amounting to 4.02, which practically does not differ from the indicators of the control group (Table 4).

Table 3. Results of egg production of laying hens

Indicators	Group				
	CG	EG1	EG2	EG3	EG4
Feed consumption per day, g	120±0.4	110±0.4	106±0.3	102±0.3	100±0.2
Feed consumption per 10 eggs, kg	1.79±0.02	1.42±0.02	1.26±0.02	1.11±0.01	1.10±0.01
Per initial laying hen, pieces	550±1.5	650±1.7	701±1.7	765±1.8	761±1.8
Total eggs produced, pcs.	7236±5.8	8737±6.2	9623±6.5	10502±6.8	10338±6.9
Oviposition intensity, % (123 days)	59.00±0.71	71.03±0.72	78.00±0.75	85.38±0.83	84.03±0.81
Egg mass yield, kg	463.6±9.0	561.4±9.0	606.9±9.1	664.1±9.5	649.4±9.2
Average egg weight, g	61.29±0.57	62.91±0,74	63.07±0.38*	63.24±0.45**	62.16±0.69

*p<0,05, **p<0,01

Table 4. Results of organoleptic evaluation of eggs (according to 5-point system, *n*=10)

Group	Aroma of the albumen	Colour of the albumen	Taste of the albumen	Degree of separation of the albumen from the yolk	Aroma of the yolk	Colour of the yolk	Taste of the yolk
CG	4.25±0.05^{c}	4.50±0.03^{b}	4.38±0.01^{c}	2.13±0.005^{a}	4.25±0.02^{c}	4.35±0.02^{b}	4.25±0.01^{c}
EG 1	4.50±0.02^{b}	4.50±0.01^{b}	4.50±0.01^{b}	1.38±0.009^{c}	4.38±0.02^{b}	4.38±0.02^{b}	4.50±0.01^{b}
EG 2	4.50±0.03^{b}	4.50±0.02^{b}	4.50±0.03^{b}	1.63±0.017^{b}	4.35±0.02^{b}	4.38±0.01^{b}	4.50±0.01^{b}
EG 3	5.00±0.04^{a}	4.63±0.01^{a}	5.00±0.01^{a}	1.25±0.010^{d}	4.50±0.01^{a}	4.50±0.01^{a}	5.00±0.01^{a}
EG 4	4.50±0.04^{b}	4.63±0.02^{a}	4.50±0.02^{b}	1.38±0.007^{c}	4.38±0.01^{b}	4.50±0.01^{a}	4.50±0.01^{b}
Mean	4.55±0.04	4.55±0.01	4.58±0.03	1.55±0.045	4.37±0.01	4.42±0.01	4.55±0.04
ANOVA	55.82***	15.48***	206.62***	1097.05***	30.36***	24.68***	754.17***

$^{***}p$<0,001

Table 5. Economic efficiency of using peat feed additive in feeding laying hens

Parameters	Group				
	CG	EG1	EG2	EG3	EG4
Number of poultry, head:					
Start of the experiment	96	96	96	96	96
The end of the experiment	92	94	96	96	95
Safety, %	93.3	96.7	100.0	100.0	96.7
Eggs produced, pcs.	7236	8737	9623	10502	10338
Revenue from sales of eggs, lei	14471.6	17474.6	19246.7	21003.8	20676.4
Feed consumption, kg	1342.4	1243.5	1210.9	1165.2	1136.4
Total costs, lei (MD)	13324.4	15810.7	17194.5	18125.30	19115.20
Profit, lei (MD)	1447.2	1963.9	2352.2	3178.6	1861.1

The analysis of egg flavour characteristics showed that laying hens of the third and fourth experimental groups showed an increased quality of production, while in laying hens of the second experimental group this quality was slightly lower. The exception is eggs from laying hens of the first experimental group, which slightly exceeded the eggs of the control group in flavour characteristics.

Tasting analysis of laying hens' products shows that the addition of additional feed peat additive to their diet contributes to a significant improvement in egg quality. The most significant impact on product quality is observed when using peat feed additive at a dose of 1.0 kg/t of mixed feed, which is reflected in the results of the third experimental group.

When studying the cost-effectiveness of organic bioregulators in laying hens' diets, a significant increase in egg production was observed in hens of experimental groups, which were supplemented with peat supplement in addition to the main feed (Table 5). In comparison with the control group, the experimental groups showed higher productivity indices. These data confirm the feasibility of using peat additive in the composition of compound feed for industrial laying hens.

The use of peat feed additive in the main compound feed for laying hens of Hy-Leni cross resulted in a significant economic effect. In the experimental groups there was an increase in profit ranging from 300.2 to 620 lei. This indicates that the introduction of peat additive not only increases the productivity of chickens, but also contributes to significant economic gain. The additional income indicates a reduction in egg production costs and an increase in egg profitability.

The conducted studies confirmed the effectiveness of using a peat feed additive to increase productivity and improve the economic performance of laying hens. The additive had a positive effect on egg production in all experimental groups, which demonstrates its potential as a means of optimizing feeding. However, the increase in total feed costs in the experimental groups, due to the cost of the additive, requires a detailed analysis of the economic return from its use. The highest economic efficiency was achieved in the third experimental group with a dosage of 1 kg/t of compound feed, where the profitability level was 17.8%, which is 6.7% higher than in the control group (Figure 1). This indicates that this dosage is optimal for achieving the maximum balance between costs and production results. A significant increase in profitability in this group is associated with an increase in egg production, which ensured maximum net profit against the background of a moderate increase in costs. The second experimental group (0.75 kg/t) showed a moderate improvement in profitability to 13.9%, which is 2.8% higher than in the control group. Despite the positive effect, the economic indicators of this group are inferior to the third, which indicates that increasing the dosage to the optimal level

allows achieving higher returns. The first experimental group, where the additive was used in a minimum dosage of 0.5 kg/t, demonstrated the lowest level of profitability among the experimental groups - 12.7%, which is 1.6% higher than in the control group. This result indicates that such a dosage is insufficient to realize the full potential of the additive, and its use in minimal quantities is not economically feasible. Exceeding the optimal dosage, as shown in the example of the fourth experimental group (1.25 kg/t), led to a decrease in profitability to 9.9%, which was lower than the level of the control group. This supports the hypothesis that excessive dosages lead to increased feed costs that are not offset by a corresponding increase in productivity.

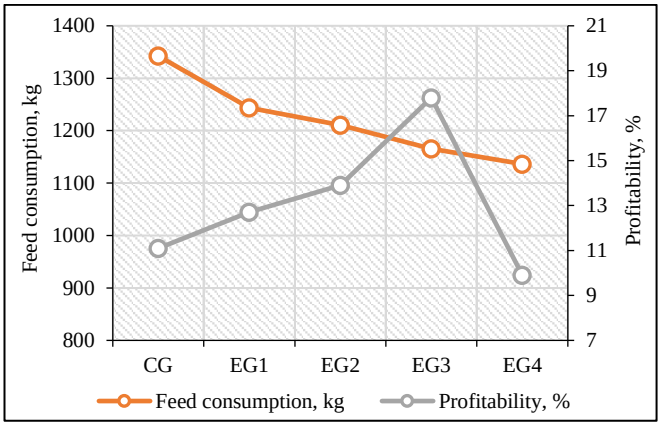

Figure 1. Dynamics of feed consumption and profitability level when using peat feed additive in laying hen diets

4. Conclusion and Suggestions

The results obtained confirm that the use of organic bioregulators in laying hens' diets not only represents an innovative approach, but is also an economically beneficial solution. Profit gains show a significant improvement in financial performance, which makes this method particularly attractive for industrial poultry farms. Thus, the introduction of feed peat additive into the main compound feed contributes to the sustainable development of poultry farming, providing both increased productivity and increased profitability of production. The addition of organic peat feed additive to the diet of laying hens, especially at a dosage of 1 kg/t (EG3), contributes to a significant increase in the productive performance of poultry. The increase in egg production by 39% and the increase in egg weight by 43.2% confirm the effectiveness of this approach. The economic benefit, expressed as an increase in profitability by 6.7%, makes the use of the additive a promising solution for increasing productivity and profitability of poultry farms.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Assessment of morphometric traits of camels using principal component analysis

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Abstract

This study assessed the morphometric traits of camels in Katsina State, Nigeria. 51 camels (24 females and 27 males) were selected randomly for the study. Data were collected on individual camels, including; heart girth (HG), abdominal girth (AG), rump height (RH), shoulder height (SH), neck length (NL) and head length (HL), and subjected to statistical analysis procedures of SPSS version 23.0.0, for descriptive statistics, phenotypic correlation, and Principal Component Analysis (PCA). Results revealed that the overall mean body weight of the camels was 230.73 kg, with females averaging 216.15 kg and males 243.70 kg. Phenotypic correlation indicated a strong positive correlation between body weight and HG (r = 0.877 for females, r = 0.911 for males; p<0.01), suggesting that HG is a reliable predictor of body weight in camels. Two principal components (PCs) extracted in male and female camels explained 77% and 84% of the total variation, respectively. For females, PC1 accounted for 57.768% of the variance and was strongly associated with HG, AG, SH, and NL. In males, PC1 explained 65.714% of the variance, with similar trait loadings. HG, AG, and SH emerged as key variables influencing overall body size, while rump height was distinct, potentially linked to mobility and endurance. These findings suggest that heart and abdominal girth, are essential indicators of body weight and conformation in camels, highlighting the potential for using these traits in selective breeding programs aimed at improving camel productivity.

Keywords: Body weight, Camel, Morphometric traits, Principal component analysis

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1. Introduction

Camels (*Camelus dromedarius*) are integral to the socio-economic and agricultural systems of arid and semi-arid regions, including northern Nigeria. They were valued for their adaptability to harsh climates, endurance, and utility in transportation, milk, and meat production (Abdelhadi & Babiker, 2020).

World Camel population is estimated to be about 35.5 million, while about 1.05 million are found within Nigeria's border (FAOSTAT, 2018). Katsina State, located in the northern part of Nigeria, has a significant camel population that contributes to the livelihoods of many pastoralists and smallholder farmers (Gafar et al., 2022). However, morphological characterization of these camels, essential for improving breeding, management, and conservation strategies, remains underexplored. Morphological traits, including body size, shape, and conformation, provide critical information for evaluating breed diversity, adaptability, and productivity (Abdulahi et al., 2021).

Principal Component Analysis (PCA), offers a robust framework for analyzing morphological traits by reducing data complexity and identifying the most informative characteristics contributing to overall variation (Girma et al., 2023).

Quantitative measurements for size and shape are essential for understanding genetic and phenotypic diversity, leading to more effective selection and breeding programs (Chineke, 2000). Morphometric traits are inexpensive measurements correlated with body weight (Mondini et al., 2009; Ajayi et al., 2012). Thus, morphometric traits could be used as markers in body weight improvement programs and body weight prediction (Musa et al., 2018)

In animal breeding, these tools are increasingly used to enhance the understanding of genetic and phenotypic diversity, leading to more effective selection and breeding programs (Salem & El-Tayeb, 2022).

