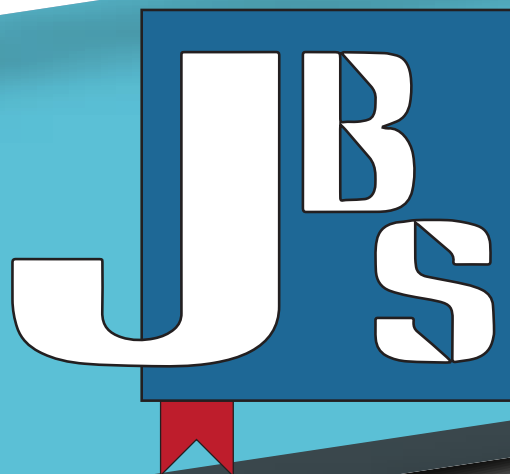


Journal of Biometry Studies

ISSN: 2791-7169

Year: 2025 - Volume: 5 - Issue: 1



Journal of Biometry Studies

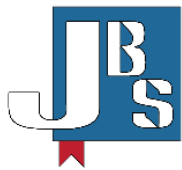
Year: 2025, Volume: 5, Issue: 1

ISSN: 2791-7169

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Journal of Biometry Studies

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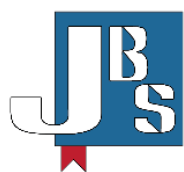
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Effects of using quinoa flour in meatballs produced from turkey meat on mineral substances, cooking and sensory properties

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Received: 19/02/2025, Accepted: 07/04/2025

Abstract

In this study, quinoa flour (0-control, 2.5% and 5%) was used instead of breadcrumbs in meatballs produced from turkey meat. At the end of the production; pH, moisture, cooking properties (cooking loss, diameter reduction and cooking yield), phenolic compounds, mineral substances and sensory analyses were performed on the samples. As a result of the analyses, it was determined that sensory parameters were not statistically affected by the use of quinoa ($p>0.05$). Similarly, quinoa use had no statistical effect on pH and moisture ($p>0.05$). However, cooking loss, diameter reduction and cooking yield were affected by quinoa use at a rate of $p<0.01$. Cooking loss and diameter reduction, which decreased with the use of quinoa, showed the highest mean values in the control group. Furthermore, the lowest cooking yield value was also determined in the control group. Among the analyzed phenolic compounds (cinnamic acid, gallic acid, tannic acid, caffeic acid, 2-5 dihydroxy benzoic acid, trans ferulic acid, rutin trihydrate, myricetin, naringenin, allagic acid, quercetin, luteolin, chrysin, apigenin, CAPE and triacetin), only gallic acid and catechin were detected in the meatballs. While gallic acid was not statistically affected by the quinoa usage rate ($p>0.05$), catechin was affected by this factor at $p<0.05$ level. Catechin showed the highest mean value in the group containing 5% quinoa. All mineral substances analyzed in the meatballs (Fe, Zn, Mg, Ca and P) were significantly affected by the quinoa usage ($p<0.01$). All of these mineral substances showed gradual increase with use of quinoa. It was concluded that quinoa could also be an alternative ingredient to breadcrumbs in meatballs produced from turkey meat.

Keywords: Turkey meat, Meatball, Quinoa, Mineral matter, Phenolic compounds

Please cite this article as follows:

Aldabak, W., & Hazar Suncak, F. Y. (2025). Effects of using quinoa flour in meatballs produced from turkey meat on mineral substances, cooking and sensory properties. *Journal of Biometry Studies*, 5(1), 1-6. <https://doi.org/10.61326/jofbs.v5i1.01>

1. Introduction

Quinoa contains the amino acid requirements of adults, according to the daily amino acid intake requirements specified by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Therefore, quinoa can be used in nutritious foods and beverages. The biological value of quinoa is 73%, which is close to beef, higher than white rice (56%), wheat (49%) and corn (36%) (Bastidas et al., 2016). Quinoa is rich food in terms of protein and has a high biological protein value as much as beef (Singh et al., 2016). Its seeds are remarkable not only for their high protein content but also their amino acid balance (Repo-Carrasco-Valencia et al., 2011; Carciochi et

al., 2014; Bastidas et al., 2016). In the report, published by FAO, WHO and United Nations University, it was stated that quinoa consumption has an amino acid profile that can meet the daily amino acid requirement of an adult such as 228% of tryptophan, 338% of lysine, 274% of isoleucine, 212% of methionine and cysteine, 320% of phenylalanine and tyrosine, 331% of threonine, 180% of histidine and 323% of valine. Quinoa plays a complementary role in the essential amino acids (especially lysine, methionine and cysteine) which are deficient in grains and legumes (Üçok et al., 2019).

Quinoa is a gluten-free grain type and a rich source of vitamins (E, C, B complex) and minerals (K, Ca, Mg, Fe,



P, Mn) (Miranda et al., 2012). On the other hand, carbohydrates constitute the majority of the dry weight of quinoa seeds. The most dominant components among these carbohydrates are starch and dietary fiber. Dietary fiber is resistant to enzymatic digestion and absorption in our digestive system; in addition to that, it usually undergoes complete or partial fermentation in the large intestine. Dietary fiber is essential for maintaining optimal digestive health and provides several functional advantages such as increasing satiety, reducing cholesterol and lipid absorption and improving the gut microbiota composition (Sharma et al., 2015). Moreover, this grain has a low glycemic index (Graf et al., 2015).

Another nutritional importance of quinoa is that it does not contain gluten. Despite the fact that consumption habits have changed worldwide, grain products still maintain their importance in nutrition. However, for some people, grains such as wheat, rye, oat and their products can cause intestinal malabsorption that can lead to celiac disease. Therefore, the only long-term solution for celiac patients is to follow a gluten-free diet (Al Shehry, 2016). It is stated that quinoa is a suitable ingredient for gluten-free diet followers who do not want to give up the foods such as bread or pasta (Singh et al., 2016). According to a study, 22 students between the ages of 18-45 were given quinoa in the form of a cereal bar once a day for 30 days. At the end of the study, it was revealed that quinoa-supported nutrition caused a decrease in triglyceride and LDL-cholesterol levels (Farinazzi-Machado et al., 2012).

Quinoa is usually mixed with wheat flour and can be used in bakery products (Enriquez et al., 2003). Many studies have been conducted on the use of quinoa in bread production (Lorenz & Coulter, 1991; Morita et al., 2001; Enriquez et al., 2003). However, studies on the use of this grain in meat products are quite limited. İnce (2019) added quinoa flour and κ -carrageenan to chicken meatballs at different rates and analyzed the product in terms of pH, moisture, aw, TBARS, L*, a*, b*, cooking efficiency, moisture retention, textural properties and sensory parameters. In another study, quinoa flour was included in the production of beef meatballs; the product was examined in terms of physicochemical, sensory and textural aspects (Bağdatlı, 2018). In the study conducted by Kuru (2021), quinoa flour was added to beef patties at different rates and the product was analyzed in terms of acrylamide and some other quality criteria. On the other hand, no study was found in the literature examining the effects of quinoa flour on the properties of patties produced from turkey meat. In addition, no study was also found examining the effects of quinoa flour on the mineral substances and phenolic compounds of turkey meatballs.

In this research, quinoa flour instead of breadcrumbs was used in meatball production from turkey meat at different rates (0-control, 2.5% and 5%). At the end of production; pH, moisture, cooking properties (cooking loss, diameter reduction and cooking yield), phenolic compound and

mineral substance analyses were applied to the meatballs. Furthermore, the products were also tested in terms of sensory properties.

2. Material and Method

2.1. Material

Ground turkey meat, ground beef fat, quinoa flour, salt and breadcrumbs used in meatball production were purchased from the Kastamonu market.

2.2. Method

Meatball production

In all groups, 84% turkey meat, 10% beef fat and 1% salt were used. In addition, 5% breadcrumbs, 2.5% breadcrumbs + 2.5% quinoa flour and 5% quinoa flour were used in the other groups, respectively. 40 g of meatballs were taken and shaped using a metal mold (6.5 cm diameter and 1 cm thickness). Sensory, mineral matter, phenolic compound, cooking properties, pH and moisture analyses were performed on the produced meatball samples.

Sensory analysis

Meatball samples were cooked and subjected to sensory analysis by 10 semi-trained panelists using a hedonic type scale (1-9). Meatballs were tested for sensory analysis in terms of color, appearance, odor, texture, taste, degree of cooking and general acceptability. 1 point was evaluated as the lowest (undesirable) score and 9 point as the highest (desirable) one.

pH

10 g of meatball sample was weighed and 100 ml of pure water was added on. Measurements were performed using a previously calibrated pH meter (Isolab, Germany) with appropriate calibration fluids. Analyses were performed in two replicates.

Moisture

10 g of meatball samples were weighed, then taken into pre-dried and tared nickel containers. After that they kept drying at 105 °C up to a constant weighing weight. As a result, the results were expressed as moisture %. Analyses were performed in two replicates.

Cooking loss, diameter reduction and cooking yield

Meatball samples were weighed both before and after cooking. In addition, the diameters of the meatballs were measured using a ruler both before and after cooking. Cooking loss (CL), diameter reduction (DR) and cooking yield (CY) were determined using the appropriate formulas. Analyses were performed in five replicates.

$$CY = \frac{A}{B} \times 100 \quad (1)$$

$$CL = \frac{A - B}{B} \times 100 \quad (2)$$

$$DR = \frac{C - D}{C} \times 100 \quad (3)$$

Where;

A: Cooked meatball weight (g),

B: Raw meatball weight (g),

C: Raw meatball diameter (cm),

D: Cooked meatball diameter (cm).

Phenolic compounds

The method proposed by Escarpa and González (2001) was applied for the extraction of phenolic compounds. 25 ml of 1% BHT solution prepared using 80% methanol was added on 5 g of sample. The samples homogenized using Ultra-turrax device (Velp Scientica, Italy) were processed for 2 hours in an ultrasonic water bath (ISOLAB Ultrasonic Water Bath, Germany) at room temperature. The samples were transferred to vials by passing through a 0.45 micron membrane filter. Phenolic compounds were determined by liquid chromatography-mass spectrometry (LC-MS/MS). While the injection volume of the device was 10 µL, the device column was Inertsil ODS4 analytical column (GL Sciences, Japan), the column diameter was 3 µM, the column size was 2.1 x 50 mm; the mobile phases in the analysis were Mobile Phase A: Water that contains 1% Formic Acid, Mobile Phase B: Methanol that contains 1% Formic Acid. The flow rate in the column was 0.4 mL/min and the column temperature was 40 °C. The calibration points were determined as 10, 50, 100, 200 and 500 ppb. Analyses were performed in two replicates.

