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THE IMPACT OF THE DIFFERENT PERIOD OF OCCURRENCE OF THE EUROPEAN BEAVER POPULATION ON ITS FEEDING BEHAVIOR AND IMPACT ON THE ENVIRONMENT*

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Key words: castor fiber, optimal foraging theory, central place foraging theory, Wigry National Park, Kampinos National Park.

Abstract

The aim of the study was to determine the influence of the period of occurrence of the European beaver (*Castor fiber* L.) population on foraging strategies and on the diversity of the species of cutting trees and shrubs. Two beaver populations at different stages of development – a younger (42yr) population from the Kampinos National Park and an older (72 yr) population from the Wigry National Park were studied. Both populations foraged according to the Optimal Foraging Theory (OFT), according to which the distance to available food depends on its size and the distance to reach it. The dominant factors modifying the foraging behavior of beavers and their foraging ranges were the presence of a dam, the availability of preferred species. The greater diversity in the areas where beaver are found may be the result of both beaver activity and the selection of more attractive areas for dam construction.

Introduction

The first beavers were introduced into Poland in the second half of the 1940s in the northeastern part of the country (ŻUROWSKI 1984, KASPERCZYK 1990, JANISZEWSKI, MISIUKIEWICZ 2012, RAKOWSKA, STACHURSKA-SWAKOŃ 2021). From the population established in this area, the species

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was reintroduced to other areas of the country. Between 2000 and 2020, the population size of this species in Poland increased 5 times (GUS 2021). Although the population continues to increase nationally, in northeastern Poland the Wigry National Park Service is beginning to observe a decline in the beaver population. When expanding into new areas, beavers primarily colonize areas rich in deciduous species. A beaver family colonizing sections of rivers rich in preferred plants initially can occupy as much as a 200m section of a watercourse. With the depletion of the food supply, the length of the section of the watercourse penetrated by beavers increases (DVORNIKOVA 1987). In extreme cases, the section of the river occupied by can reach several kilometers (DEZHKIN et al. 1986). Beavers show a preference not only for species, but also for the diameter of gnawed trees. The most common diameter reported in the literature is less than 10 cm (ŻAK 2001, MARGALETIĆ et al. 2006, BRATCIKOV 2007, MISIUKIEWICZ et al. 2016, MAHONEY and STELLA 2020, JACKOWIAK et al. 2020, JUHÁSZ et al. 2023).

There have been a number of studies and observations in Poland and around the world related to changes in bite intensity with increasing distance from the edge of a reservoir or watercourse. In Poland, the species in search of which beavers moved furthest from the water was poplar, and the average distance of movement for this species was 62 m. The animals also traveled relatively far in search of hazel and willow, for which the average distance was 34 and 25 m, respectively. Beavers traveled the shortest distance (4 m) to forage on alder (BOROWSKI and BORKOWSKI 2003, 2004). In the Wigry National Park, which has one of the oldest beaver populations in Poland, beavers used to roam up to several hundred meters from the shore in search of aspen (BOREJSZO and SKÓRZYŃSKA 1991). According to STOFFYN-EGLI and WILLISON (2011), 95% of trees are cut within 50 meters of the shore. FRYXELL (2001) showed that more than 50% of gnawed stems were within 10 meters of the water. BARNES and DIBBLE (1988) show that beavers penetrate an area up to 60 meters from the water in search of food.

Many researchers are attempting to develop a model to predict the foraging behavior of animals based on the theory of the central foraging site. In the case of beavers, this theory is related to foraging in terms of not only the species of trees and shrubs but also their size, distance, energetic inputs in felling trees and transport of food to water (ORIAN and PEARSON 1979, SCHOENER 1979, MCGINLEY, WHITHAM 1985, FRYXELL and DOUCET 1991). According to this theory, animals should be more selective near the center of their stand, and in order to maximize the profit associated with the energy expended, beavers should reach for thicker trees at distances farther from their feeding and burrows. According to GALLANT et al. (2004),

food selectivity depends on the quality of the habitat. In fertile habitats (with a higher proportion of deciduous species), beavers show greater selectivity than in poorer habitats (with less deciduous species). According to these studies, in high-quality areas, as the distance from the pond increased, beavers cut fewer trees but the trees were larger. Some authors suggest that the relationship between size and distance should be inverse, especially when "the prey is larger than the predator" (JENKINS 1980). A number of studies have documented a decrease in the thickness of gnawed plants as distance from shore increases (JENKINS 1980, BELOVSKY 1984, BUSHER 1996, HAARBERG and ROSELL 2006, RAFFELA et al. 2009). The results of these studies are consistent with the optimal foraging theory (OFT), which states that the distance from the available food, and the time spent acquiring it, depends on the size of the food and the distance that must be traveled to reach it. In the literature, one can also find studies that indicate that there is no correlation between the diameter of felled trees and the distance from the water (BRATCIKOV 2007). Other factors such as habitat richness (GALLANT et al. 2004), predator pressure (BASEY and JENKINS 1995, MYSŁAJEK et al. 2019) or humans (JACKOWIAK et al. 2020) can also influence selectivity.