Despite the economic and cultural significance of camels in northern Nigeria, particularly in Katsina State, limited scientific research exists on the morphological traits that define camel populations in the region. Previous studies have either focused on camel productivity or general management practices without paying adequate attention to the detailed analysis of morphological variations. This gap in knowledge hinders the development of targeted breeding and conservation strategies that could improve the productivity and sustainability of camel populations in the area.

PCA has been used to evaluate different phenotypic characters by other authors; in Uda Sheep (Salako, 2006), hairy sheep (Lopez-Carlos et al., 2010), Djallonke sheep in northern Ghana (Birteeb et al., 2012), Yankasa sheep (Yakubu, 2013), Zulu sheep (Mavule et al., 2013) and Thalli sheep in Pakistan (Akbar et al., 2022). However, the application of multivariate statistical methods, such as Principal Component Analysis, to characterize these morphological traits is largely unexplored on camels in Nigeria. Without such analysis, the ability to identify and preserve key genetic resources for future generations may be compromised.

Morphological characterization is a fundamental step in understanding the genetic diversity and adaptability of livestock species. The application of PCA in this context provides a data-driven approach to identifying critical morphological traits that can influence breeding and selection processes (Kebede et al., 2021). In a region where camels play a crucial role in agriculture and pastoral livelihoods, such as Katsina State, the need for efficient and scientifically informed breeding strategies is paramount. This study is justified by the growing demand for sustainable livestock management in response to environmental changes and increasing pressure on agricultural systems (Musa et al., 2023). It is therefore crucial to describe the phenotypic characteristics of camel populations in Katsina state, Nigeria, using the FAO guidelines (FAO, 2012). By identifying key morphological traits that correlate with productivity and adaptability, this research can contribute to enhancing camel production systems and ensuring the long-term sustainability of camel populations in Katsina State. Therefore, this study uses multivariate PC analysis to assess the variability and relationships among body measurements, and to deduce components that describe these traits.

2. Material and Methods

2.1. Locations of the study area

This study was carried out in two LGAs in Katsina state, namely, Charanchi (12°43'N and Longitude 7°44'E) and Mai'Adua (latitude 13°8'N and longitude 8°13'E), Northern region Nigeria. The description of the study location was earlier given by Rotimi et al. (2023).

2.2. Experimental animals

The study involved 51 (24 females and 27 males), apparently healthy and non-pregnant camels, randomly selected across the study area. Data collected included; heart girth (HG), abdominal girth (AG), rump height (RH), shoulder height (SH), neck length (NL) and head length (HL). Data were measured following FAO (2012) descriptors standard using simple measuring tapes. Body weight was estimated using the Barymetric weight estimation formula of Yagil (1994).

2.3. Statistical analysis

Data obtained were subjected to statistical analyses using the statistical procedures of SPSS version 23.0.0 (IBM SPSS 23.0.0).

Principal Components

The principal components are the new variables obtained by projecting the original variables onto the eigenvectors.

$$PC = X * V$$

Where,
PC: the matrix of principal components,
X: the data matrix,
V: the matrix of the eigenvectors.

Explained Variance

The explained variance (*EV*) is the proportion of variance explained by each principal component.

$$EV = \left(\lambda_i / \sum \lambda_j\right) * 100$$

Where,
EV: Explained variance,
λ_i: the eigenvalue corresponding to the ith principal component,
$\sum \lambda_j$: the sum of all eigenvalues.

Orthogonality

The principal components are orthogonal to each other, meaning that their covariance is zero.

$$\text{cov}\left(PC_i, PC_j\right) = 0 \text{ for } i \neq j$$

Data set validity for PCA analysis was evaluated using Kaiser-Meyer-Olkin and Bartlett's sphericity tests. Eigenvalue values ≥1.000 were used to select the number of components to retain.

Table 1. Descriptive statistics of body weight and body measurements of the pooled population of camels

Traits	Sex	N	Mean	SE	*p*
Body weight (kg)	Female	24	216.15	15.07	0.233
	Male	27	243.70	16.80	
	Overall	51	230.73	11.43	
Heart girth (cm)	Female	24	169.00	4.86	0.522
	Male	27	173.30	4.55	
	Overall	51	171.28	3.30	
Abdominal girth (cm)	Female	24	143.95	3.45	0.076
	Male	27	153.72	4.06	
	Overall	51	149.12	2.75	
Rump height (cm)	Female	24	171.07	4.54	0.707
	Male	27	168.35	5.44	
	Overall	51	169.63	3.56	
Shoulder height (cm)	Female	24	167.14	4.10	0.235
	Male	27	173.30	3.16	
	Overall	51	170.40	2.56	
Neck length (cm)	Female	24	127.23	7.51	0.666
	Male	27	131.78	7.30	
	Overall	51	129.64	5.19	
Head length (cm)	Female	24	51.53	1.64	0.549
	Male	27	53.04	1.88	
	Overall	51	52.33	1.25	

3. Results and Discussion

Table 1 shows the results of the descriptive statistics of body weight (kg) and morphometric traits (cm) of the camels. The results show a non-significant (p>0.05) difference in values obtained for female and male camels. However, the overall mean values obtained for the traits were 230.73 kg (BWT), 149.12 cm (AG), 171.28 cm (HG), 169.63 cm (RH), 170.40 cm (SH), 129.64 cm (NL) and 52.33 cm (HL). Results obtained in this study agree with earlier reports by Rotimi et al. (2023)

3.1. Correlations

Table 2 shows that all the correlation values between body weight and linear body parameters and among the linear body measurements were positive, for both female and male camels. In female camels, the highest correlation value was observed between body weight and HG (0.877) while the lowest exists between body weight and HL (0.420). Similarly in male camels, the highest correlation value was obtained between body weight and HG (0.911) with the lowest existing between body weight and TL (0.125). In the pooled data, the highest was also obtained between body weight and AG (0.866). In same trend, the lowest was observed between body weight and RH (0.416). This implies that selection for HG and AG can result in rapid improvement in body weight in both female and male camels. Correlations among the linear body measurements were all positive. The result is similar to the records of Rotimi et al. (2023) and Kebede et al., (2022), who also obtained positive values for the linear body measurements in their study. Other researchers also reported positive correlation values in chickens (Yosef et al., 2014).

3.2. PC Factor Analysis

The result of the KMO measure was found to be adequately high with values ranging from 0.722 to 0.809 for females, males and the pooled data (Table 3). However, Kebede et al. (2022) reported a higher value of KMO (0.94) in their study. A KMO measure of ≥0.60 indicates that the data set is adequate for PCA analysis (Eyduran et al., 2010).

3.3. Eigenvalues, percentage of total variances and communalities

Table 4 shows the total variance of the observed traits explained by each of the PCs after varimax rotation of the component for female, male and the pooled data. In both female camels, two (2) PCs were extracted with eigenvalues of 3.466 (PC1) and 1.153 (PC2), explaining total variability of about 76.992% of the total variances. PC1 explained 57.768% of the total variances while PC2 explained about 19.224% of the total variances. Similarly in male camels, two (2) PCs were also extracted with eigenvalues of 3.943 (PC1) and 1.084 (PC2), contributing about 84.503% total variability. PC1 explained 65.714% while PC2 explained 18.059% of the total variability. From the pooled data, only one (1) PCs was extracted with eigenvalues of 3.610 and about 60.166% total variance explained.

Table 2. Phenotypic correlations between body weight and body measurements by sex in camels

Sex	Traits	Body weight	Heart girth	Abdominal girth	Rump height	Shoulder height	Neck length
Female	Heart girth	0.877**	---				
	Abdominal girth	0.830**	0.745**	---			
	Rump height	0.720**	0.583**	0.575**	---		
	Shoulder height	0.804**	0.517**	0.526**	0.815**	---	
	Neck length	0.610**	0.456*	0.642**	0.525**	0.428*	---
	Head length	0.420*	0.312	0.503*	0.006	0.145	0.305
Male	Heart girth	0.911**	---				
	Abdominal girth	0.847**	0.695**	---			
	Rump height	0.416*	0.434*	0.167	---		
	Shoulder height	0.877**	0.718**	0.821**	0.427*	---	
	Neck length	0.645**	0.479*	0.793**	0.018	0.683**	---
	Head length	0.740**	0.691**	0.715**	0.294	0.761**	0.668**
Both sex	Heart girth	0.892**	---				
	Abdominal girth	0.844**	0.708**	---			
	Rump height	0.511**	0.484**	0.289*	---		
	Shoulder height	0.833**	0.614**	0.681**	0.573**	---	
	Neck length	0.629**	0.471**	0.719**	0.217	0.550**	---
	Head length	0.622**	0.532**	0.636**	0.183	0.468**	0.518**

***Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).*

Table 3. Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) and Bartlett's test

Test		Female	Male	Pooled data
KMO		0.722	0.809	0.794
Bartlett's Test	Chi-square	71.423	105.754	153.601
	df	15	15	15
	p	<0.001	<0.001	<0.001

Table 4. Variance contributions of each trait in camels

Traits	Female			Male			Pooled data	
	PC1	PC2	Communality	PC1	PC2	Communality	PC1	Communality
Heart girth	**0.675**	0.373	0.670	0.389	0.152	0.752	0.335	0.687
Abdominal girth	**0.708**	0.060	0.851	**0.810**	0.305	0.879	**0.763**	0.798
Rump height	0.124	**0.761**	0.908	0.068	**0.682**	0.920	-0.044	0.305
Shoulder height	**0.847**	0.138	0.776	-0.133	0.021	0.864	-0.062	0.707
Neck length	**0.812**	-0.010	0.567	**0.603**	0.349	0.841	0.438	0.583
Head length	0.489	-0.579	0.848	**0.847**	-0.174	0.770	**0.862**	0.530
Eigenvalue	3.466	1.153	-	3.943	1.084	-	3.610	-
Variance contribution (%)	57.768	19.224	-	65.714	18.059	-	60.166	-
Cumulative variance contributions (%)	-	76.992	-	-	83.773	-	60.166	-

Communalities show the proportion of variance in each variable explained by the extracted PCs. In the female camels, the communality values ranged from 0.567 (NL) to 0.908 (RH). However, in male camels, the communality values were 0.752 (HG) to 0.920 (RH) while in the pooled data, the communality ranged from 0.305 (RH) to 0.798 (AG). These values further indicate that the data set is adequate for PCA. The ranges of communality values obtained are within the acceptable communality values for PCA (0.60 - 0.80). communalities are crucial in assessing the adequacy of the PCA model and the retained components' ability to capture the variability of the original data.