Mineral matters

The samples dried at 105 °C and weighed as 0.25 g were transferred into teflon beakers. Then, 10 ml of HNO₃ (67% v/v) was added to the meatball samples and organic burning process was performed in the microwave at 210 °C for 30 min using the Run-Food method. After that, ultrapure water was added on the samples that were left cooling at room temperature in order to complete the volume up to 25 ml. The samples filtered through the microfilter were processed so that no particles remained in them. Concentrations of the metals were measured in the ICP-OES (Spectro Blue, Germany) device. Multielement standard stock solution (Merck, Germany) was used in the preparation of calibration standards for the ICP-OES device. Measurements against the blank for each element were performed. Analyses were performed in three replicates.

Statistical analysis

In the study, quinoa flour ratio was selected as the factor and the trial was carried out as 2 replications depending on complete randomization. The data found to be significant ($p < 0.01$ or $p < 0.05$) in the variance analysis

applied to the results that were obtained from the analyzes were compared using Duncan's Multiple Comparison test.

3. Results and Discussion

As seen in Table 1, the use of quinoa flour had no statistically significant effect on any sensory parameter ($p > 0.05$). In other words, according to the panelists, the use of quinoa showed no change in the sensory properties of the meatballs such as traditional taste, appearance, texture, color, odor and general acceptability (Figure 1). This result shows that quinoa flour can be used instead of breadcrumbs in meatball production from a sensory perspective. In a study investigating the effects of quinoa flour on some properties of chicken meatballs, the use of quinoa flour increased the texture scores of sensory parameters, while decreasing the scores of appearance, odor, taste and color. Researchers have also emphasized that moisture and fat content can change the sensory characteristics of cooked products, especially affecting texture and taste parameters (Meral et al., 2022). In fat-reduced chicken meatballs produced with quinoa flour and κ-carrageenan, sensory analysis was not statistically affected by these factors (İnce, 2019).

Table 1. The effect of using quinoa in turkey meatball on sensory properties

Sensory parameters	Quinoa Flour Ratio (%)			Sig.
	0-Control	2.5	5	
Color	6.85±1.53 ^a	6.95±1.23 ^a	6.60±1.35 ^a	NS
Appearance	7.20±1.32 ^a	7.25±1.12 ^a	6.80±1.32 ^a	NS
Odor	7.05±1.39 ^a	7.10±1.29 ^a	6.85±1.27 ^a	NS
Texture	7.15±1.66 ^a	7.00±1.75 ^a	6.80±1.47 ^a	NS
Taste	7.10±1.52 ^a	6.90±1.77 ^a	6.40±1.23 ^a	NS
Cooking level	7.10±1.68 ^a	7.00±1.86 ^a	6.50±1.67 ^a	NS
General acceptability	7.10±1.59 ^a	7.15±1.46 ^a	6.45±1.61 ^a	NS

^a: Same letters indicate no statistical difference ($p > 0.05$) in each line, NS: not significant.

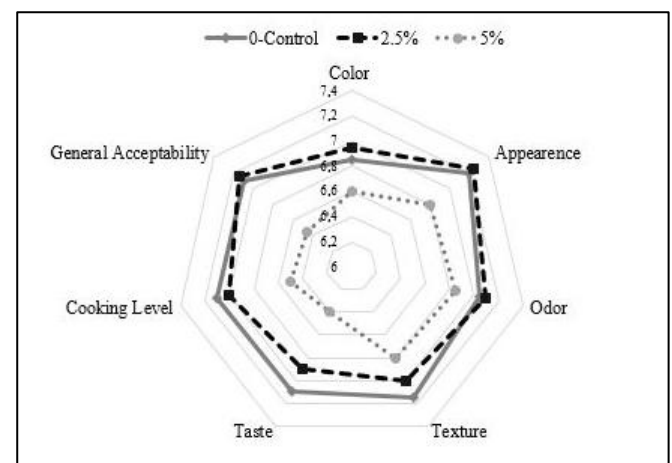


Figure 1. Sensory analysis of meatball with quinoa flour

The use of quinoa flour in meatball production did not cause a statistical change in pH and moisture values (Table 2). Kuru (2021) found that the pH value in meatballs produced from beef increased with the increase in the amount of quinoa flour and there were statistical changes in moisture value. In the fat-reduced chicken meatballs produced with quinoa flour and κ -carrageenan, the highest pH value was determined in the control group and the moisture value was not affected by the quinoa usage rate (İnce, 2019). The use of quinoa flour in chicken meatballs caused statistical changes in pH value during storage (Meral et al., 2022). Bağdatlı (2018) determined that the moisture value increased with the use of quinoa flour in meatballs produced from beef, but there was no change in pH value. Cooking loss, diameter reduction and cooking yield values were affected by the use of quinoa flour at the $p<0.01$ level. The use of quinoa flour had a positive effect on these values. The cooking loss value showed the highest average value of 12.06 in the control group. In addition, the highest average diameter reduction value was determined in the control group meatballs. There was a decrease in cooking loss and diameter reduction values with the use of quinoa flour. On the other hand, the lowest average cooking yield value was determined in the control group. Cooking yield increased with the use of 2.5% and 5% quinoa flour in the samples (Table 2). Cooking yield is one of the most important factors affecting the water retention capacity of myofibrillar proteins during cooking (Jiang et al., 2024). Thus, yield is closely related to the shrinkage of the product. This also affects consumer preference (Meral et al., 2022). In the study conducted by Jiang et al. (2024), it was determined that quinoa protein increased the cooking yield of meatballs produced from pork. Meral et al. (2022) determined that quinoa flour increased the cooking yield in chicken meatballs produced using quinoa. In the reduced-fat chicken meatballs produced

with quinoa flour and κ -carrageenan, the lowest average cooking yield was determined in the control group. These results show that the use of quinoa flour in meatball production improves cooking properties and contributes to the reduction of economic losses. In a study conducted on beef meatballs, it was determined that the highest cooking loss value was in the 2% quinoa group and the lowest value was in the 6% quinoa group (Kuru, 2021).

Phenolic compounds, which are secondary metabolites of plants, have at least one hydroxyl group containing an aromatic hydrocarbon ring (Agarwal et al., 2023). Xanthenes, flavonoids, quinines, phenolic acids, phenols, cumarines, phenylpropanoids and lignans are phenolic compounds found in plants. On the other hand, quinoa also contains some important phenolic compounds such as quercetin and kaempferol. Quinoa seeds show antioxidant properties due to the phenolic compounds they contain (Balakrishnan & Schneider, 2022; Agarwal et al., 2023). Meatballs produced using quinoa were analyzed for phenolic compounds (cinnamic acid, gallic acid, tannic acid, caffeic acid, 2-5 dihydroxy benzoic acid, trans ferulic acid, rutin trihydrate, myrcetin, naringenin, allagic acid, quercetin, luteolin, chrysin, apigenin, CAPE and triacetin). Of the analyzed phenolic compounds, only gallic acid and catechin was within the detectable limits. The failure to detect other phenolic compounds is probably due to their amounts being below the calibration value. Gallic acid was not affected by the quinoa usage rate ($p>0.05$). On the other hand, catechin, another phenolic compound determined in meatballs, was statistically affected by the use of quinoa and showed the highest average value in the group with 5% quinoa (Table 2). This situation proves that the use of quinoa resulted in increase of phenolic compounds in meatballs.

Table 2. The effect of using quinoa in turkey meatball on pH, moisture, cooking properties, gallic acid and catechin

Analyses	Quinoa Flour Ratio (%)			Sig.
	0-Control	2.5	5	
pH	6.55±0.05 ^a	6.61±0.02 ^a	6.62±0.04 ^a	NS
Moisture (%)	58.23±0.59 ^a	57.55±0.34 ^a	58.05±0.83 ^a	NS
Cooking loss (%)	12.06±1.82 ^a	10.01±1.05 ^b	9.35±0.98 ^b	**
Diameter reduction (%)	6.07±3.62 ^a	3.09±2.76 ^b	1.67±2.61 ^b	**
Cooking yield (%)	87.94±1.82 ^b	89.99±1.05 ^a	90.65±0.98 ^a	**
Gallic acid (ppb)	8.75±0.20 ^a	8.55±0.71 ^a	9.12±0.16 ^a	NS
Catechin (ppb)	5.03±4.37 ^b	5.15±3.51 ^b	12.35±3.29 ^a	*

^{a-b}: Different letters indicate statistical difference ($p<0.05$) in each line, *: $p<0.05$, **: $p<0.01$, NS: not significant.

Quinoa has a higher mineral content than many other grains. While minerals such as P, K and magnesium are found in the embryo of quinoa, calcium and phosphorus in the pericarp (outer shell) are associated with the pectic compounds of the cell wall. Sulfur is distributed homogeneously in the quinoa embryo. Quinoa can be an

alternative food source for anemia caused by iron deficiency due to its high soluble iron content (Arneja et al., 2015). In addition, it has been stated that it contains higher amounts of calcium, phosphorus, magnesium, iron, zinc, potassium and copper compared to other grains (Ruales & Nair, 1993). The mineral

substances of meatballs produced using quinoa flour are given in Table 3. Quinoa flour usage had a very significant ($p<0.01$) effect on all analyzed mineral substances. Fe, Zn, Mg, Ca and P showed the lowest

mean values in the control group with 588.740, 300.843, 4.170, 2.019 and 34.751, respectively. The highest mean values were found in the groups produced using 5% quinoa flour.

Table 3. The effect of using quinoa in turkey meatball on mineral matters

Mineral matter	Quinoa Flour Ratio (%)			Sig.
	0-Control	2.5	5	
Fe (ppb)	588.740±69.554 ^c	647.106±62.350 ^b	703.914±108.030 ^a	**
Zn (ppb)	300.843±20.403 ^c	376.707±29.406 ^b	311.293±24.134 ^a	**
Mg (ppm)	4.170±0.257 ^c	5.028±0.124 ^b	5.146±0.030 ^a	**
Ca (ppm)	2.019±0.028 ^c	2.272±0.044 ^b	2.840±0.634 ^a	**
P (ppm)	34.751±1.202 ^c	36.349±1.805 ^b	39.121±0.228 ^a	**

^{a-c}: Different letters indicate statistical difference ($p<0.05$) in each line, **: $p<0.01$.

4. Conclusion

The use of quinoa flour in meatballs did not affect the sensory parameters. This shows that the use of quinoa does not affect the desired taste of the meatball and can be used in terms of sensory aspects. On the other hand, quinoa flour reduces cooking loss and diameter reduction values and increases cooking yield, improving the cooking properties of the product and providing economic gain. In addition, the mineral substances of the groups where quinoa flour is used show higher values than the other groups and this contributes to the nutritional value of the meatball. It was also concluded that quinoa is a good alternative to bread crumbs, especially for the production of gluten-free meatballs.

Acknowledgements

This study was supported by TÜBİTAK 2209A project.

Conflict of interest

The authors declare no conflict of interest.

Ethical Approval

This article does not require ethics committee approval.