The purpose of our study was to indicate whether the different period of colonization of the areas influences the selective foraging of rodents near the center of their habitat, and what differences exist between the parks' vegetation, especially in terms of species diversity of woody vegetation in beaver sites.

Material and Methods

Research works were located in the Wigry National Park (WNP) and Kampinos National Park (KNP). A population from the WNP established in the 1950s and a population from the KPN established from individuals brought from the KPN in 1980 (ŻUROWSKI 1984, KASPERCZYK 1990, JANISZEWSKI, MISIUKIEWICZ 2012, RAKOWSKA, STACHURSKA-SWAKOŃ 2021). The KPN population is classified as stabilizing (younger population, 42 years since introduction), while the WPN population is classified as aging (older population, 72 years since introduction).

Within each park, six experimental plots (banks of rivers, canals and lakes) were selected where traces of beaver foraging were found, and an additional three research plots (with beaver dams) were selected (Figure 1).

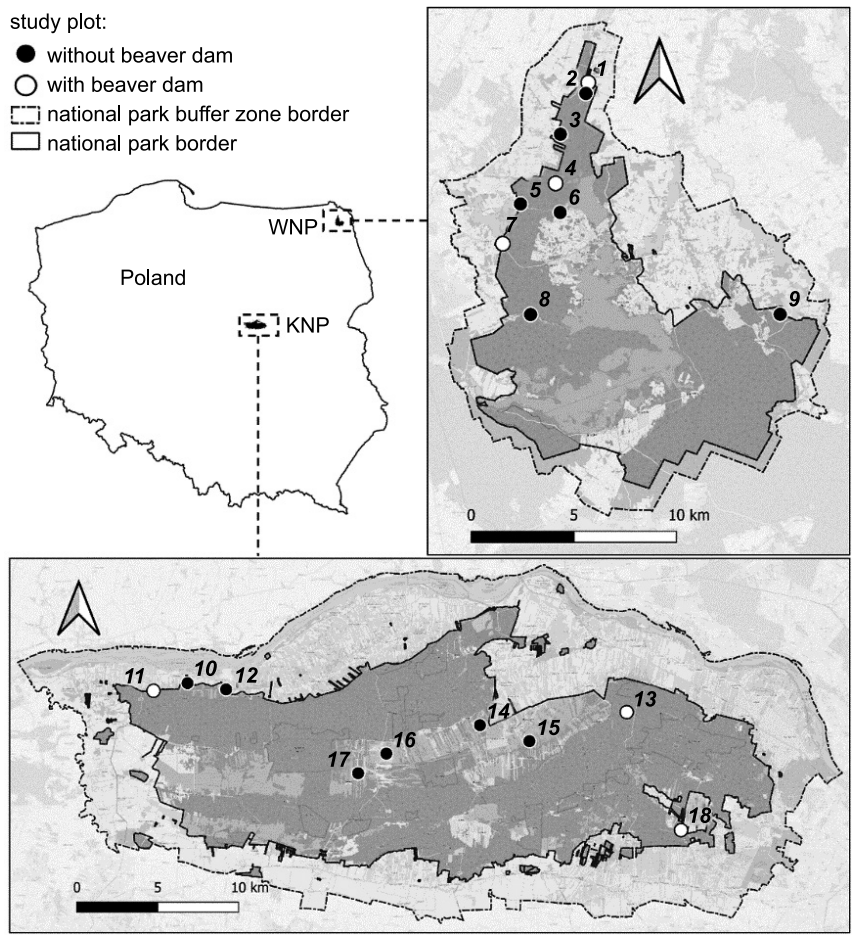

Fig. 1. Location of survey plots in Wigry National Park (WNP, older population, 72 years) and Kampinos National Park (KNP, younger population, 42 years)

Research plots from the area of the Wigry National Park were mainly located in the northern part of the Park (Wiatrołuża, Kamionka, Maniówka, Samlanka, Kamionka and Czarna Hańcza rivers). One plot was located in the eastern part of the WNP (Gremzdowka river). One survey plot was also located in the WPN area near the Suchar I water reservoir. Inside KNP, the plots were drowning located in northwestern (Kromnowski canal), central and eastern (Łasica, Ł9, Wilcza Struga canals) and south-eastern (Struga canal) parts of the Park.

Plots were drawn from historical databases and current information provided by the park managers. From the presence of watercourses, the most common habitats in the survey plots were wet and marshy habitats.

The most common plant communities were Fraxino-Alnetum (WNP) and Carici elongatae-Alnetum (KNP). More detailed descriptions of the measurement plots are included in the Appendix (Table 1.1).