3.4. PC loadings

Table 4 shows the PC loadings of each linear body measurements in the camels. In female camels, PC1 loaded highly on HG, AG, SH and NL while PC2 loaded highly

on RH only. Factor loadings heavily on HG, AG, SH, and NL in camels suggesting that these variables are strongly related to overall body size or conformation. HG, AG, and SH are strongly correlated, suggesting a relationship between body circumference and overall size. NL is also related, potentially indicating a connection between neck proportions and body size. Body size is crucial for camel's thermoregulation, mobility, and load-carrying capacity. HG and AG may be related to digestive efficiency and energy reserves, thereby influencing body size whereas, shoulder height and neck length might influence the camel's ability to browse and reach food sources. PC2 factor might be labeled as Rump Size or Hindquarters Development" factor, suggesting that RH is a distinct characteristic, separate from overall body size and may be related to muscle mass, strength, or fat reserves in the hindquarters. RH could influence the camel's locomotion and mobility (e.g., stride length, agility), load-carrying capacity and endurance.

Other researchers have used PCA to examine different phenotypic traits; in Uda Sheep (Salako, 2006), Djallonke sheep in northern Ghana (Birteeb et al., 2012), Yankasa sheep (Yakubu, 2013), Zulu sheep (Mavule et al., 2013) and Thalli sheep in Pakistan (Akbar et al., 2022).

4. Conclusion

This study highlighted that HG, AG, and SH had strong correlations with body weight, particularly HG which showed the highest correlations in both male (r = 0.911; p<0.01) and female (r = 0.877; p<0.01) camels.

Principal component analysis (PCA) revealed that in both male and female camels, two principal components explained a much proportion of the total variability. In females, PC1 and PC2 accounted for 57.77% and 19.22% of the variance, respectively, while in males, these components explained 65.71% and 18.06% of the total variance. Traits such as HG, AG, SH, and neck length (NL) contributed heavily to PC1 in both sexes, indicating that these traits are key indicators of body size and overall conformation in camels.

The positive correlations observed between body weight and morphometric traits suggest that selection based on HG and AG can result in rapid improvement of body weight in camels. These findings are consistent with earlier reports and confirm that these traits are reliable predictors of body size in camels.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Colchicine-induced polyploidy and morphological changes in wild *Silene compacta* Fischer: Potential as an ornamental plant in Türkiye

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Abstract

This study aimed to induce polyploidy in *Silene compacta* Fischer by applying varying colchicine doses and soaking durations while evaluating resultant changes in plant growth and morphology. *Silene compacta* seeds growing naturally in Erzurum and its surroundings were treated with different colchicine doses (0.01%, 0.05%, 0.1%, 0.2% and 0.4%) and soaking durations (6, 12, 24, 48 and 72 hours). In addition, two different application methods (dripping on the shoot tip of the seedlings and application to the root tip meristem regions) were also tested. The control group received only pure water. As a result of our study, the toxic effect of colchicine at low doses (0.01% and 0.05%) in root application was not lethal and the plants survived 100%. However, higher doses and prolonged applications (e.g. 0.2% and 0.4%) led to plant death. Application to the shoot tip was more toxic, especially high doses (e.g. 0.2% and 0.4%) caused plant death. Colchicine treatments affected stomatal number and stomatal size. In root applications, stomatal width increased at 0.01%, 0.05% and 0.1% doses, but decreased at higher doses. In shoot tip treatments, the highest stomatal number was determined at 0.01% dose. Stomatal length decreased with increasing dose and this decrease was significant compared to the control group. The optimal soaking duration for the increasing stomatal length was determined as 12 hours for both root and shoot tip applications. As a result, it was found that the *S. compacta* plants kept at 0.1% colchicine dose for 12 hours in both treatment methods were likely to be tetraploid. Also, it was predicted that various *S. compacta* cultivation materials with improved properties that can be used as main materials in future breeding programs can be developed.

Keywords: *Silene compacta*, Colchicine, Polyploidy

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1. Introduction

The native flora of Türkiye is relatively rich in the genus Silene. *Silene compacta* Fischer, widely distributed in Türkiye, shows significant potential as an ornamental plant, due to its striking pink flowers. In this context, it is important to improve the characteristics of local plant species without disturbing the adaptation of traditional varieties to the growing area. For the cultivation of ornamental plants, especially species for which little breeding has been done, various biotechnological interventions such as polyploidization, haploids, mutation breeding and in vitro soma clonal variations can accelerate reproduction and selection of new mutants. Among all these methods, mutation breeding and polyploidy are used to develop new varieties and obtain different genetic variations in plants.

To accelerate the development of new varieties and achieve agronomic traits unattainable through conventional breeding, non-traditional approaches such as mutation and polyploidy are increasingly emphasized.

Polyploidization, chromosome folding, can be achieved using various chemicals. Colchicine is the most widely used chemical to induce polyploidy. The effects of colchicine have been tested in many studies to obtain tetraploid plants. It has been found that the stems of polyploid plants are thicker, the leaves are large and dark colored, the roots are strong and spread wider than diploids, and the flowers, pollen and seeds are larger than diploids (Motosugi et al., 2002; Sattler et al., 2016).

The mutation effect can be easily seen in ornamental plants in terms of changes in flower color, shape and size (Ari et al., 2015). Polyploids have been successfully bred in many ornamental plants in the last few decades (Sajjad et al., 2013). Polyploidy breeding is an effective method compared to mutation breeding and conventional hybridization, which is easy to use in a short time and increases germplasm availability (Niu et al., 2016). Polyploidy leads to intensification of flower color, increase in flower size and change in plant shape (Sajjad et al., 2013). Besides increasing the size of various vegetative parts in tetraploid plants, it can alter growth habits, sterility and sometimes increase cold hardiness (Dibyendu, 2010).

Colchicine not only helps to double chromosomes, but also causes mutation in plants. Colchicine is usually applied as an aqueous solution. It is recommended to make a fresh aqueous solution before application (Kumar & Rani, 2013). Colchicine concentrations for seed treatment usually range from 0.1% to 0.8%, but high doses cause malformation and reduce the production of tetraploid plants. Therefore, it is recommended to use colchicine in concentrations as low as possible (Pirkoohi et al., 2011). Colchicine is highly toxic to plants. Therefore, low doses with long exposure time are considered safe to reduce its toxic effect and increase the rate of polyploid production (Sajjad et al., 2013).

One of the simplest, easiest and most effective methods is to use a large number of seedlings with small and actively growing meristematic tissues, depending on the plant species. Seedlings can be dipped/soaked or apical meristems can be immersed in different concentrations of anti-mitotic agent solution at different exposure times or frequencies. Furthermore, a specific protocol is required for each plant species. Seneviratne et al. (2002) in African violet (*Saintpaulia ionantha*), leaf bases (stem part) were immersed in colchicine solution at concentrations of 0.025, 0.04, 0.04, 0.05, 0.05, 0.06, 0.1% and for 18, 23.5, 27, 43, 47 and 117 hours to obtain plants with different flowers. Morphologically different plant size, leaf shape and size, flower color, shape and size, and mutations attributed to polyploidy were reported in the new plants obtained at different doses and times. These polyploid plants can also be taken as a new variation or a genotype that can be used in future breeding programs for crop improvement. Due to the enormous importance of polyploidy, many economically important crops have been artificially induced, with the highest success reported in the ornamental plants industry. Chromosome doubling through colchicine has been achieved in many ornamental plants such as lily, salvia, phlox, gladiolus, petunia and marigold using different application methods (Manzoor et al., 2019). These studies, in which colchicine was applied on a large number of ornamental plants and successful results were obtained, were generally carried out in in-vitro environments. The research was carried out under greenhouse conditions. In addition, the method applied in the studies is generally applied to the seed and shoot tip meristem region. In this study, colchicine was applied to the shoot tip meristem region and root tip meristem region and the effects of these two application regions were also revealed. In the literature review, no study on the use of colchicine to obtain polyploid plants of our local plant species *S. compacta* was found.

This study was carried out with the aim of obtaining polyploid plants and selecting the best quality plants with the most suitable characteristics and realizing their propagation in order to bring this species, which has a very high potential to be an ornamental plant, into the sector and to be more demanded in terms of ornamental plant properties. Determination of the most appropriate dose and soaking duartion for this species is the most important goal of the research.

2. Material and Methods

The research was carried out in the research greenhouse, in the Atatürk University Faculty of Agriculture (in Türkiye). The latitude of the research site is 39°53′57,4″N and longitude is 41°14′14,8″ E. The height of the research area is 1890 m above sea level.

In the study, the seeds of *Silene compacta* Fischer grown naturally in Erzurum (Türkiye) and its surroundings were collected from the determined locations during the seed ripening period and were used as plant material (Figure 1).

The peat used as growing medium in the experiment was obtained with a grain diameter of 0-40 mm, pH value (CaCl2) approximately 5.2-6.0, porosity weight 95-99%, volume weight (dry state) <55-90 g/l and organic matter content (dry state) 95-99%. A mixture of garden soil, sand and peat was prepared and filled into pots. The seeds were cold-dressed until the seedling stage to stimulate germination and grown in vials. Colchicine treatments were applied to the plants and was transplanted into pots after the seedling stage. Plants in the control group were treated with pure water.

The stratification treatments were carried out by placing the seeds on moistened blotting papers (towel napkins) in the refrigerator conditions set to +4 °C for 64 days. The moisture content of the blotters and the germination of the seeds were checked at regular intervals. In cases where humidification was necessary, the lost moisture was added by spraying water (Figure 2).

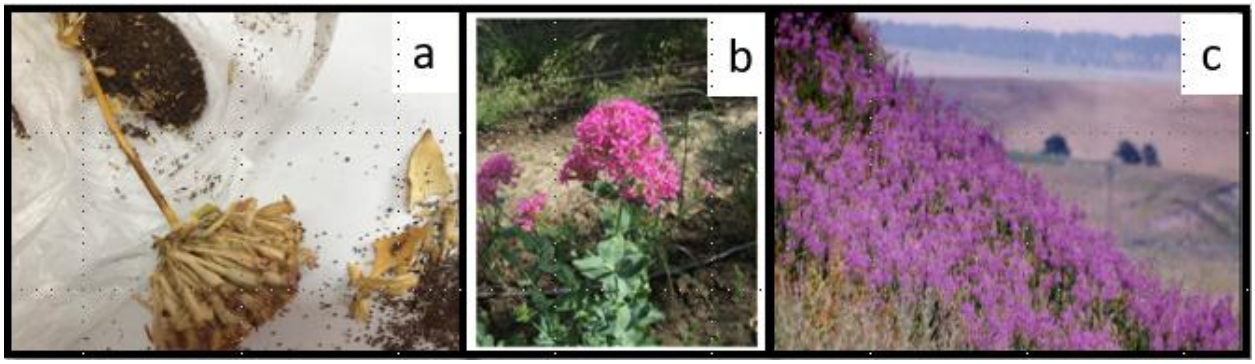

Figure 1. (a) View of *S. compacta* Fischer seeds (August) at the mature stage and seeds (Original); (b) Natural appearance of *S. compacta* Fischer (Draghia et al., 2013); (c) (Özer et al., 2009)

Figure 2. Seeds placed on moistened blotting papers (towel napkins) (a,b), rolled (c), placed in transparent bags, left open for ventilation (d) and folded

The doubled seeds were removed and cleaned in tap water and then transplanted in greenhouse conditions into peat-containing viols (70 wells). By following irrigation procedures regularly, these germinated plantlets were grown in viols (seedling growing container) and brought to seedling size (Figure 3).