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A bibliometric analysis of scientific publications on the freshwater crayfish *Pontastacus leptodactylus* in Türkiye: Trends and developments based on the Web of Science database

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Received: 07/04/2025, Accepted: 11/06/2025

Abstract

In this study, a bibliometric analysis is presented that examined 228 publications concerning *Pontastacus leptodactylus* (*Astacus leptodactylus*) in Türkiye, published between 1989 and 2024 and indexed in the Web of Science (WoS) database. The findings reveal that the number of publications increased from the 2000s onward, peaking between 2010 and 2022, but declining in 2023–2024. Research articles (216=94.7%) constitute the main body of academic output, while other document types, such as review articles (7) and conference papers (2), remain limited. Among the most prolific authors, Harlioğlu, Muzaffer Mustafa (43 publications) leads, followed by Harlioğlu, Ayşe Gül (17) and Mazlum, Yavuz (16), with research primarily concentrated in the categories of Fisheries (72), Marine and Freshwater Biology (51), and Environmental Sciences (36). Firat University (73) emerged as the leading institution, with notable contributions from Gazi University (23) and Munzur University (22). The most cited studies include Harlioğlu and Harlioğlu (2004) with 77 citations and Kokko et al. (2021) with 62 citations. Keyword analysis highlights the centrality of terms such as *Astacus leptodactylus* (70), crayfish (69), and growth (19), encompassing topics like reproduction, oxidative stress, and artificial neural networks. The findings indicate that research in Türkiye into *P. leptodactylus* is predominantly focused on biological, ecological, and environmental aspects; however, greater efforts are needed to enhance international visibility and strengthen interdisciplinary collaboration. Future studies on issues such as disease, the effects of invasive species, stock protection, climate change and habitat loss will be critical for the sustainable management and scientific contributions of the species.

Keywords: Narrow-clawed crayfish, Scientific productivity, Citations, VoSviewer

Please cite this article as follows:

Aydin, H. (2025). A bibliometric analysis of scientific publications on the freshwater crayfish *Pontastacus leptodactylus* in Türkiye: Trends and developments based on the Web of Science database. *Journal of Biometry Studies*, 5(1), 7-20. <https://doi.org/10.61326/jofbs.v5i1.02>

1. Introduction

Freshwater crayfish (Decapoda: Astacidae) are considered among the most ecologically and economically significant invertebrates inhabiting freshwater ecosystems. Furthermore, they represent a strategic resource for the fisheries and aquaculture sectors, making substantial contributions to food security and local economies. *Pontastacus leptodactylus* (*Astacus leptodactylus*), commonly found in Türkiye's freshwater ecosystems, is also known by various names, including the Turkish crayfish, Danube crayfish, or Galician crayfish (Harlioğlu, 1996, 2004; Farhadi & Harlioğlu, 2018).

Crayfish within the Astacidae family are classified into four genera: *Pacifastacus*, which is endemic to North America, and *Astacus*, *Pontastacus*, and *Austropotamobius*, which are native to Europe (Boštjancic et al., 2021). *P. leptodactylus*, a species of European origin, has a broad natural range extending from Europe to eastern Russia and parts of the Middle East (Kouba et al., 2014; Harlioğlu, 2004; Berezina et al., 2021). Due to its economic importance, the species has also been introduced into various Western European countries for aquaculture purposes (Harlioğlu, 2008; Harlioğlu & Farhadi, 2017).



Türkiye was one of the leading countries supplying *P. leptodactylus* to European markets between 1970 and 1986 (Harlioğlu & Holdich, 2001; Harlioğlu & Harlioğlu, 2004; Aydın et al., 2012). However, since 1985, overfishing and the crayfish plague caused by *Aphanomyces astaci* have led to significant declines in natural stocks. Crayfish harvesting, which amounted to 7,936 tons in 1984, steadily decreased in subsequent years, dropping to 304 tons by 1992 (Harlioğlu & Harlioğlu, 2006; Aydın et al., 2012). From 1995 onward, a partial recovery in stocks was observed, with harvest levels reaching 2,317 tons in 2004. However, this increase was not sustained, and harvesting declined again in the following years. According to the latest data, 662 tons of crayfish were harvested in 2022, and 736 tons in 2023 (Anonymous, 2023, 2024; Mazlum et al., 2025). Although the crayfish plague has had significant impacts on stocks, both historically and in recent times, it is reported that the current population has largely remained stable (Souty-Grosset et al., 2006; Gherardi & Souty-Grosset, 2017; Kokko et al., 2012, 2018).

P. leptodactylus is a species that, in addition to being harvested from natural waters, can also be cultured, and its production ranks fifth globally in crayfish production (Özturan et al., 2022; Nuc et al., 2023). In recent years, there has been an increase in studies aimed at enhancing the reproductive efficiency of this species, its aquaculture, and the reinforcement of local crayfish populations affected by diseases, including the crayfish plague (Harlioğlu et al., 2012, 2013a, 2013b, 2014, 2017, 2018; Farhadi et al. 2018; Farhadi & Harlioğlu, 2019).

Fisheries science has been experiencing rapid development worldwide in recent years, shaped by a combination of environmental, economic, technological, and societal factors. Primarily, global population growth and the increasing demand for food have rendered fishery products an increasingly important source of protein, thereby heightening interest in fisheries science (Pauly et al., 2022). In addition to the global increase in publications in the fields of fisheries and aquaculture, research using bibliometric analysis of these publications has also begun to emerge (Natale et al., 2012; Jaric et al., 2012; Radael et al., 2014; Aksnes and Browman, 2015; Syed et al., 2019; See et al., 2021; Bujas et al., 2023; Sheikh et al., 2024; Qin et al., 2024; Saramah & Falahatkar, 2024). The number of publications concerning the bibliometric analysis of crayfish-related studies remains extremely limited. Fetzner (2009) published an article within the scope of the Freshwater Crayfish Symposium Books, addressing the impact factor (IF) and various bibliometric indicators of publications on crayfish. Mihaela et al. (2022) examined more than 700 articles about crayfish published in 189 different journals over the past thirty years, evaluating keywords, main research topics, institutional and author collaboration networks, as well as co-citation and co-occurrence analyses. Azra et al. (2023) investigated the

trends and development of crayfish research through scientific measurement analyses, focusing on published literature, authors, affiliations, international collaboration networks, and co-citation datasets. In a more recent study, Ion et al. (2024) developed a web platform presenting real-time global mapping of freshwater crayfish and their pathogens. However, despite extensive literature reviews, a specific bibliometric analysis targeting the species *P. leptodactylus* has not yet been documented in the literature.

Publishing scientific articles in journals indexed by the Web of Science (WoS) is important in terms of academic credibility, increased citation counts, and international recognition. These journals ensure the maintenance of scientific quality through rigorous peer-review processes and enable research to reach a broad academic audience. Furthermore, they offer advantages for academic promotion, scientific incentives, and international collaboration.

The aim of this study was to conduct a bibliometric analysis of articles about *P. leptodactylus* published in WoS from 1989 to 2024 with a Turkish affiliation. By examining all WoS-indexed documents affiliated with Türkiye, the study sought to identify influential authors, journals, institutions, key trends, and collaboration networks, thereby providing insights into the national scientific, economic, and environmental significance of this species. Furthermore, through the analyses conducted, the study aimed to identify gaps in the existing literature, encourage future research collaborations, and contribute to a deeper understanding of the conservation and aquaculture potential of *P. leptodactylus* in light of historical and ongoing challenges, such as overfishing and diseases.

2. Material and Methods

In this study, bibliographic data were retrieved from the Web of Science (WoS) Core Collection, including the Science Citation Index Expanded (SCIE), Conference Proceedings Citation Index – Science (CPCI-S), and Emerging Sources Citation Index (ESCI). The search was conducted on March 15, 2025, covering the publication period between 1989 and 2025. An advance search was adopted and used the following keywords for searching and retrieving data “*Pontastacus leptodactylus*” or “*Astacus leptodactylus*”. The query path was: “*Astacus leptodactylus*” (All Fields) or “*Pontastacus leptodactylus*” (All Fields).

The results were refined by selecting “Türkiye” in the country rankings, and there were no restrictions on the language of the articles. The bibliographic data related to *P. leptodactylus* or *A. leptodactylus* included publication trends, publication types, top WoS categories, most productive authors, top journals, top affiliations, most cited publications, highly collaborative authors, highly cited articles and most used keywords. A total of 228 bibliographic records were retrieved and bibliographic

records were analyzed and mapped using the VoSviewer version 1.6.20 (<https://www.vosviewer.com/>). Additionally, Microsoft Excel 2016 was employed for the preparation of graphical representations.

3. Results

3.1. Number of articles published by year

Figure 1 presents the annual distribution of academic articles published by authors from Türkiye on *P. leptodactylus* and indexed in the WoS database. The first study about *P. leptodactylus* with a Turkish affiliation was titled “Identification of the Pathogenic Fungus Causing Destruction to Turkish Crayfish Stocks (*Astacus leptodactylus*)”, and was conducted by Rahe

and Soylu (1989). No further studies were recorded from 1989 to 2000; however, an increase in the number of publications was observed starting in the 2000s. A significant rise in publications occurred after 2010, with the highest levels reached in 2018, 2020, and 2022, when the number of articles peaked at 18 per year.

3.2. Publication types

The majority of identified studies consisted of research articles (216). In addition, there were review articles (7) and proceedings papers (2). A smaller number of publications included a meeting abstract (1), editorial material (1), and a correction (1). These data indicate that the studies were largely based on original research (Figure 2).

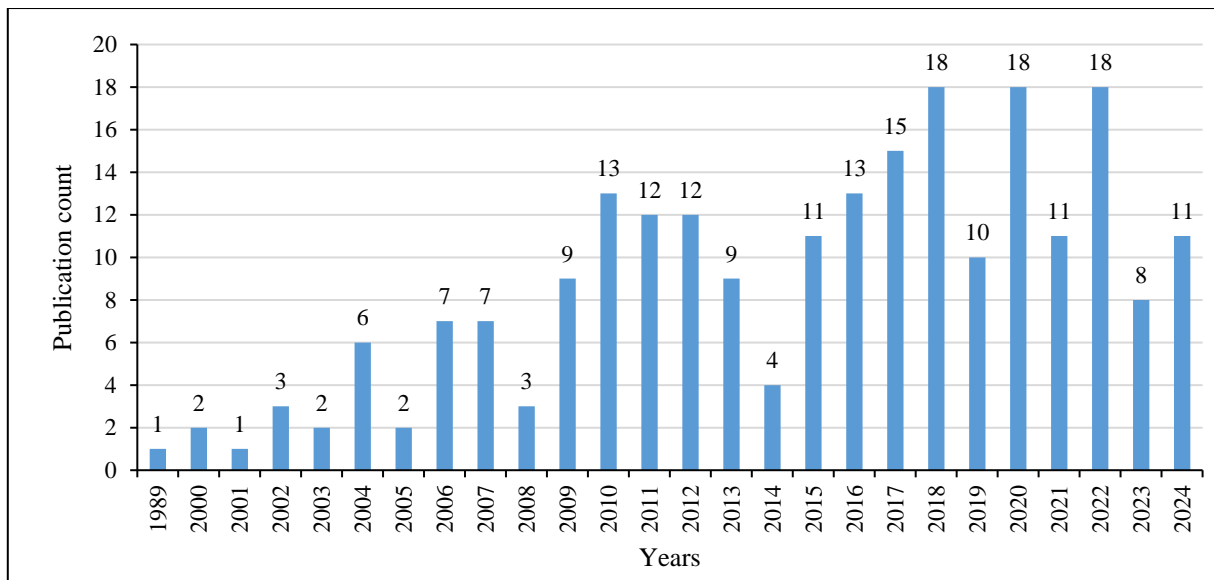


Figure 1. The number of publications on *P. leptodactylus* in WoS between 1989 and 2024 from Türkiye

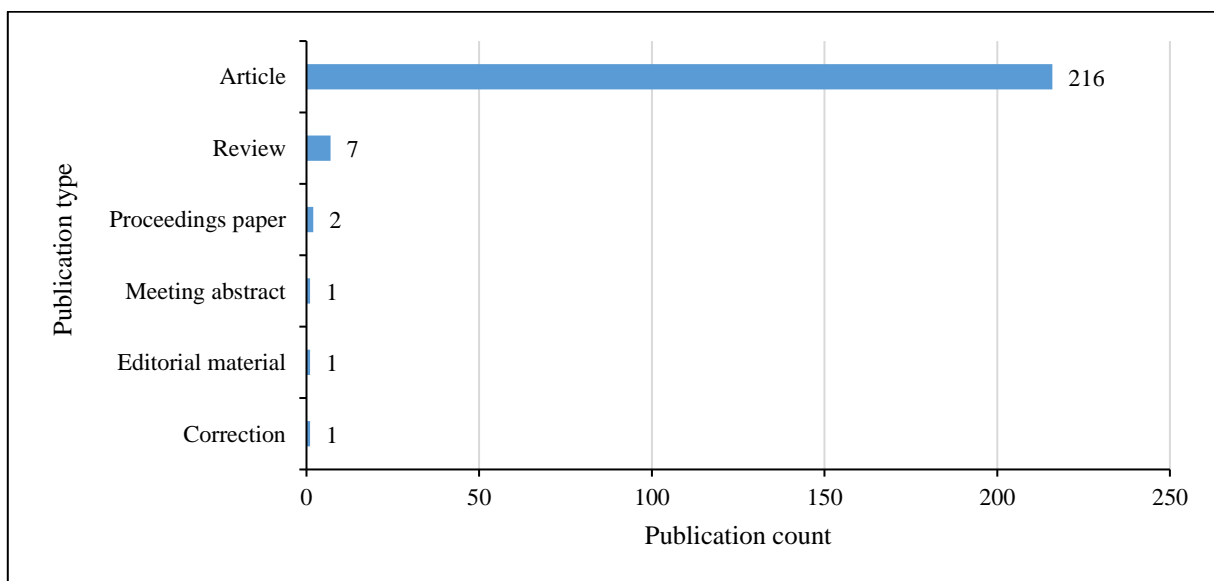


Figure 2. Distribution of publication types and their counts about *P. leptodactylus* in Türkiye

3.3. Most productive authors

The top 15 most productive authors of publications about *P. leptodactylus* are shown in Figure 3. The researcher with more than twice as many publications as other Turkish authors was Harlıoğlu, Muzaffer Mustafa. He was followed by Harlıoğlu, Ayşe Gül and Mazlum, Yavuz. Other authors with more than 10 publications included Aksu, Önder, Günal, Aysel Çağlan, and Berber, Selçuk. Other researchers have contributed to the literature with publication counts ranging between six and nine articles. Furthermore, a large number of researchers with lower publication counts have also made contributions to the field. These data indicate that the studies are concentrated around certain researchers while also demonstrating broad academic participation.

3.4. Web of Science categories

Research into *P. leptodactylus* conducted in Türkiye is categorized under various scientific fields in WoS. The highest number of publications is concentrated in the categories of Fisheries, Marine and Freshwater Biology, and Environmental Sciences. These are followed by Veterinary Sciences, Zoology, and Biochemistry and Molecular Biology. Although fewer in number, studies have also been conducted in areas such as Toxicology, Food Science, Agriculture, Ecology, and Reproductive Biology. This distribution suggests that research into *P. leptodactylus* is primarily focused on aquatic products, environmental sciences, and biology, while also showing an interdisciplinary spread (Figure 4).

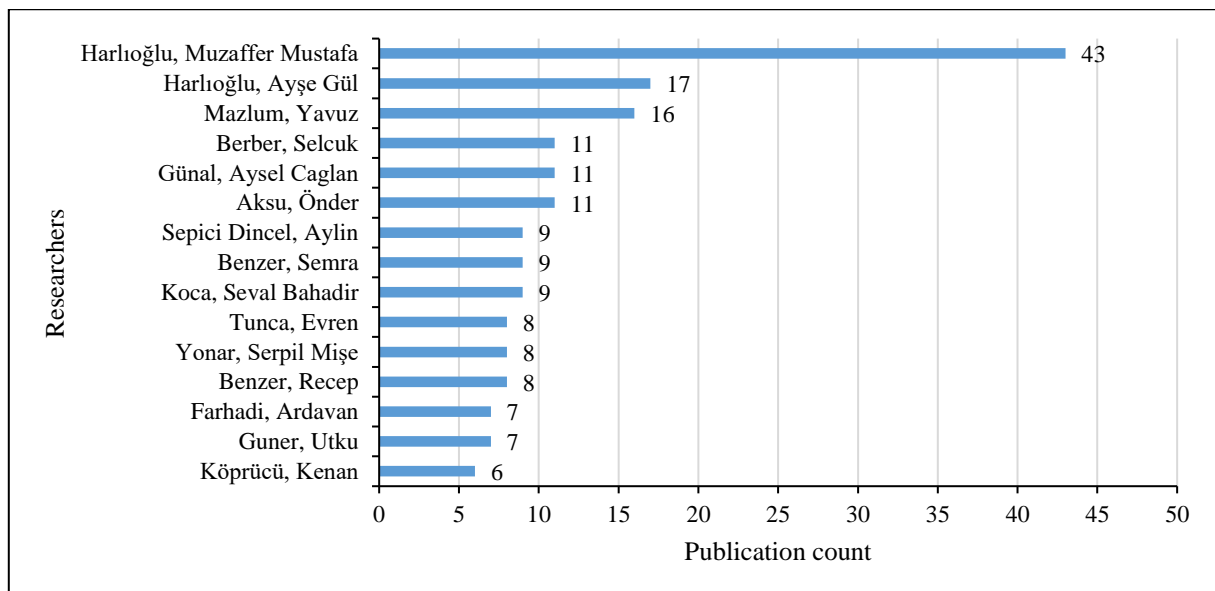


Figure 3. The top 15 most productive authors involved in *P. leptodactylus* publications in Türkiye

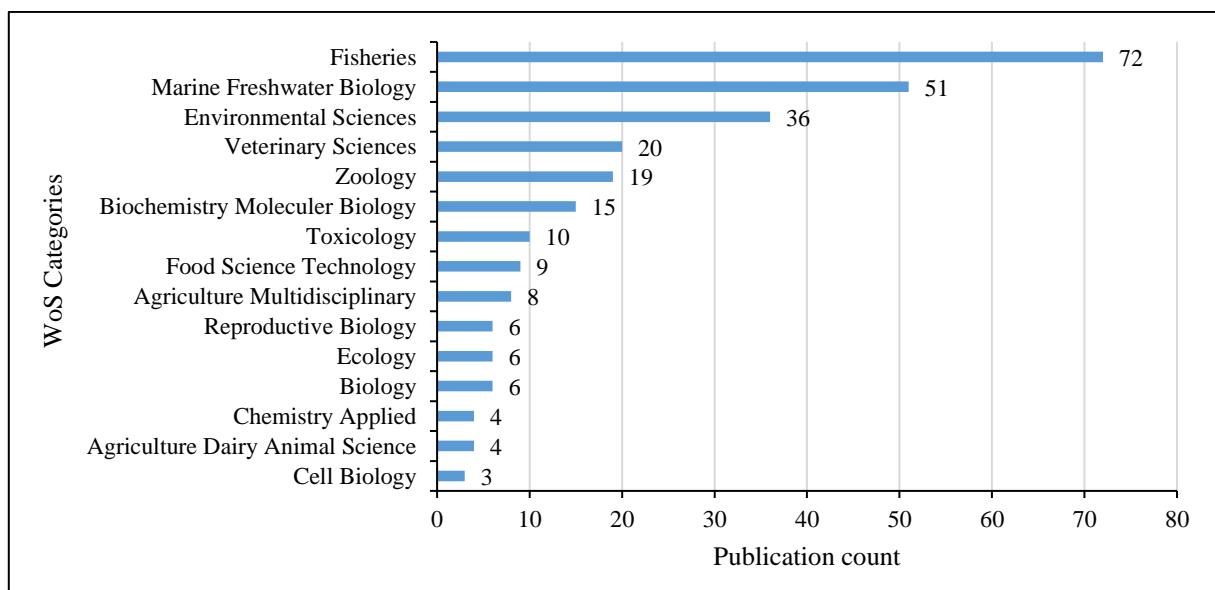


Figure 4. Number of publications on *P. leptodactylus* according to top 15 of WoS categories

3.5. Affiliations

In Türkiye, the institution with the highest contribution to research into *P. leptodactylus* is Fırat University, followed by Gazi University and Munzur University. Istanbul University, Süleyman Demirel University, and Ankara University are also among the major contributors. In addition, the Ministry of Agriculture and Forestry and its affiliated institutes, various research centers, and international universities, including Harvard University, the Technical University of Denmark and the University of Eastern Finland, have also contributed to the studies. Overall, the top 15 universities and public research institutions in Türkiye that have made the most contributions to the research are shown in Figure 5.

3.6. Publication titles

In Türkiye, among the journals with the highest number of publications about *P. leptodactylus*, the Fresenius Environmental Bulletin stands out, followed by Crustaceana, Aquaculture International, and Aquaculture Research. The Turkish Journal of Fisheries and Aquatic Sciences and Su Ürünleri Dergisi host studies originating from Türkiye. In addition, many national and international journals focusing on environmental sciences, veterinary medicine, ecotoxicology, and aquaculture have also published research. This finding highlights the multidisciplinary nature of research into *P. leptodactylus* (Figure 6).