Colchicine was dissolved in 1% Dimethyl sulfoxide (DMSO) (Yang et al., 2006). Five different (0.01%, 0.05%, 0.05%, 0.1%, 0.1%, 0.2% and 0.4%) colchicine doses were applied to the shoot tip meristem region of the seedlings by placing cotton wool impregnated with colchicine for five different times (6, 12, 24, 48 and 72 hours) and covered with aluminum foil. In these treatments, the cotton impregnated with colchicine was changed daily. The root tip meristem region treatment was done by immersing in solutions prepared with the same dose and soaking durations. Control plants were treated with pure water only (Ma et al., 2014; Sattler et al., 2016). After 6, 12, 24, 48 and 72 hours, the plants of the treatment groups were transplanted into pots filled with growing medium prepared with a mixture of garden soil, sand and peat in equal proportions. The treatments were arranged according to the randomized plots experimental plan with 3 replicates and 10 seedlings in each replicate (Figure 4).

The experiment was completed 100 days after the application of colchicine and the following observations and measurements were made:

Plant height (cm): Plant height of all plants grown after colchicine applications to the growing tip and root tip of the seedlings from the soil to the longest shoot tip of the plant was determined in cm with the help of a ruler.

Plant stem diameter (mm): The stem diameters of all plants grown after colchicine applications to the growing tip and root tip of the seedlings, 5-6 cm above the root collar, were determined in mm with the help of a digital caliper.

Number of leaves (number/plant): The number of leaves per plant was determined by counting the number of leaves of all plants grown after the application of colchicine to the growing tip and root tip of the seedlings.

Number of branches (number/plant): The number of branches per plant was determined by counting the number of branches of all plants grown after colchicine applications to the growth tip and root tip of the seedlings.

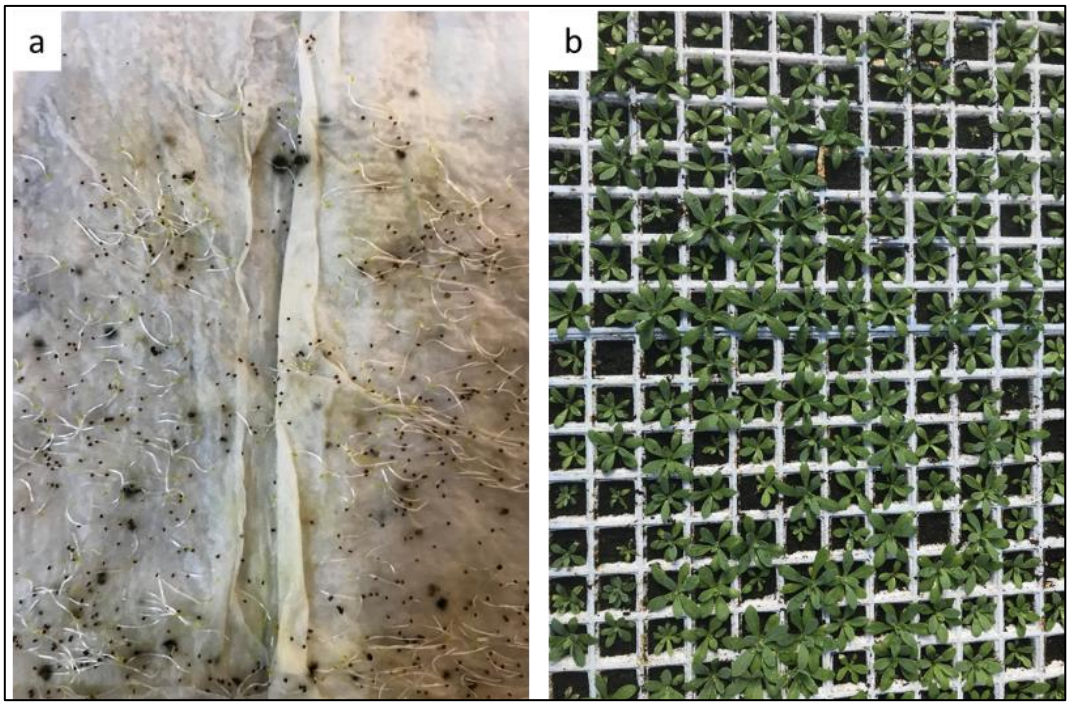

Figure 3. Germination and emergence of seeds after stratification (a); view of seedlings after germination (b)

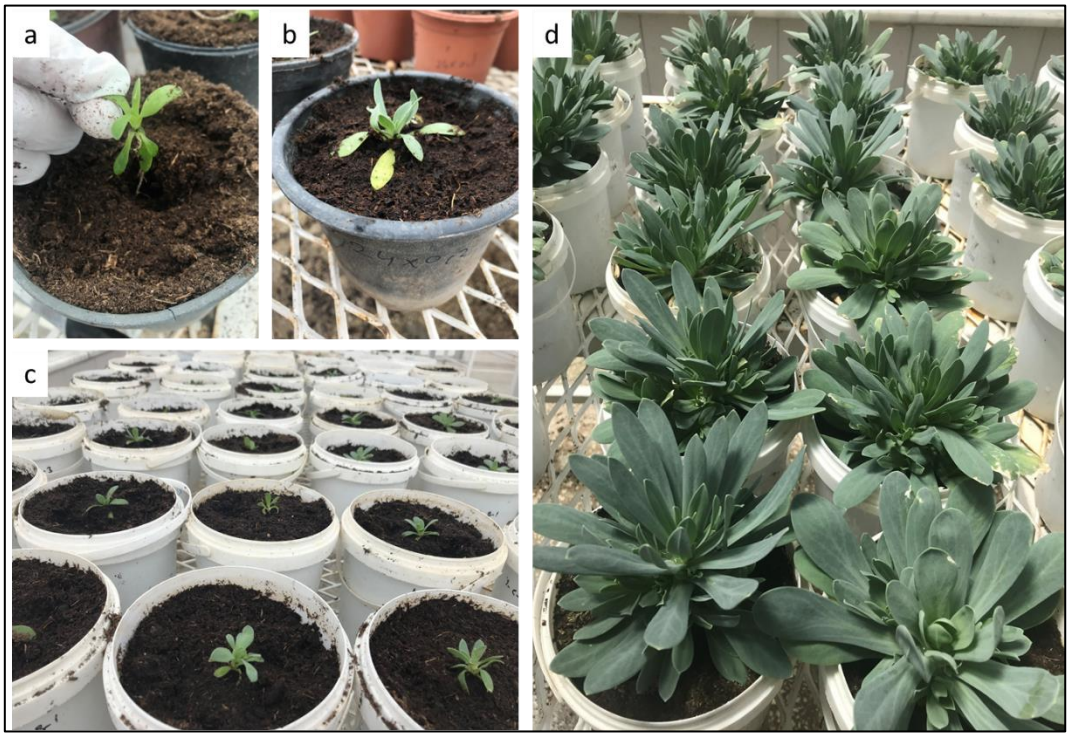

Figure 4. Stages of plant growth after colchicine application in the research greenhouse

Leaf length (cm): The lengths of the leaves taken from the leaves growing in the middle part of the seedlings after the application of colchicine to the growing tip and root tip of the seedlings were determined in cm with the help of a ruler.

Leaf width (cm): The width of the leaves taken from the leaves growing in the middle part of the seedlings after the application of colchicine to the growing tip and root tip of the seedlings was determined in cm with the help of a ruler. The measurement of plant height, plant stem diameter, number of leaves, number of branches, leaf length and leaf width parameters is described above. Also, these measurements were made according to the methods described by Błażewicz-Woźniak et al. (2021) and Dikbas et al. (2023).

Stomatal number (pcs mm^{-2}): Stomata were measured on the leaves of chemically mutated *S. compacta* plants. In order to determine the number of stomata, 3 leaves were selected from each plant. Nail polish method (Akal, 2001) was used for stomatal counts. According to the method, mono nitro cellulose was applied on the underside of the leaves and kept until it dried thoroughly. Then the lower epidermis was peeled off with this material and placed on a coverslip and the number of stomata in an area of 1 mm2 was counted and recorded for three different parts of the leaf using a 20-magnification objective and a 10-magnification ocular micrometer using a 'Net Micrometer' (Figure 5).

Stomatal width and length (µm): The width and length of the stomata of the specimens, whose lower epidermis was stripped and placed on coverslips, were measured with a 100-magnification objective and a 10-magnification ocular micrometer by taking 3 readings for three different parts of each leaf (Figure 6).

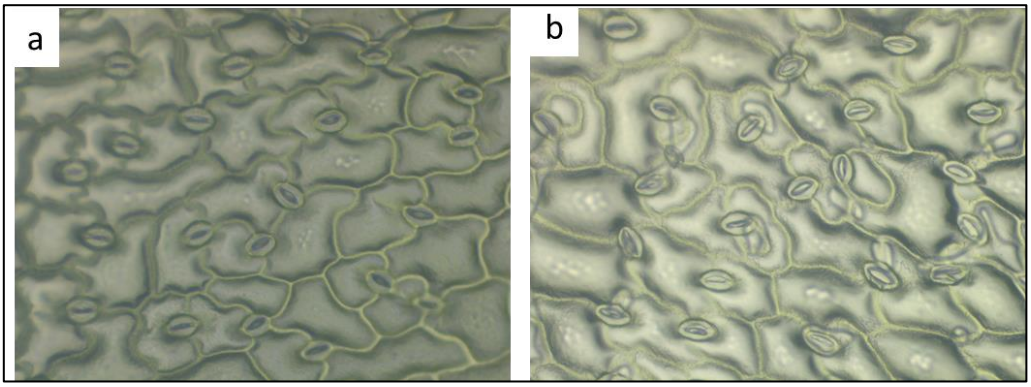

Figure 5. Examples of images used to determine the number of stomata in an area of 1 mm^2 using a 2- magnification objective and a 10-magnification ocular micrometer on the leaf

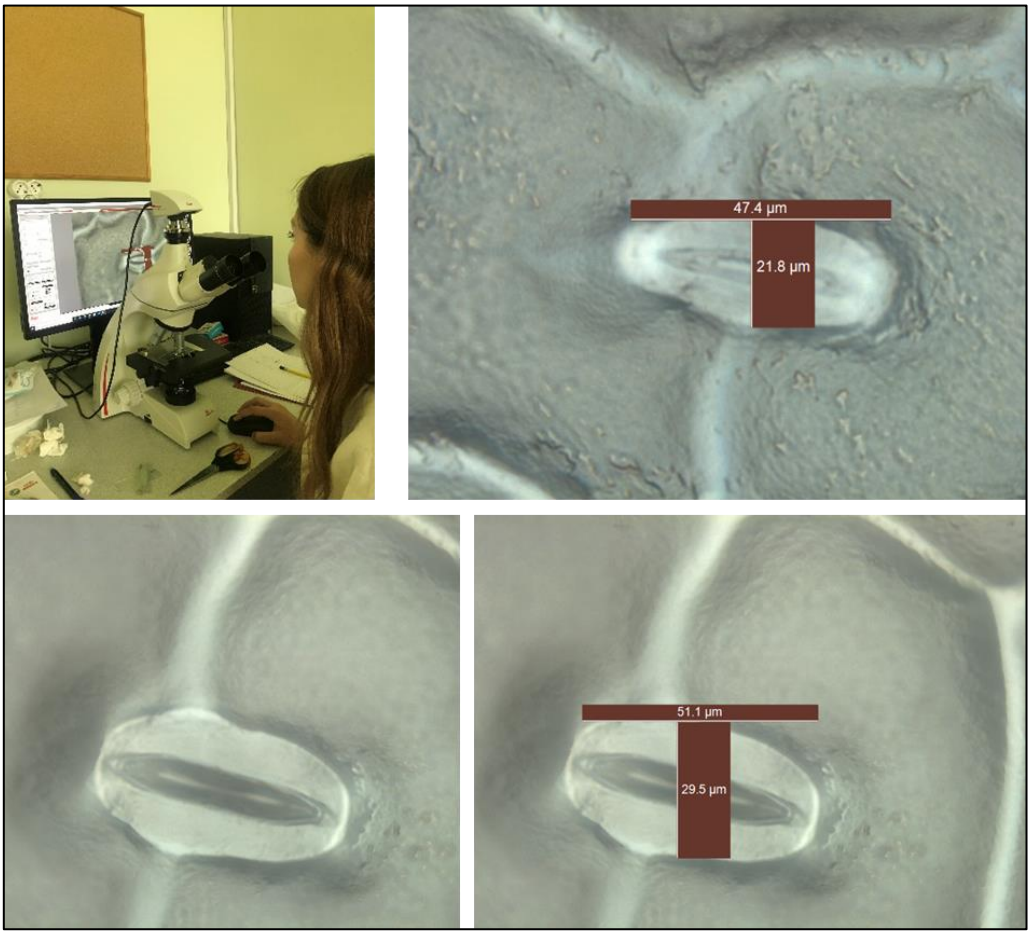

Figure 6. Determination of stomatal width and length using a 100-magnification objective and a 10-magnification ocular micrometer

The data obtained as a result of colchicine applications were compared with Duncan multiple comparison test in SPSS 20.0 statistical program (SPSS Inc, Chicago, IL, USA) at $p<0.05$ significance level.

3. Results and Discussion

Silene compacta Fischer plants were treated with colchicine and plant height was measured after 100 days. For rooting tip meristem application, the mean plant height was lowest at 4.92 cm with 0.4% colchicine dose, while the highest plant length was 9.94 cm with 0.05% colchicine dose. The application of 0.05% colchicine dose, in which the highest plant height was obtained, was in the same statistical group as the control application at $p<0.05$ significance level (Table 1). For the rooting tip meristem application, the lowest plant stem diameter was 3.80 mm at 0.4% colchicine dose and the highest plant stem diameter was 8.20 cm at 0.0% colchicine dose (control treatment). This application, in which the highest plant stem diameter was obtained, was in the same statistical group as the 0.01% and 0.05% colchicine doses applications. In the root tip meristem application, the number of leaves decreased from 32.40 leaves per/plant in the control treatment to 12.57 leaves per/plant at 0.4% colchicine dose (Table 1). The number of branches varied between 0.60 and 3.83 number plant-1 branches per plant according to the treatments; the highest number of branches was obtained from 0.05% colchicine dose in root tip meristem application. In the general evaluation of the treatment doses, all treatment doses except 0.05% colchicine dose were in the same statistical group and there was no significant difference between them in terms of the number of branches. The effect of root tip meristem application and different doses on leaf length was not statistically significant. As a result of the evaluation made in the experimental group applied from the root, it was determined that the leaf width varied between 0.74 and 1.91 cm according to the applications. The highest leaf width was obtained from the 0.05% dose application. In the general evaluation of the application doses, the 0.05% colchicine dose was the control, 0.01%, 0.05% and 0.1% doses were in the same statistical group and no significant difference was determined between them in terms of leaf width (Table 1).

The lowest plant height was 5.26 cm at 0.4% colchicine dose and the highest plant height was 11.07 cm at 0.05% colchicine dose in the shoot tip meristem application method. It was determined that plant height decreased as the doses and soaking durations of application increased regardless of the type of application (Table 2). In a study conducted by Gupta and Koak (1976), tetraploid plants obtained after 0.01% colchicine applied to *Zinnia elegans* plants were compared with diploid zinnia plants. According to this comparison, tetraploid zinnias were shorter than diploids. The results reported by these researchers were in parallel with the results of our study.

The lowest plant stem diameter was 3.57 mm at 0.4% colchicine dose and the highest plant stem diameter was 7.93 mm at 0.0% colchicine dose (control treatment) in the shoot tip meristem application method. It was determined that the plant stem diameter decreased as the application doses and times increased regardless of the application type (Table 2).

In a study conducted by Gupta and Koak (1976), tetraploid plants obtained after colchicine applied to Zinnia elegans plants had thinner main stem diameter than diploid zinnia plants. The results reported by these researchers are compatible with the results of our study. The different doses of colchicine applied from the shoot tip became shorter with the increase in the soaking duration. In general, the morphology of the plants decreased with increasing soaking duration and dose. In this case, more than 0.2% of colchicine was toxic to the plants. This toxic effect of the dose increased with the soaking duration.

Plant growth and development depends on the amount of assimilate produced by photosynthesis. The amount of assimilate is mainly undertaken by the leaves, which is the main site of photosynthesis in plants. In other words, plant growth and development are related to the number of leaves. In the study, the number of leaves decreased with the increase in colchicine doses as a result of the treatments. While the highest number of leaves was obtained from the control treatment, the lowest number was determined in the highest colchicine dose treatment. This decrease in the number of leaves also increased with the increase in colchicine doses and soaking durations. In both root tip (Table 1) and shoot tip application method (Table 2), the number of leaves decreased with increasing doses and soaking durations. At the highest soaking durations in the study, 48 and 72 hours (and at the highest colchicine doses) plants died. 48 and more than 48 hours of soaking durations not only caused a decrease in the number of leaves but also slowed down the growth of the plants. In the shoot tip meristem application, the highest number of leaves was 29.97 leaves per plant and the lowest number of leaves was 13.00 leaves per plant (Table 2). The explanation for this situation is that overdose of colchicine can be fatal by toxic effect on plants (Table 1, Table 2). As a result of lateral branching, plants can fill the pot volume and create a more compact texture.

This situation can be successfully created by increasing the leaf area and with the lateral branch. In this study, the leaves of plants with a high number of lateral branches had narrow and thin leaves. Therefore, less lateral branching may positively affect the leaf area of the plant. The lowest number of lateral branches was determined at 0.4% colchicine dose and the highest number of lateral branches was determined at 0.05% dose. Based on this result, colchicine applied at low levels was effective in increasing the number of lateral branches, and as the dose increased, both the number of lateral branches decreased and dwarf appearance occurred in the plant. In general, plants died at

Table 1. The effects of root tip meristem application method, different doses (%) and soaking durations of colchicine

			Soaking durations in colchicine					
		Doses	*6 hours*	*12 hours*	*24 hours*	*48 hours*	*72 hours*	Mean
Root tip meristem application method	Plant height (cm)	Control	9.57 c**	9.63 ns	9.94 a*	9.59 a***	8.82 a***	9.51 AB***
		0.01	11.97 a	9.48	10.60 a	8.00 b	7.53 ab	9.51 AB
		0.05	12.87 a	10.37	9.80 a	8.01 b	8.67 a	9.94 A
		0.1	12.83 a	10.47	9.03 a	6.83 c	6.75 b	9.18 B
		0.2	11.10 ab	10.17	9.25 a	0.00 d	0.00 c	6.10 C
		0.4	9.37 c	8.75	6.50 b	0.00 d	0.00 c	4.92 D
		Mean	11.28 A***	9.81 B	9.19 C	5.41 D	5.30 D	
	Plant stem diameter (mm)	Control	8.20 ns	7.87 ns	8.87 ns	8.54 a***	7.54 b***	8.20 A***
		0.01	8.27	7.99	7.44	5.50 c	9.29 a	7.70 A
		0.05	8.63	9.03	5.77	6.53 b	8.68 ab	7.73 A
		0.1	7.44	6.83	7.82	4.56 d	4.86 c	6.30 B
		0.2	7.65	8.22	5.66	0.00 e	0.00 d	4.31 C
		0.4	8.83	5.61	4.56	0.00 e	0.00 d	3.80 C
		Mean	8.17 A***	7.59 A	6.69 B	4.19 B	5.06 B	
	Number of leaves (number plant^{-1})	Control	31.33 ab*	33.67 a**	34.67 a**	32.33 a***	30.00 a***	32.40 A***
		0.01	34.67 a	33.67 a	24.00 b	19.00 bc	26.00 ab	27.47 B
		0.05	32.67 ab	32.00 a	21.33 b	18.67 c	27.67 a	26.47 B
		0.1	30.67 ab	29.00 a	25.67 b	20.33 b	21.00 b	25.33 B
		0.2	29.00 bc	27.33 a	20.00 b	0.00 d	0.00 c	15.27 C
		0.4	25.33 c	18.50 b	19.00 b	0.00 d	0.00 c	12.57 D
		Mean	30.61 A***	29.03 A	24.11 B	15.06 D	17.44 C	
	Number of branches (number plant^{-1})	Control	2.33 b**	0.67 ns	1.33 ns	1.33 a*	0.67 ns	1.28 B***
		0.01	1.67 b	2.33	1.33	0.00 b	0.00	1.07 B
		0.05	7.33 a	3.33	2.67	0.67 ab	0.33	2.87 A
		0.1	1.00 b	1.00	0.67	0.33 b	0.00	0.60 C
		0.2	1.00 b	2.00	2.00	0.00 b	0.00	1.00 B
		0.4	1.67 b	3.00	1.00	0.00 b	0.00	1.13 B
		Mean	2.50 A***	2.06 A	1.50 B	0.39 D	0.17 C	
	Leaf length (cm)	Control	8.33 ns	8.00 ns	8.67 ns	9.03 a***	8.03 a***	8.41 ns
		0.01	10.92	7.73	7.97	6.50 b	7.10 ab	8.04
		0.05	10.33	8.33	7.50	6.90 b	7.67 a	8.15
		0.1	9.00	8.50	6.83	6.77 b	5.85 b	7.39
		0.2	8.67	7.33	6.40	0.00 c	0.00 c	4.48
		0.4	7.27	6.75	5.33	0.00 c	0.00 c	3.87
		Mean	9.09 ns	7.78	7.12	4.87	4.78	
	Leaf width (cm)	Control	1.47 ns	1.33 ns	1.30 ns	1.40 a***	1.17 a***	1.33 AB*
		0.01	1.37	1.63	1.43	1.10 b	1.10 a	1.33 AB
		0.05	1.37	4.73	1.13	1.17 b	1.17 a	1.91 A
		0.1	1.33	1.30	1.13	1.10 b	1.00 a	1.17 AB
		0.2	1.70	1.23	0.80	0.00 c	0.00 b	0.75 B
		0.4	1.30	1.25	1.13	0.00 c	0.00 b	0.74 B
		Mean	1.42 AB**	1.91 A	1.16 B	0.79 B	0.74 B	

ns: not significant at $p>0.05$, *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ are statistically significant at the probability level. There is no difference at the 5% significance level between means indicated with the same letter.