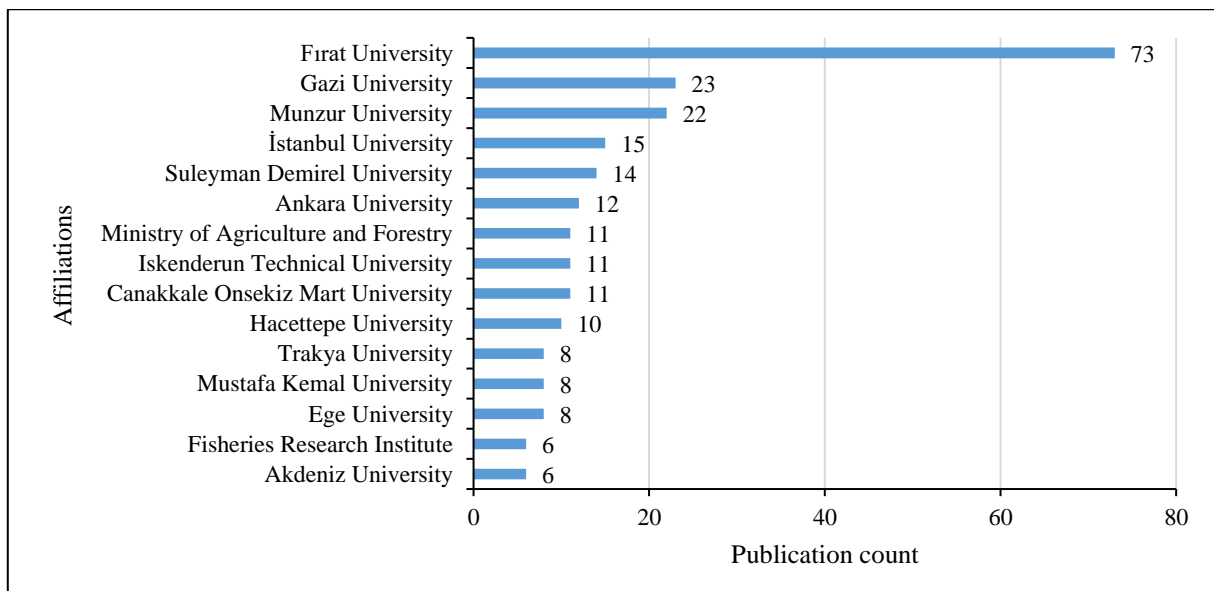


Figure 5. The top 15 affiliations with the most publications involved in *P. leptodactylus* in Türkiye

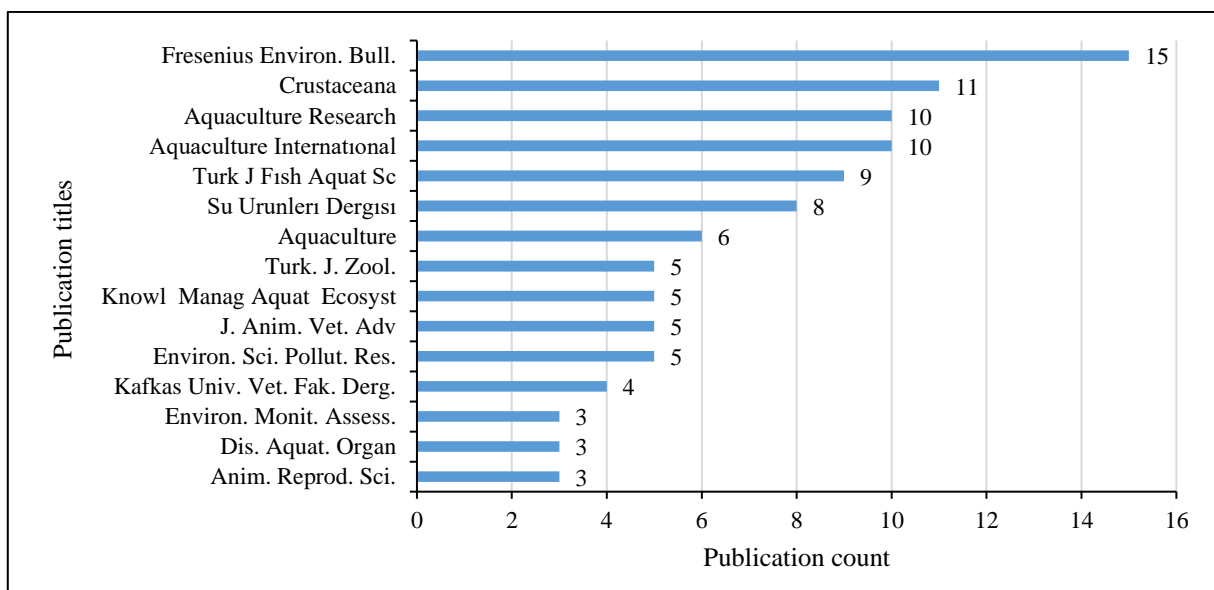


Figure 6. The top 15 journals and publication numbers in which studies on *P. leptodactylus* are most published

3.7. Most cited publications

According to WoS data, of the 228 articles published on *P. leptodactylus* between 1989 and 2024, 14 articles were not cited at all, while 193 articles were cited between 1 and 77 times. The most cited publication was Harlioğlu (2004a) with 77 citations, Kokko (2012) was second with 62 citations, and Svoboda (2012) was third with 58 citations. The most cited articles are shown in Table 1.

3.8. Citation network of publications

The bibliographic citation network of publications of research into *P. leptodactylus* (1989-2024) in Türkiye is shown in Figure 7. This figure, created using WoSviewer, based on data retrieved from WoS on March 15, 2025, presents a network analysis of bibliographic citation across the 228 publications. Publications that received at least one citation were included in the visualization. Citation network analysis has revealed the relationships between key studies in the field and the most influential publications. The map shows that Harlioğlu (2004a) occupies a central position, with studies such as Harlioğlu (2008a), Kokko (2012), and Harlioğlu (2017c) also receiving high citation counts. The colour scale indicates that early studies serve as fundamental citation sources, and new research is built upon these works. Particularly, the strong connections between Harlioğlu (2004a),

Harlioğlu (2008a), and Kokko (2012) highlight that these studies are reference points within a specific research trajectory.

3.9. Analysis of the most used keywords

In the 228 documents identified, 525 different keywords were found, with the most frequently used keywords listed in Table 2. When examining the frequency and total link strength of the keywords used in the publications, *Astacus leptodactylus* (70) and *crayfish* (69) were the most frequently used terms, with their total link strengths reaching the highest values of 289 and 290, respectively (Figure 8). These results indicate that these keywords played a central role in the research, with most studies being centered around these concepts. Among the other keywords, *growth* and *survival* (19), *freshwater crayfish*, *oxidative stress*, and *reproduction* (10) are prominent. These terms suggest studies of biological processes and environmental factors. Furthermore, the use of the word *Türkiye* 19 times emphasizes a specific focus on the ecological context of the species in Türkiye. Keywords such as *Pontastacus leptodactylus*, *artificial neural networks*, and *decapoda* have been used less frequently, yet these are important components of specific research fields. These data demonstrate that research into *P. leptodactylus* tends to focus on both biological and environmental factors in Türkiye, with these areas being extensively studied through the use of keywords.

Table 1. Ranking of the most cited top 20 publications on *P. leptodactylus* in WoS in Türkiye

Rank	First Author*	Documents	Citations ▼	Link
1	Harlioğlu (2004a)	Harlioğlu and Harlioğlu (2004)	77	50
2	Kokko (2012)	Kokko et al. (2021)	62	11
3	Svoboda (2012)	Svoboda et al. (2012)	58	9
4	Harlioğlu (2008a)	Harlioğlu (2008)	49	30
5	Harlioğlu (2017c)	Harlioğlu and Farhadi (2017)	38	27
6	Barım (2010)	Barım and Karatepe (2010)	37	8
7	Duman (2016)	Duman and Kaya, (2016)	37	1
8	Harlioğlu (2004b)	Harlioğlu and Harlioğlu (2004)	36	31
9	Yıldız (2004a)	Yıldız and Banlı (2004)	36	7
10	Rahe (1989)	Rahe and Soylu (1989)	36	23
11	Harlioğlu (2006a)	Harlioğlu and Harlioğlu (2006)	36	16
12	Mazlum (2011b)	Mazlum et al. (2011)	34	6
13	Parrillo (2017)	Parrillo et al. (2017)	34	0
14	Akhan (2014)	Akhan et al. (2014)	32	10
15	Varol (2018a)	Varol and Sünbül (2018)	32	6
16	Harlioğlu (2018)	Harlioğlu et al. (2018)	32	12
17	Güner (2007)	Güner (2007)	29	12
18	Benli (2007)	Benli et al. (2007)	29	3
19	Harlioğlu (2004c)	Harlioğlu et al. (2004)	28	20
20	Kurun (2010)	Kurun et al. (2010)	27	10

*The top 20 most cited articles in the table were automatically obtained from the VoSviewer application, and only the first author's name has been retrieved. The correct sources are displayed in the "Documents" column.

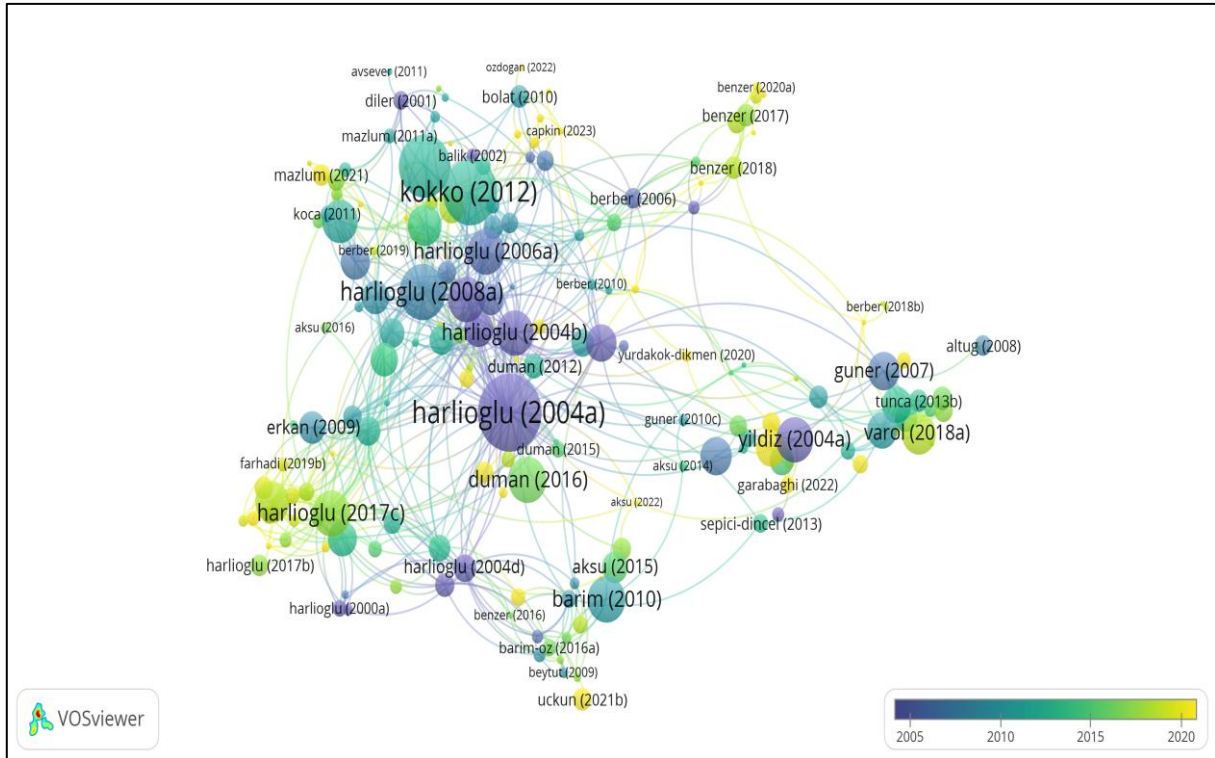


Figure 7. Publications featuring *P. leptodactylus* with at least 1 or more citations between 1989–2024 in Türkiye

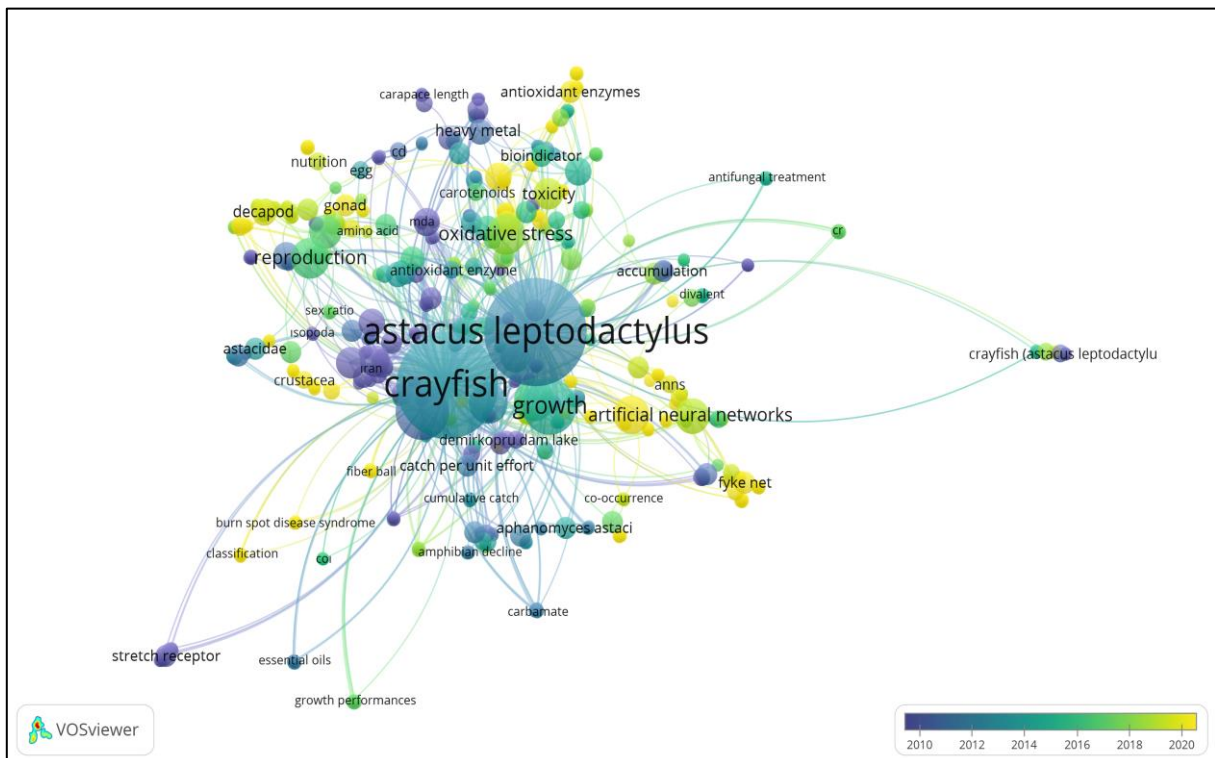


Figure 8. Analysis of keywords in published documents on *P. leptodactylus*

Table 2. Most frequently used keywords and link strength in published documents on *P. leptodactylus*

Rank	Keywords	Occurrences ▼	Total Link Strength
1	<i>Astacus leptodactylus</i>	70	289
2	Crayfish	69	290
3	Growth	19	77
4	Türkiye	19	91
5	Survival	11	48
6	Freshwater crayfish	10	43
7	Oxidative stress	10	41
8	Reproduction	10	47
9	<i>Pontastacus leptodactylus</i>	9	34
10	Artificial neural networks	8	28
11	Decapoda	7	29
12	Distribution	6	30
13	Harvest	6	32
14	Juvenile	6	28
15	Toxicity	6	26

4. Discussion

Research on freshwater crayfish has garnered significant global attention due to their ecological roles and economic value. Among these species, *Pontastacus leptodactylus* is widely recognized as one of the most economically important, particularly in European and Eurasian contexts. This study presents a bibliometric analysis of WoS-indexed publications on *P. leptodactylus* affiliated with Türkiye between 1989 and 2024.

The distribution of these publications affiliated with Türkiye over the years provides valuable insights into national research trends and the evolution of scientific interest in this species (Figure 1). Studies about *P. leptodactylus* began with only one publication in 1989 but showed a significant increase from the early 2000s onward. Notably, between 2010 and 2022, there was a remarkable rise in the number of publications, with annual publication counts generally exceeding 10, peaking at 18 in 2018. However, despite the importance of the species, the decline in the number of publications in 2023 and 2024 may be attributed to factors such as the impact of the COVID-19 pandemic or the redirection of resources and research interest toward other fields. According to WoS data, a significant decrease in the number of publications on *P. leptodactylus* has been observed in European countries in the last two years compared to previous periods. This trend mirrors the recent decline in the number of publications observed in Türkiye.

Although numerous national studies on *P. leptodactylus* have been published in recent years (Cilbiz et al., 2020; Özkök et al., 2021; Baltacı et al., 2023; Kan et al., 2024; Aydın & Serdar, 2024; Gençay et al., 2024), inclusion in the WoS database increases international visibility and contributes to academic impact through peer review, citation indexing, and funding eligibility. Promoting WoS-

indexed publication of Türkiye-originated research on this species is crucial for global recognition of its ecological and economic importance.

An analysis of the document types identified in the present study revealed that academic output was predominantly focused on research articles, highlighting the primary objective of contributing original research to the scientific literature. Other document types included review articles, and a few conference papers, corrections, editorial comment, and a meeting abstract. The low number of conference papers suggests a need to enhance the international visibility of Türkiye-affiliated researchers at academic events. While academic production in Türkiye is largely built on a strong research foundation, increasing the number of review articles and conference papers would contribute to both the synthesis of scientific knowledge and the enhancement of international visibility.

An analysis of the identified documents showed a total of 201 researchers contributing to this field from Türkiye. A few of these researchers contributed a significant proportion of all the articles. These were Muzaffer Mustafa Harlioğlu, Ayşe Gül Harlioğlu, and Yavuz Mazlum. M.M. Harlioğlu and A.G. Harlioğlu, as the leading authors, have primarily conducted research on the freshwater crayfish *Astacus leptodactylus*, focusing on topics such as reproductive biology, nutrition, aquaculture, population status, the impacts of invasive species, and harvesting practices (Harlioğlu, 1996; Harlioğlu & Harlioğlu, A.G. 2004; Harlioğlu, 2004; Harlioğlu et al. 2004; Harlioğlu & Harlioğlu, 2006; Harlioğlu, 2008; Harlioğlu et al. 2012; Harlioğlu et al. 2013a; Harlioğlu et al. 2013b; Harlioğlu et al. 2014; Harlioğlu et al. 2017; Harlioğlu & Farhadi 2017; Harlioğlu et al. 2018). Ranked third, Yavuz Mazlum has primarily conducted aquaculture-oriented research on *Pontastacus leptodactylus*, focusing on growth performance, nutritional strategies, environmental influences, body composition, and capture characteristics (Mazlum, 2007; Bolat et al. 2010; Tufan et al. 2012; Berber & Mazlum, 2016; Öksüz & Mazlum, 2016; Şirin & Mazlum, 2016; Mazlum et al. 2017; Mazlum et al. 2021). Other authors contributed around 5% of the output including Aksu Ö, Günel A.Ç, and Berber S. (Figure 3). Furthermore, the WoS database indicates that 80 authors have contributed a single publication, 60 authors have two publications, 15 authors have three, 20 authors have four, and 7 authors have five publications each. These findings suggest that research into *P. leptodactylus* is concentrated around specific individuals, with M.M and A.G Harlioğlu particularly standing out in this field. Variations in publication counts may be attributed to researchers' areas of expertise or collaborative efforts within the scientific community.

The WoS categorization of studies into *P. leptodactylus* was primarily focused on aquatic ecosystems, environmental factors, and the economic value of the species. Notably, the high number of publications in the

fields of *Fisheries* and *Marine & Freshwater Biology* underscores the ecological and economic significance of *P. leptodactylus* in freshwater habitats. The prominence of the *Environmental Sciences* category is probably a reflection of the growing research interest in the effects of water quality and habitat changes on the species. Studies under the categories *Veterinary Sciences*, *Zoology*, and *Biochemistry & Molecular Biology* focus on the physiology, genetic structure, and diseases of *P. leptodactylus*, further highlighting the species' biological and ecological importance. Furthermore, research in the fields of *Toxicology* and *Food Science & Technology* focused on human consumption, safety, and nutritional value (Figure 4). While studies in other disciplines were more limited, subjects explored included reproductive biology, ecological role, and relationship of *P. leptodactylus* with agricultural activities. Overall, studies into *P. leptodactylus* from Türkiye primarily concentrated on environmental factors and economic value. In the future, increasing research into topics such as the effect of climate change, habitat loss, and sustainable harvesting will be important for the conservation and management of this species.

In the present study, Fırat University made the highest contribution followed by Gazi University and Munzur University. Other significant contributors included Istanbul University, Süleyman Demirel University, and Ankara University. Moreover, Çanakkale Onsekiz Mart University, İskenderun Technical University, and the Turkish Ministry of Agriculture and Forestry contributed >10 studies to this field. Ege University, Mustafa Kemal University, and Trakya University made moderate contributions, while Hacettepe University contributed ten studies. Akdeniz University and the Fisheries Research Institute also contributed some studies (Figure 5). This distribution showed that research on *P. leptodactylus* is concentrated in specific universities, but this is probably associated with the small number of active authors in this field being affiliated with specific institutions. The interest of various academic and public institutions in Türkiye in this subject provides a significant academic framework for the conservation of freshwater ecosystems and biodiversity. In the future, fostering research through greater institutional involvement and interdisciplinary collaborations will contribute to a more comprehensive understanding of the species' ecological, genetic, and biological dynamics.

The 228 studies included in this analysis were published in 96 different journals indexed in the WoS database. The journal with the highest number of published articles was *Fresenius Environmental Bulletin*. Journals such as *Crustaceana*, *Aquaculture International*, and *Aquaculture Research* also stand out in this field with these four journals publishing almost a quarter of the 228 studies. Local academic journals such as the *Turkish Journal of Fisheries and Aquatic Sciences* and *Su Ürünleri Dergisi*

were also notable (Figure 6). This distribution supports the notion that the studies were primarily concentrated in the fields of environmental sciences, aquaculture, and zoology. The publication of these studies in international journals demonstrates that research on *P. leptodactylus* in Türkiye had appreciable visibility within the global academic community. In the future, an increase in interdisciplinary studies and publications in high-impact journals will contribute to scientific advances in this field.