Table 2. The effects of shoot tip meristem application method, different doses (%) and soaking durations of colchicine

			Soaking durations in colchicine					
		Doses	*6 hours*	*12 hours*	*24 hours*	*48 hours*	*72 hours*	Mean
Shoot tip meristem application method	Plant height (cm)	Control	9.57 ^{ns}	9.53 b***	8.89 b**	9.00 c***	6.00 c***	8.60 C***
		0.01	9.28	12.55 a	13.00 a	9.95 a	10.07 a	10.97 A
		0.05	10.40	12.03 a	12.75 a	10.50 a	9.67 ab	11.07 A
		0.1	11.57	11.87 a	10.40 b	7.95 b	8.67 b	10.07 B
		0.2	11.17	11.83 a	8.78 b	0.00 d	0.00 d	6.36 D
		0.4	9.65	7.67 c	9.00 b	0.00 d	0.00 d	5.26 E
		Mean	10.27 B***	10.91 A	10.47 AB	6.23 C	5.74 C	
	Plant stem diameter (mm)	Control	8.87 b**	7.87 ^{ns}	8.87 ^{ns}	7.90 a***	6.13 a***	7.93 A***
		0.01	9.25 b	7.27	8.64	6.64 b	4.31 b	7.22 A
		0.05	9.78 ab	7.92	8.69	6.22 b	4.43 b	7.41 A
		0.1	10.03 ab	8.65	7.98	6.13 b	5.43 a	7.62 A
		0.2	10.62 a	7.77	8.53	0.00 c	0.00 c	5.38 B
		0.4	7.18 c	4.78	5.90	0.00 c	0.00 c	3.57 C
		Mean	9.29 A***	7.38 C	8.11 B	4.48 D	3.38 E	
	Number of leaves (number plant^{-1})	Control	31.33 a**	33.33 a***	31.33 ^{ns}	31.33 a***	22.50 a***	29.97 A***
		0.01	34.67 a	30.33 a	25.33	21.67 b	20.67 a	26.53 B
		0.05	34.33 a	31.33 a	30.00	22.00 c	13.00 b	26.13 BC
		0.1	32.67 a	29.67 a	26.50	22.50 c	9.67 c	24.04 C
		0.2	33.33 a	21.00 b	24.33	0.00 d	0.00 d	15.73 D
		0.4	21.00 b	19.33 b	24.67	0.00 d	0.00 d	13.00 E
		Mean	31.22 A***	27.50 B	27.06 B	16.25 C	10.97 D	
	Number of branches (number plant^{-1})	Control	1.00 b*	1.67 b*	1.00 b*	2.67 a**	2.00 ^{ns}	1.67 C***
		0.01	2.00 b	5.00 a	3.00 b	2.00 a	2.33	2.87 AB
		0.05	2.67 b	5.00 a	6.00 a	2.50 a	3.00	3.83 A
		0.1	0.33 b	5.00 a	2.00 b	0.00 ab	3.00	2.07 BC
		0.2	5.33 a	1.67 b	1.33 b	0.00 b	0.00	1.67 C
		0.4	1.50 b	2.00 ab	2.67 b	0.00 b	0.00	1.23 C
		Mean	2.14 BC***	3.39 A	2.71 AB	1.19 C	1.72 BC	
	Leaf length (cm)	Control	8.33 ^{ns}	9.00 ab***	9.37 ab*	7.20 ab***	5.53 a ***	7.89 A***
		0.01	8.62	10.33 a	9.50 a	7.00 a	6.33 a	8.36 A
		0.05	8.57	10.33 a	9.75 a	8.00 b	3.50 b	8.03 A
		0.1	9.17	9.33 ab	7.95 abc	5.85 c	3.67 b	7.14 B
		0.2	9.33	8.60 b	7.58 bc	0.00 d	0.00 c	5.10 C
		0.4	7.90	5.80 c	6.57 c	0.00 d	0.00 c	4.05 D
		Mean	8.65 A***	8.90 A	8.48 A	4.68 B	3.17 C	
	Leaf width (cm)	Control	1.47 ^{ns}	1.63 ^{ns}	1.57 ^{ns}	0.97 a***	1.45 a***	1.42 A***
		0.01	1.60	1.43	1.20	1.33 a	1.07 b	1.33 AB
		0.05	1.50	1.53	1.25	1.40 b	0.55 c	1.25 AB
		0.1	1.40	1.40	1.25	1.45 b	0.63 c	1.23 B
		0.2	1.63	1.33	1.50	0.00 c	0.00 d	0.89 C
		0.4	1.40	1.17	0.97	0.00 c	0.00 d	0.71 D
		Mean	1.50 A***	1.42 AB	1.29 B	0.86 C	0.62 D	

^{ns}: not significant at $p>0.05$, *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ are statistically significant at the probability level. There is no difference at the 5% significance level between means indicated with the same letter.

the highest doses of 48 and 72 hours (0.2% and 0.4%). In terms of the number of lateral branches, this formation decreased significantly as the soaking duration increased (Table 1, Table 2). The application of 0.01% colchicine solution to *Impatiens balsamina* L. seedlings was able to induce polyploidy in a study conducted by Wiendra et al. (2011). Observation on morphological traits showed that colchicine treatment increased plant height, stem diameter, leaf length and number of branches (Wiendra et al., 2011). The results of Wiendra et al. (2011) and the increase in the number of branches at 0.05% colchicine dose obtained in our study can be explained. Treatments from the shoot tip of colchicine significantly ($p \leq 0.001$) shortened the leaf length in terms of both dose and soaking duration (Table 2). It was reported by Compton et al. (1996) that chloroplast density, ovary diameter, petal-anther diameter of male flowers and leaf length/width ratio are good indicators of plant ploidy. The evaluation of the leaf length parameter was also carried out for this purpose. According to the results obtained, it was determined that 0.05% and 0.01% doses of colchicine application from the shoot tip increased the leaf length (Table 2). In applications made from the shoot tip, the lowest leaf width was determined at 0.4% colchicine dose; the highest leaf width was determined at 0.0% dose. Based on this result, high-level colchicine application reduced leaf width; as the dose increased, both leaf width decreased and dwarf appearance occurred in the plant. In general, plants died at the highest doses (0.2% and 0.4%) of 48 and 72 h soaking durations. Leaf width increased as the waiting period increased at the colchicine dose, and the decrease in leaf width increased (Table 2). It was reported by Compton et al. (1996) that chloroplast density, ovary diameter, petal-anther diameter of male flowers and leaf length/width ratios are good indicators in determining plant ploidy.

Polyploid plants have a special place in the breeding of ornamental plants. The typical characteristics of polyploidy, such as dark green coloration and large, showy flowers, are of significant importance in the field of ornamental plants. In root tip meristem application, the treatments that resulted in the lowest number of stomata compared to the control group plants were those with 0.2% colchicine for 72 hours and 48 hours, as well as 0.4% colchicine for 48 and 72 hours. While an average of 18.73 stomata were counted on the leaves of the control group plants, the application of 0.2% colchicine resulted in 9 stomata mm^{-2}, and the application of 0.4% colchicine resulted in 9.67 stomata/mm^2. The evaluation of the treatments applied to the shoot tip showed that, in terms of the overall application doses, the highest number of stomata per unit area was 20.20 stomata, observed with a 0.01% colchicine dose, while the lowest number was determined with the highest colchicine doses (Table 3). As it is known, the number of stomata of mutated plants is less compared to the control group plants. Xing et al. (2011) applied colchicine to the seeds of Catharanthus roseus (L.) G. Don and in their stomatal studies, they found that the stomatal size and density of stomata were higher in tetraploid lines than in the control group, and the total stomatal area in tetraploid plants was 1.76% ± 0.01%, while it was 1.24% ± 0.02% in control group plants. Tepe et al. (2002) found that a relationship between stomatal number and chromosome number can be established. The stomatal numbers obtained from this study can be used as a preliminary key to identify folded plants.

Stomatal density per leaf unit area, length and width of stomatal guard cells are used as morphological markers to determine ploidy level in many plant species. In general, the size of leaf stomatal guard cells increases with increasing ploidy level, while stomatal density per leaf unit area decreases (Yen etal., 2010). As a result of flow cytometry analysis, it was determined by Doğan (2017) that the plantlets determined to be polyploid also showed differences in the number, diameter and length of stomata in the 4-hour application of 0.1% colchicine and confirmed polyploidy. Çimen et al. (2016) reported that the number of stomata per mm2 was denser in diploid plants than in tetraploid plants when the leaf stomatal characteristics of clementine 22D mandarin plants at diploid ploidy level and tetraploid ploidy level obtained by colchicine application were examined. The number of stomata obtained as a result of the present research decreased according to the increasing colchicine dose, while the soaking durations did not show a significant difference compared to the control.

As it is known, the stomatal diameters of the mutated plants are higher compared to the control group plant. Xing et al. (2011) applied colchicine to the seeds of *Catharanthus roseus* (L.) G. Don in their study. In their stomatal studies, they found that the stomatal size and density in tetraploid lines were higher than those in the control group. Padoan et al. (2013) reported that triploid *Clementine mandarin* plants had larger stomatal sizes, both in length and width, compared to diploid plants. Similarly, Sharif et al. (2013) noted that the stomatal sizes of tetraploid individuals obtained from certain citrus rootstocks were larger than those of the diploid ones. In root applications, when compared to the control group in terms of stomatal width, the treatments with 0.01%, 0.05%, and 0.1% colchicine doses showed the highest stomatal width (with average values of 33.36, 33.39, and 35.61 µm, respectively). In shoot tip applications, the highest values of stomatal width compared to the control group were observed with the 0.01%, 0.05%, and 0.1% colchicine doses, while a decrease was noted with the highest colchicine dose (Table 3). This decrease can be explained by the toxic effect of the high dose. The optimal soaking duration for increasing stomatal width was found to be 24 hours, as shown in Table 3.