The most cited study was authored by Harlioğlu and Harlioğlu in 2004, with 77 citations and a link strength of 50. Other prominent studies included Kokko et al. (2021) and Svoboda et al. (2012). In addition, Harlioğlu (2008) and Harlioğlu and Farhadi (2017) also provide noteworthy contributions (Table 1). These data indicate that studies by Harlioğlu are among the most influential in the field and have a broad academic impact. While the majority of the research focuses on biology, ecology, and aquaculture, the fact that studies from various years continue to receive citations highlights the scientific importance and ongoing relevance of the topic. In the future, an increase in such high-impact studies will contribute to the development of more comprehensive and interdisciplinary approaches in *P. leptodactylus* research.

The citation network of publications was examined, and the results are presented in Figure 7. This citation network map shows the relationships between key studies in the field and the most influential publications. The study by Harlioğlu (2004a) occupied a central position and had strong connections with other publications in the field. Similarly, Harlioğlu (2008a), Kokko (2012), and Harlioğlu (2017c) also figured prominently, were cited relatively frequently and were thus interconnected with many other studies. The color scale used on the map illustrates the distribution of publications over the years. Notably, studies conducted between 2005 and 2010 are predominantly represented in blue and purple tones, while more recent publications are depicted in green and yellow tones (Figure 7). This indicates that early studies in the field are regarded as foundational citation sources, with newer research building upon them. Strong connections between certain publications were observed. In particular, the robust links between the works of Harlioğlu (2004a), Harlioğlu (2008a), and Kokko et al. (2012) suggest that these publications serve as key references within a specific research trajectory. On the other hand, more recent studies (e.g., Benzer, 2020; Uçkun, 2022) exhibit fewer connections, implying that these publications offer new contributions to the field and may strengthen their position within the network over time as they accumulate more citations.

The most frequently used keywords suggested research focusing on the biological, ecological, and environmental characteristics of the species. Keywords such as *Astacus leptodactylus* and *crayfish* indicate that the core focus of these studies is on the species *P. leptodactylus*, while

research concentrating on biological processes such as growth, survival, and reproduction points to a significant trend toward understanding the species' life cycle and its sensitivity to environmental factors. Furthermore, *ecotoxicological* topics like *oxidative stress* and *toxicity* emerge as key areas of study, exploring the impacts of environmental pollutants on the species. Keywords such as *freshwater crayfish* and *Pontastacus leptodactylus* provide insights into the species' biogeography, while the use of modern modelling techniques using *artificial neural networks* demonstrated an innovative approach to understanding ecological parameters such as growth, survival, and reproduction (Table 2, Figure 8). Moreover, topics such as *distribution* and *harvest* are considered in studies examining the population dynamics of the species and its relationship with environmental conditions. These findings reveal that research on *P. leptodactylus* in Türkiye adopts a multidisciplinary approach, encompassing not only biological characteristics but also ecotoxicology, environmental factors, and advanced modelling techniques, thereby offering significant contributions to future studies.

5. Conclusion

This study reveals that research on *Pontastacus leptodactylus* in Türkiye, as indexed in the Web of Science (WoS) database, has made noteworthy contributions to the global scientific literature, particularly within the domains of biology, ecology, aquaculture, and ecotoxicology. The bibliometric analyses demonstrate a notable increase in publications over the years, reaching its peak in 2018. Prominent scholars such as Harlioğlu M.M. have played a pivotal role in the development of the field, as evidenced by their frequently cited publications and central positions within citation networks. The concentration of studies in disciplines such as fisheries, marine freshwater biology, and environmental sciences underscores the species' ecological and economic importance, while the use of innovative approaches like artificial neural networks highlights the adoption of multidisciplinary methods. Despite these advancements, the recent absence of publications in 2023 and 2024, alongside the relatively low number of review articles and conference proceedings, highlights a need for greater efforts toward international visibility and comprehensive knowledge synthesis. Moving forward, increasing interdisciplinary collaborations, targeting high-impact journals, and addressing emerging challenges, such as climate change and sustainable harvesting, will be key to advancing the conservation and management of *P. leptodactylus* and enhancing its scientific and economic significance on a global scale.

Conflict of interest

The author declares 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 manuscript.

Ethical Approval

The author declares that formal consent is not required for this type of study.

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Effects of zinc and copper oxide nanoparticles on sugar beet (*Beta vulgaris* L.)

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Received: 30/05/2025, Accepted: 20/06/2025

Abstract

In recent years, the development of nanotechnology and the results of many researches in this field have shown that it contributes significantly to germination, growth, development and product yield of plants. In addition, nanoparticle applications have been shown to positively affect cell structure and cell function on physiological and biochemical mechanisms in the regulation of adverse effects caused by various environmental stresses. In this study, zinc oxide (ZnO) and copper oxide (CuO) nanoparticle were applied to the seeds of sugar beet (Bernache) variety and the effects of changes in germination, weight and length measurements in radicle and plumule were investigated. It was determined that the seeds responded differently to both nanoparticle. In our study, copper oxide pretreatment caused an increase in radicle and plumula length. In terms of weights, it was observed that copper oxide 1800 mg/l application caused a significant increase in shoot and root wet weight. As a result, it was determined that copper oxide 1800 mg/l pretreatment had a positive effect on all parameters examined.

Keywords: Nanoparticle, Sugar beet, Copper, Zinc

Please cite this article as follows:

Sefaoğlu, F., Ünal, G. B., & Kaynar, D. (2025) Effects of zinc and copper oxide nanoparticles on sugar beet (*Beta vulgaris* L.). *Journal of Biometry Studies*, 5(1), 21-25. <https://doi.org/10.61326/jofbs.v5i1.03>

1. Introduction

In recent years, the use of nanotechnology in agricultural production has become an area of increasing interest. Especially metal-based nanoparticles are considered as a new generation of biostimulants that can positively affect plant physiology when applied at low doses. Since these particles have larger surface area and high reactivity compared to ionic elements, they can penetrate plant cells faster and more effectively (González-Melendi et al., 2008). In this context, zinc nanoparticles (ZnO NPs) are the nano form of the element zinc, which is vital in plant metabolism. ZnO NPs are involved in many basic physiological processes such as the production of photosynthetic pigments, regulation of the antioxidant system, enzyme activity and maintenance of cell membrane stability (Cakmak, 2000; Dimkpa & Bindraban, 2016).

Studies have shown that ZnO NP application promotes plant growth and alleviates ion toxicity by increasing root and shoot development (Mahajan & Kaushal, 2018). On the other hand, some studies show that these nanoparticles can affect not only physical development but also metabolic processes in plants. Kaveh et al. (2013) revealed that silver nanoparticle (Ag NP) treatment modulated stress responses by increasing antioxidant enzyme activities in *Arabidopsis thaliana*, indicating that nanoparticles may be effective in plant defense systems. Mahajan and Kaushal (2018) emphasized that zinc-based fertilizers provide a more effective uptake compared to traditional zinc fertilizers, so similar or higher yields can be obtained with lower doses. It is also reported that ZnO NPs may have positive effects on plant microbiota (González-Melendi et al., 2008). The intracellular uptake mechanisms of nanoparticles suggest that they may function not only as nutrient carriers but also as



biostimulants and protective agents in plant stress management.

In this study, it was aimed to determine the effects of zinc oxide (ZnO) and copper oxide (CuO) nanoparticles applied at different doses on the germination process and early seed development of sugar beet (*Beta vulgaris* L.) seeds.

2. Material and Method

Seed trials were carried out in the acclimatization room. The seeds to be used in the experiment were selected from sugar beet seeds of similar size with plump and robust structure. The seeds were surface sterilized before use; this was done with 1% sodium hypochlorite solution for 10 minutes, then washed with pure water and dried on filter papers at room temperature. The experiment was carried out according to the "Coincidence Plots Experimental Design" with three replications of nanoparticle pretreatments.

In germination trials, nanoparticle pretreatment of sugar beet seeds was carried out by using 4 different dosages of zinc (0, 600, 1200 and 1800 mg/l) and distilled water at 20 ± 1 °C in dark environment for 18 hours at appropriate concentrations. After treatment, the seeds were dried and stored at +4 °C before use. Sugar beet seeds were placed in petri dishes, each of which was covered with two layers of blotting paper, with 20 seeds. Control and only nanoparticle treated groups were added 5 ml of pure water.

The germination process was monitored in the dark at 22 ± 1 °C for 7 days. The seeds were checked every day and seeds reaching a root length of 1 mm were considered germinated. During the control, contaminated seeds were removed from the medium and recorded. A total of 60 seeds were sown for each group and calculations were made accordingly.

Data were analyzed by one-way ANOVA using SPSS software. Differences were evaluated at 5% significance level.

3. Results and Discussion

3.1. Germination rate

The effect of ZnO NP and CuO NP doses on germination rate was found statistically significant ($p < 0.01$, Table 1). There was no significant change in germination rate values with increasing CuO NP application doses (0, 600, 1200, 1800 mg/l), and the highest value among CuO concentrations was obtained from 600 mg/l application. Similar situation was observed between ZnO NP concentrations and the highest value was obtained from 600 mg/l application. In addition, 1800 mg/l dose showed inhibitory effect on germination rate in both nanoparticles (Table 2). Kunjam et al. (2015) found that high concentration of ZnO metal had a negative effect on seed germination. When the studies conducted with nanoparticles were examined; it was observed that different plant species reacted differently

to different types of nanoparticles at different concentrations (Zhao et al., 2021).

3.2. Germination duration

The effect of nanoparticle doses on germination period was found to be insignificant (Table 1). The effect of 600, 1200 and 1800 mg/l CuO NP applied to sugar beet seeds on germination period was negative. ZnO application doses (600, 1200 and 1800 mg/l) decreased the germination time by 22.0%, 10.4% and 18.3%, respectively. CuO NP application decreased the germination period by 27.3%, 6.0% and 23.1%, respectively. The lowest germination time occurred in 600 mg/l ZnO and 1800 mg/l CuO NP treatments (4.26 and 4.46 days, respectively), while the longest germination time was obtained from the control group (5.46 days) without any nanoparticle application. Kunjam et al. (2015) determined that high concentration of ZnO metal had a negative effect on seed germination.

3.3. Root width

The effect of nanoparticle doses on root width was insignificant (Table 1). The effect of 600, 1200 and 1800 mg/l ZnO and CuO NP applied to sugar beet seeds on germination width was negative. Root diameter affects the structural strength and water carrying capacity of the root system. According to the average values, root diameter varied between 0.524-0.661 mm. In both nanoparticles applied in the study, the highest root diameter values were obtained in the control group (0.661 mm), while the lowest root diameter values were obtained in ZnO NP treatment at 1800 mg/l and in copper oxide treatment at 600 mg/l concentration. It can be said that ZnO and CuO NP treatments did not cause a significant and regular increase in root diameter, but caused some variations in terms of tissue thickening in the roots. The effect of ZnO NP on root diameter did not vary greatly according to the doses; therefore, it is difficult to mention a direct and significant effect of ZnO NP on root diameter.