In the present study, as the dose amount increased, stomatal length decreased and this decrease was statistically significant compared to the control. It was determined that 12 hours was the most appropriate soaking duration for the increase in stomatal length in root applications; 6 and 12 hours were determined as the most appropriate soaking duration in shoot tip applications. As it is known, the stomatal length of the mutated plants is higher compared to

the control group plants. Maghbel (2015) observed that stomatal length was 128.01 nm and 181.86 nm, respectively, in 24-hour application of 0.05% and 0.1% colchicine for *Glycyrrhiza glabra* L. var. glandulifera plant, while it was determined to be 84.7 nm in the control group. For *Carthamus tinctorius*, the stomatal length was 132.67 nm and 123.83 nm in 0.05% and 0.1% colchicine 24 h treatment, respectively, while the control group was 99.44 nm (Maghbel 2015).

Table 3. The effects of different application methods, different doses (%) and soaking durations of colchicine on stomatal number, stomatal width and stomatal length

			Soaking durations in colchicine					
		Doses	*6 hours*	*12 hours*	*6 hours*	*48 hours*	*6 hours*	Mean
Root tip meristem application method	Stomatal number (pcs mm^{-2})	Control	16.67 ^{ns}	17.67 ^{ns}	18.33 bc**	20.00 c***	21.00 a***	18.73 B***
		0.01	23.00	27.00	24.33 a	24.67 b	21.00 a	24.00 A
		0.05	25.50	22.00	24.00 a	24.00 b	18.67 a	22.83 A
		0.1	18.33	15.00	23.00 ab	36.33 a	21.00 a	22.73 A
		0.2	11.33	16.67	17.00 c	0.00 d	0.00 b	9.00 C
		0.4	18.33	15.50	14.50 c	0.00 d	0.00 b	9.67 C
		Mean	18.86 A***	18.97 A	20.19 A	17.50 A	13.61 B	
	Stomatal width (µm)	Control	22.23 ^{ns}	22.23 b***	22.23 ***c	22.57 d***	21.90 c***	22.23 B***
		0.01	22.83	23.20 b	34.38 b	45.38 a	41.02 a	33.36 A
		0.05	27.23	24.20 b	29.60 bc	43.43 b	42.50 a	33.39 A
		0.1	25.43	30.67 a	55.27 a	31.83 c	34.85 b	35.61 A
		0.2	27.2	21.73 b	45.00 a	0.00 e	0.00 d	18.79 C
		0.4	30.67	33.80 a	47.25 b	0.00 e	0.00 d	22.34 B
		Mean	25.93 B***	25.97 B	38.96 A	23.87 BC	23.38 C	
	Stomatal length (µm)	Control	37.23 c**	34.57 ^{ns}	39.23 b***	37.48 a***	34.82 ab***	36.67 A***
		0.01	36.99 c	42.73	47.57 a	25.51 c	23.95 b	35.35 A
		0.05	41.83 bc	50.27	46.73 a	27.33 b	25.73 b	38.38 A
		0.1	43.40 bc	56.73	24.67 d	26.56 bc	39.50 a	38.17 A
		0.2	55.30 a	52.3	30.15 c	0.00 d	0.00 c	27.55 B
		0.4	47.23 ab	54	29.15 c	0.00 d	0.00 c	26.08 B
		Mean	43.66 B***	48.43 A	36.25 C	19.48 D	20.67 D	
Shoot tip meristem application method	Stomatal number (pcs mm^{-2})	Control	16.67 ab**	19.67 ab*	21.33 a**	16.33 b***	23.67 a***	19.53 A***
		0.01	19.67 a	23.00 a	15.00 bc	19.00 a	24.33 a	20.20 A
		0.05	14.00 bc	20.67 ab	14.50 bc	10.00 c	23.00 a	16.43 B
		0.1	18.67 a	19.33 ab	12.50 c	22.00 ab	19.33 b	18.79 A
		0.2	12.33 c	15.33 c	18.00 b	0.00 d	0.00 c	9.13 C
		0.4	17.50 ab	12.00 bc	16.33 b	0.00 d	0.00 c	9.17 C
		Mean	16.47 B***	18.33 A	16.50 B	11.22 D	15.06 C	
	Stomatal width (µm)	Control	22.23 b*	23.23 c**	23.60 b***	53.73 b***	34.39 c***	31.44 B***
		0.01	25.87 ab	28.99 bc	46.98 a	52.34 a	44.70 b	39.77 A
		0.05	28.07 a	26.93 bc	47.25 a	51.25 a	45.43 ab	39.79 A
		0.1	30.57 a	26.27 bc	53.10 a	34.39 c	48.70 a	37.57 A
		0.2	25.67 ab	33.67 b	52.85 a	0.00 d	0.00 d	22.44 C
		0.4	30.05 a	43.73 a	50.40 a	0.00 d	0.00 d	24.84 C
		Mean	27.08 D***	30.47 BC	45.26 A	31.95 B	28.87 CD	
	Stomatal length (µm)	Control	37.23 c**	35.57 c*	36.48 a**	25.93 b***	25.04 b***	32.05 C***
		0.01	48.53 ab	46.20 abc	29.16 b	34.37 a	34.07 a	38.47 AB
		0.05	56.43 a	49.17 abc	29.15 b	35.15 a	34.14 a	40.81 A
		0.1	53.60 ab	41.77 bc	31.15 b	25.04 b	28.25 b	36.30 B
		0.2	44.97 bc	54.33 ab	27.75 b	0.00 c	0.00 c	25.41 D
		0.4	57.55 a	58.30 a	27.03 b	0.00 c	0.00 c	28.58 D
		Mean	49.72 A***	47.56 A	30.06 B	20.08 C	20.25 C	

^{ns}: not significant at $p>0.05$, *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ are statistically significant at the probability level. There is no difference at the 5% significance level between means indicated with the same letter.

In addition, autotetraploid plants could be obtained from the application of 0.5% colchicine to the growth tip of basil (*Ocimum basilicum*) seedlings at the cotyledon development stage. Tetraploid plants showed larger stomata, pollen, increased number of chloroplasts in guard cells and decreased stomatal density compared to diploids (Omidbaigi et al., 2010). In the present study, it was concluded that the plants kept in 0.1% dose of colchicine for 12 hours in both treatments could be tetaploid plants in terms of stomatal length, stomatal number and stomatal length parameters (Table 3). However, it was stated that stomatal examinations before determining the ploidy level were beneficial in reducing the cost of flow cytometry (Aydın et al., 2021).

4. Conclusion

In this study, due to the increasing interest in the use of native plant species in planting designs and landscaping arrangements, it was tried to obtain tetraploid individuals of *Silene compacta* Fischer, which is rich in the native flora of Erzurum (Türkiye), by applying colchicine to the root tip and shoot tip of young seedlings at different soaking durations and doses.

The highest doses of colchicine applied both from the root and shoot tip were found to have a lethal effect on the plants at the highest soaking durations. In general, high doses and prolonged application of colchicine caused a decrease in the viability rate. In conclusion, as a result of this study, which aimed to obtain larger leaves, flowers and plants, it was determined that the most effective concentration of colchicine for polyploid induction of *S. compacta* was 0.1%, the application time was 12 and 24 hours and there was no statistically significant difference between the application methods and success could be achieved with the use of both application methods. With this study, it is also suggested that a variety of *S. compacta* breeding material with improved characteristics can be developed that can be used as parent material in future breeding programs.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Determination of chemical composition and biological activity of flaxseed (*Linum usitatissimum*) essential oil

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Abstract

The essential oil was obtained from flaxseed (*Linum usitatissimum*) through cold press oil machine of ripe seeds. The chemical composition of the flaxseed essential oils was analyzed by GC-MS. The results revealed that the chemical composition of flaxseed essential oil was found as 9,12-Octadecadienoic acid (Z, Z)-(33.16%), Tributyl acetylcitrate (15.31%), 9,12,15-Octadecatrienoic acid (Z, Z, Z)-(15.28%), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z)-(12.72%), and Ethanol, 2-(9,12-octadecadienyloxy)-(Z, Z)-(9.54%) were found as major compounds followed by Ethyl Linoleolate (3.64%), Tricyclo [6.4.0.0 (3,7) and dodecane (2.04%)].These chemical compounds identified has general biological activities (Antioxidant, Antimicrobial Activity ,anti-inflammatory, Nematicide, Antihistaminic Antieczemic, Insectifuge). As a result of this study, it can be suggested that flaxseed essential oil in the biological application.

Keywords: Flaxseed (*Linum usitatissimum*), Essential oil, GC-MS, Biological activity, Chemical composition

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1. Introduction

One of the earliest crops to be farmed, flaxseed (*Linum usitatissimum* L.) is still widely planted for food, oil, and fiber (Jhala, & Hall, 2010). In 2022, the average global production of flaxseed was 3,973,931.78 tons (FAO, 2023). Because of its quick polymerization, flaxseed oil is a great source of linolenic acid, an omega-3 fatty acid, with normal levels of 55% of the oil (Oomah, 2001). This makes it perfect for paints, varnishes, and inks. Flaxseed is being used as a functional food due to the growing desire for edible oil sources that contain notable percentages of omega-3 fatty acids. In order to enhance the health and reproductive performance of animals, flaxseed is also added to their feed (Salem, 2022; Turner et al., 2014).

Flaxseed is consumed in a variety of forms, such as whole seeds, ground whole seeds, flaxseed oil, partially and fully defatted flaxseed meal (usually from solvent extraction), flaxseed hulls, flaxseed extracts, and partially defatted flaxseed meal (usually from expeller pressing) (Ganorkar & Jain, 2013, Kaur et al., 2018). These goods are all linked to particular positive health outcomes.

The main purpose of the current study is to determine the chemical composition and biological activities of flaxseed (*Linum usitatissimum*) essential oil.

2. Material and Methods

2.1. Plant material and extraction of essential oil

Flaxseeds (*Linum usitatissimum*) were obtained from a local supermarket in Kastamonu, Türkiye then the seed has been cold-pressed. To this end, flaxseeds were dried at a temperature lower than 40°C and then grounded. After grinding, the oils were collected in oil collecting chutes by pressing them without any exposure to heat. After the oil was squeezed, the pulp was discarded and the oil was left to rest for 5 days. The solid layer in the rest oil is sent to the paper or cloth filters without moving. At this stage, the

filtered oils get clarity and kept at room temperature until used.

2.2. Determination of chemical components by GC-MS Analyze

To identification of chemical components, oil Sample was analyzed by GCMS QP 2010 Ultra (Shimadzu) equipped with a Rtx-5MS capillary column (30m·0.25 mm; coating thickness 0.25 μm).