3.4. Root length

In the study, the effect of different doses of nanoparticles on stem length was found to be positive and statistically significant ($p < 0.01$, Table 1). The highest root length was obtained in 600 mg/l ZnO NP application with 67.47 cm. These findings show that ZnO NP supports root growth at low and medium doses, but this effect decreases at high doses. Similar situation was determined in CuO NP application and the highest and lowest root lengths were obtained from 1800 mg/l (81.60 mm) and control group (34.47 mm), respectively. It is known that nanoparticle applications have a negative effect on plant growth after a certain dose and may even be toxic (Rizwan et al., 2017; Sarkar et al., 2023).

3.5. Root weight

According to the results of the study, the effect of ZnO and CuO NPs application on root weight was found statistically significant ($p < 0.01$, Table 1). The highest root weight was measured as 0.0156 and 0.0158 g in 1800mg/l CuO NP and 600mg/l ZnO NP treatments, respectively. This indicates that ZnO supports the effect of NP to increase root biomass. The medium dose of ZnO NP significantly promoted the mass development of the root system. As a matter of fact, it was found that 50 mg/l dose of ZnO NP positively affected growth and development and increased root growth by 28.4% among 25 and 50 mg/l ZnO NP doses applied in the study (Ulhasan et al., 2023). In another study, it was determined that foliar application of ZnO NP at different doses (0, 20, 50, 80 mg/l) significantly improved root and shoot weights up to 50 mg/l in wheat under salt

stress (Lalarukh et al., 2022). In the same study, it was reported that ZnO NP applications increased plant resistance and growth by stimulating the formation of osmolytes and increasing nutrient uptake.

3.6. Shoot length

Shoot length is one of the important parameters for growing healthy and high-quality seedlings. There is a positive relationship between shoot length and plant growth traits such as number of leaves, shoot dry and wet weight (Vazirimehr & Rigi, 2014; Jini & Joseph, 2017). The longest shoot was 50.3, 52.9 and 55.9 mm in 600, 1200 and 1800 mg/l ZnO NP treatment, respectively (Table 1). However, it is known that nanoparticle applications have a negative effect on plant growth after a certain dose and may even be toxic (Rizwan et al., 2017; Sarkar et al., 2023).

Table 1. The effects of ZnO and CuO NPs treatment on germination rate, germination period, root width, root length, root weight and shoot length of sugar beets

Nanoparticle	Dose (mg/l)	Germination rate (%)	Germination period (day)	Root width (mm)	Root length (mm)	Root weight (g)	Shoot length (mm)
CuO	Control	60.9 ^f	5.46	0.605	34.47 ^c	0.0088 ^{bc}	41.20 ^{bc}
	600	94.7 ^{ab}	4.26	0.661	56.43 ^b	0.0095 ^{bc}	50.29 ^a
	1200	74.0 ^e	4.89	0.586	77.53 ^a	0.0124 ^{ab}	52.88 ^a
	1800	89.5 ^c	4.46	0.535	81.60 ^a	0.0156 ^a	55.89 ^a
ZnO	600	97.2 ^a	3.97	0.524	67.47 ^{ab}	0.0158 ^a	43.38 ^{ab}
	1200	92.2 ^b	5.13	0.544	24.20 ^c	0.0063 ^c	32.19 ^d
	1800	86.3 ^d	4.20	0.560	30.74 ^c	0.0095 ^{bc}	37.32 ^{cd}
Average		85.0	4.62	0.570	53.20	0.0110	44.74

3.7. Shoot diameter and shoot weight

According to the results of the study, the effect of nanoparticle dose application on shoot diameter was found to be statistically significant ($p < 0.01$). Shoot diameter is important in terms of stem thickness and mechanical resistance of the plant. Shoot diameter values varied between 0.68-0.88 mm in nanoparticle applications. 1800 mg/l ZnO dose produced the lowest shoot diameter (0.68 mm) and the highest shoot diameter value was obtained from 600 mg/l CuO NP application dose (Figure 1). High dose ZnO NP had a negative effect on shoot diameter, but this effect was not supported by all parameters.

When the analysis of variance was examined, it is seen that the effect of nanoparticle dose application on shoot weight is significant ($p < 0.01$). In this study, the highest shoot weight was observed in 1800 mg/l ZnO NP application with 0.060 g. The lowest shoot weight was obtained from 1200, 1800 mg/l ZnO NP application and control group (Figure 1). This indicates that the medium dose is the optimum level for shoot growth. The 1200 mg/l dose increased shoot biomass and optimized upper

plant growth. It can be said that this dose acts as a “supportive buffer”.

3.8. Leaf length and leaf weight

While nanoparticle applications had a significant effect on leaf length, the effect of zinc and copper nanoparticle applications on leaf weight was significant ($p < 0.01$). Figure 2 shows that the highest and lowest leaf length values were obtained from 600 mg/l copper oxide nanoparticle application and the lowest values were obtained from 1200 and 1800 mg/l zinc oxide application, respectively. In terms of leaf weight, copper and zinc, although there was a numerical difference between the weight values of zinc oxide nanoparticle application, this difference was statistically insignificant. The highest and lowest weight values were obtained from 600 and 1200 mg/l zinc oxide application and the highest value was obtained from 1800 mg/l copper application. It was observed that 1800 mg/l dose of CuO NP supported leaf growth in terms of biomass and length, but the effect of zinc oxide nanoparticles at the same dose was limited. This may be related to an increase in cell size or water-holding capacity.

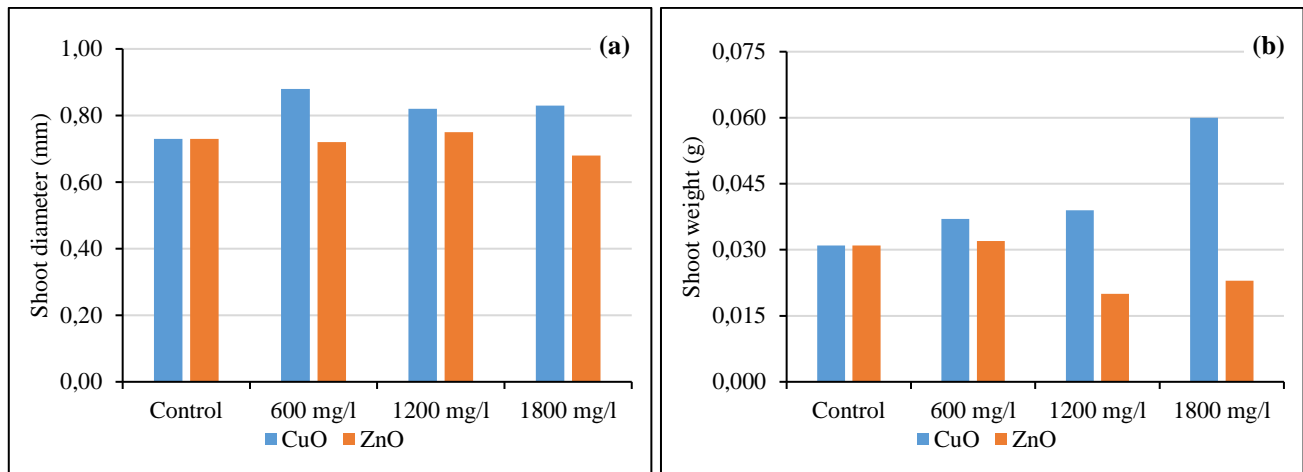


Figure 1. The effects of ZnO and CuO NPs treatment on (a) shoot diameter and (b) shoot weight of sugar beets

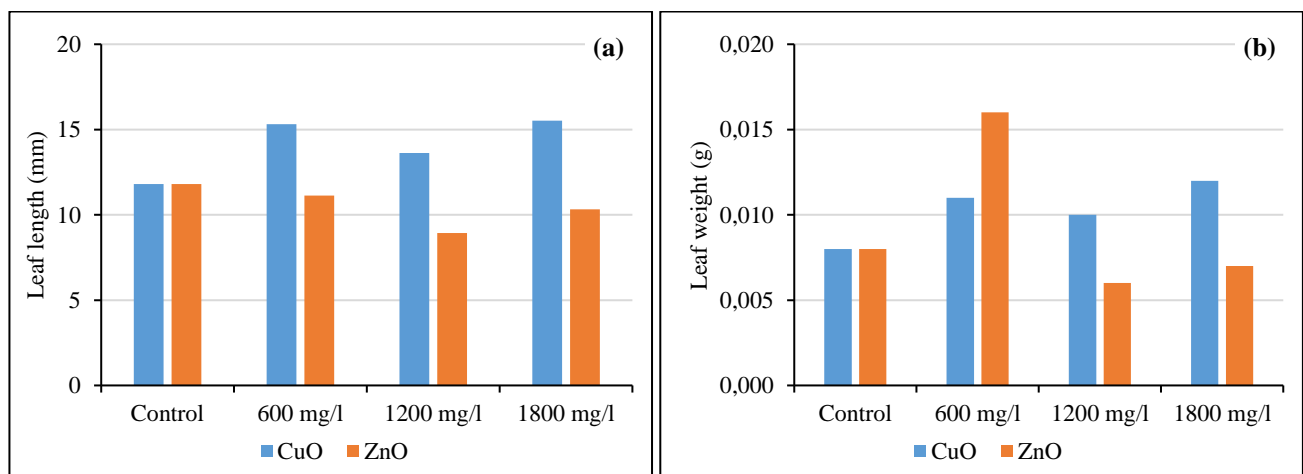


Figure 2. The effects of ZnO and CuO NPs treatment on (a) leaf length and (b) leaf weight of sugar beets

4. Conclusion

In this study, morphological and physiological changes in sugar beet (*Beta vulgaris* L.) seeds exposed to different nanoparticle concentrations were investigated in detail. It was found that the doses of copper and zinc nanoparticles, especially zinc oxide nanoparticles, partially improved the germination properties of sugar beet seeds. It was observed that zinc oxide and copper oxide nanoparticles had positive effects on many of the characters examined and this effect increased in many parameters depending on the increasing concentration levels. In order to transfer such studies to practice, it is important to carry out studies with different NPs and at different doses and to evaluate them with economic analyses.

Acknowledgement

This study was presented at the 5th International Congress on Engineering and Life Science held in Pitești/ROMANIA on September 10-12, 2024.

Conflict of interest

The authors declare no conflict of interest.

Ethical Approval

This article does not require ethics committee approval.

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Acknowledgements

The funding organization(s) and/or person(s) who indirectly contribute to the study should be thanked. Corresponding author is responsible for ensuring that the organization(s) and/or persons given in the Acknowledgements agree to be named.

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