The essential oil components were identified by comparing their relative retention times and mass spectra with those of authentic samples (analytical standards from Aldrich, Acros and Fluka; purity ≥ 97%). Sample solutions were prepared in n-hexane (GC grade, Merck) at 1.0% (w/w).

A summary of the working conditions of the gas chromatography-mass spectrophotometer device are given in Table 1.

Table 1. The effects of root tip meristem application method, different doses (%) and soaking durations of colchicine

Feature	Conditions
Column	RTX-5MS Capillary column (30 m; 0,25 mm; 0,25 μm)
Carrier gas	Helium
Column oven temperature	90˚C
Injection temperature	250˚C
Pressure	90 kPa
Injection mode	Split
Split ratio	10
Injection volume	1µL
Oven temperature program	5 minutes at 90 C, 4 C min-1 increments from 90 C to 250 C, 5 min at 250 C
Interface temperature	250˚C
Ion source temperature	200˚C

3. Results and Discussion

3.1. Chemical composition

Table 2 and Figures 1 shows the results pertaining to the GC-MS chromatogram analyses of flaxseed oil. The compounds are listed in order of their elution time on the column. 31 compounds were detected in flaxseed oil.

The results revealed that 9,12-Octadecadienoic acid (Z, Z)-(33.16%), Tributyl acetylcitrate (15.31%), 9,12,15-Octadecatrienoic acid (Z,Z,Z)-(15.28%), 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z)-(12.72%), and Ethanol, 2-(9,12-octadecadienyloxy) (Z, Z)-(9.54%) were found as major compounds followed by Ethyl Linoleolate (3.64%), Tricyclo [6.4.0.0(3,7) and dodecane (2.04%)].

3.2. Biological activities

Previous studies have demonstrated that 20 of 31 of flaxseed oil compounds has a variety of pharmacological and biological functions (Table 3). 9,12-Octadecadienoic acid is the predominant ingredient and essential bioactive ingredient in flaxseed oil, and its health benefits and pharmacological activities have been confirmed according to a large number of animal and clinical experiments (Zheng et al., 2020, Gharibzahedi & Smith, 2021; Shenoy, et al., 2022; Nasr et al., 2024; Attia et al., 2024).

Dietary fiber, protein, and fat are all abundant in flaxseed. According to chemical analysis, flaxseed typically had 30-40% oil, 20-25% protein, 20-28% total dietary fiber, 4-8% moisture, and 3-4% ash. The inclusion of physiologically active food components in the oil provides vitamins A, B, D, and E, as well as minerals and amino acids, which may have health advantages beyond basic nutrition. In recognition of flaxseed's high concentration of dietary fiber, natural phenolic antioxidants, alpha-linolenic acid (ALA), and important omega-3 fatty acids, its use in food and food products has been growing daily. One of the main sources of phytochemicals is now flaxseed (Shahzad et al., 2006). These chemical compounds (phenolic acids, cinnamic acids, flavonoids and lignins) are antioxidants as well as affect the cell growth and viability (Salem et al., 2023; Lakwani & Salem, 2024). According to Amin and Thakur (2014), flaxseed has significant potential as an alternative source of phenolic compounds and is a vital supply of high-quality protein and soluble fiber.

The most abundant source of lignans in diet is flaxseed, where secoisolariciresinol diglucoside (SDG) predominates. Plant lignans are a biologically significant class of phenolic chemicals (Salem & Moammer, (2024). Few studies on the stability of lignans during the food process have demonstrated that SDG levels did not change when flaxseed-containing breads and cookies were made (Cardoso et al., 2012). One of the components, dietary fibers, reduces serum cholesterol and flattens the blood glucose profile, similar to guar gum, oat gum, and other viscous fibers (Jenkins, 1995). Flavonoids have antibacterial, anticancer, anti-inflammatory, and mildly hypersensitive effects of oxidative cell damage in linseed (Pruthi, 2007; Taştan & Salem, (2021).

The potential bioactivities and health benefits of flaxseed oil are summarized and discussed in detail below Table 3.

Table 2. Results of chemical composition analysis of flaxseed oil

No	R.Time	Peak Area (%)	Name of the compound
1	10.027	0.80	Hydroperoxide, 1-ethylbutyl (CAS)
2	10.405	0.89	Pentane, 3-ethyl-2,4-dimethyl- (CAS)
3	10.791	0.15	3-Hexen-2-ONE
4	11.450	0.09	Myrcene
5	11.742	0.39	Heptandienal <2,4-trans,trans->
6	12.285	0.08	Heptandienal <2,4-trans,trans->
7	12.875	0.32	dl-Limonene
8	22.983	0.21	7-Methylene-9-oxabicyclo [6.1.0]non-2-ENE
9	23.094	0.09	2,4-Decadienal, (E, E)- (CAS)
10	23.197	0.10	Tridecane
11	23.913	0.40	endo-Dicyclopentadiene dioxide
12	43.170	0.22	Palmitic acid
13	46.216	0.09	10,13-Octadecadienoic acid, methyl ester
14	46.374	0.31	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-
15	47.049	1.40	2-Ethylhexyl methyl isophthalate
16	47.295	1.40	Tetradecalactone <delta->
17	47.717	22.85	9,12-Octadecadienoic acid (Z, Z)-
18	47.948	7.88	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-
19	48.215	9.54	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)-
20	48.295	10.31	9,12-Octadecadienoic acid (Z, Z)-
21	48.620	15.28	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-
22	50.192	15.31	Tributyl acetylcitrate
23	50.845	4.84	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z ,Z)-
24	51.135	2.04	Tricyclo [6.4.0.0 (3,7)] dodecane
25	51.322	3.57	Ethyl Linoleolate
26	52.055	0.14	2H-Pyran-2-one, tetrahydro-6-tridecyl-
27	54.637	0.14	Bicyclo[10.1.0]tridec-1-ene
28	54.745	0.24	DI-(9-Octadecenoyl)-Glycerol
29	54.854	0.54	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)-
30	55.175	0.21	2-Bromotetradecane
31	56.893	0.17	Ethyl Linoleolate

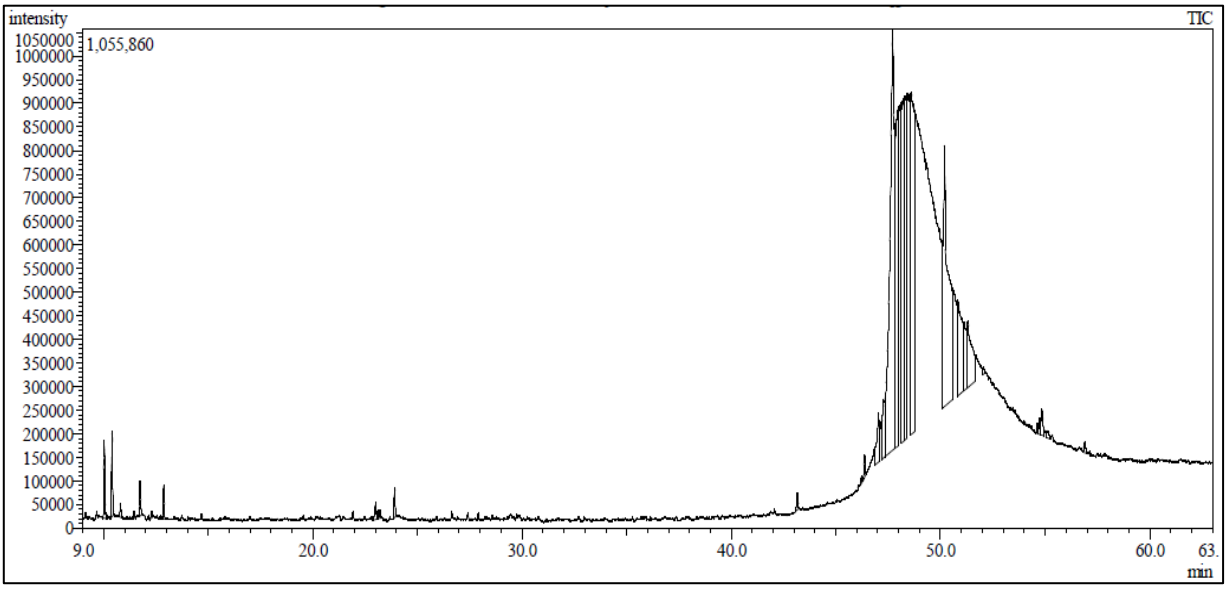

Figure 1.The chromatogram from the GC-MS analysis of the flaxseed oil

Table 3. The chemical compounds identified from the flaxseed oil and their general biological activities

No	Name of the compound	Reported Biological Properties	References
1	Myrcene	Antimicrobial	Inoue et al. (2004)
2	Heptandienal <2,4-trans,trans->	Antimicrobial	Wang et al. (2018)
3	dl-Limonene	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer	Erasto & Viljoen (2008)
4	2,4-Decadienal, (E,E)- (CAS)	Has negative effects on marine invertebrate larval survival	Caldwell et al. (2005)
5	Tridecane	Antibacterial	Sreedharan et al. (2019)
6	Palmitic acid	Anti-inflammatory	de Souza et al. (2018)
7	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	Anti-inflammatory, Hypocholesterolemic, Antihistaminic	Srinivasan et al. (2013)
8	9,12-Octadecadienoic acid (Z, Z)-	Anti-inflammatory, Anti-arthritic	Lalitharani et al. (2009)
9	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-	Antioxidant, Antimicrobial, Activity, Anti-inflammatory, Nematicide, Antihistaminic, Antieczemic, Insectifuge	Al-Gara et al. (2019)
10	Ethanol, 2-(9,12-octadecadienyloxy) -, (Z, Z)-	Antibacterial, Anti-inflammatory	Hase et al. (2017)
11	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	Antimicrobial	Al-Gara et al. (2019)
12	Tributyl acetylcitrate	Anti-bacterial, Antioxidant, Anti-inflammatory	Al-Rubaye et al. (2017a, 2017b)
13	Ethyl Linoleolate	Antioxidant	Masuda et al. (2006)
14	2H-Pyran-2-one, tetrahydro-6-tridecyl-	Antidiabetic, Gastro intestinal, Antibacterial, Antioxidant, Mitogenic, Anticancer	Ramya et al. (2015)
15	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)-	Antioxidant, Antimicrobial	Fatema et al. (2019)
16	2-Bromotetradecane	Antioxidant, Antimicrobial	Sasikumar et al. (2020)

4. Conclusion and Future Perspectives

Finally, it can be suggested that the essential oils of flaxseed have strong biological activities such as Antioxidant, Antimicrobial Activity, anti-inflammatory, Antihistaminic Antieczemic, Insectifuge Antibacterial, anti-inflammatory. The flaxseed essential oils may find industrial applications as natural preservatives and antimicrobial agents in cosmetics and food industries.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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