Ten transects located perpendicular to the axis of the watercourse were established along the river in each study plot. The transects were distanced 20 meters apart from each other (Figure 2). Each transect was divided into 10-meter sections 4 meters wide. The number of sections per transect was determined by the presence of traces of beavers. If occlusions or damage to trees and shrubs were not present no further measurements were made on a given transect. In the plots with a beaver dam present, transects 5 and 6 were located near the dam, while the remaining transects were located in the area upstream of the dam, transects 1–4, and downstream of the dam, transects 7–10.

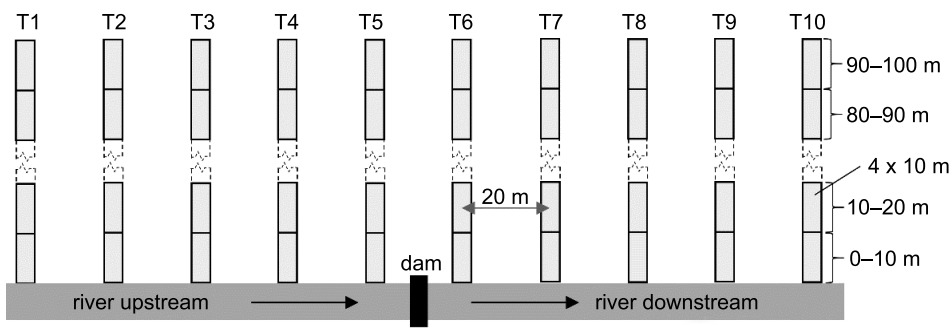

Fig. 2. Sampling scheme for assessing beaver foraging and floristic characteristics of sample plots. Arrow water flow direction

The measurements mainly used the methodology proposed by O'CONNELL et al. (2008), with some modifications, including the height at which tree and shrub thicknesses were measured. In each subplot, woody plants were inventoried by species, divided into three degrees of damage: *S* – stumps after trees and shrubs, *D* – damaged standing trees, and *U* – undamaged trees or shrubs. Thickness was measured at a height of 20 cm from the ground level, divided into three classes: 1 (<10 cm), 2 (11–30 cm) and 3 (>31 cm).

Data on the dimensions of the dam – length, thickness and height – were collected on measuring plots where beaver dams occurred. Information about the width of the river (the section behind the dam) and the beaver pond (the section before the dam) was also recorded. Information on the width of the river or canal was collected at each transect.

The collected data was analyzed in terms of the beaver's foraging range from the riverbank, preference for gnawed species, and diversity of

trees and shrubs. Preferences were analyzed in terms of the number of stumps gnawed and damaged trees, but also in terms of the basal area of gnawed trees and shrubs. The basal area reflects the volume of felled trees relatively well and is commonly used in Polish forestry as a volume index equivalent. In describing the results, there were also used the sum of the diameters of gnawed or damaged trees in the survey plot and the average diameter of gnawed wood.

There were compared the effects of population age (WNP – older population vs. KNP – younger population) and the dam on foraging range, number and diameters of gnawed and damaged trees, and species diversity of trees and shrubs. We performed the same analysis for the immediate vicinity of the dam (Upstream, Dam, Downstream). The Margalef (R) and Shannon-Wiener (H) diversity indices and Simpson's Dominance (C) were used to assess species diversity of trees and shrubs (Table 1). As beaver populations differed significantly in terms of foraging range, comparisons were made for the entire length of the transect and for the section in the immediate vicinity of the river or channel (0–10 m).

Table 1

Ecological indices

Index	Equation	Legend
Margalef (R)	$R = \frac{S_1}{\log N}$	S_1 – number of species N – number of all trees, shrubs on plot, transect n_i – number of trees, shrubs of a given species on plot, transect
Shannon-Wiener (H)	$H = -\sum (pi) \cdot (\log pi)$ $pi = \frac{n_i}{N}$	
Simpson (C)	$C = \sum \left(\frac{n_i}{N} \right)^2$	

The analyses used non-parametric tests – Mann-Whitney U test for two independent samples and Kruskal-Wallis ANOVA with Dunn Bonferroni post-hoc test for comparisons of more than two variants. Spearman's rank correlation coefficient was used in correlation analyses. Data were processed using an Excel spreadsheet and the PQ Stat statistical package.

Results

It was found no differences between the characteristics of the dams between analysed populations of beaver (Table 2). It was also shown that the rivers and channels on which the dams were built were also characterized by a similar width in both experimental parks (3.7–4.0 m), and beaver pond had a similar surface area too (450.0–666.7 m^2).

Table 2

Main features of the river and the dam

Feature	Younger population	Older population	Total
	average		
Width of dam [m]	7.7^{A}	10.3^{A}	9.0
Height of dam [m]	1.0^{A}	0.7^{A}	0.9
Thikness of dam [m]	1.3^{A}	1.2^{A}	1.2
Volume of dam [m^3]	14.2^{A}	9.6^{A}	11.9
Width of river upstream over a distance of 50 meters from dam [m]	13.3^{A}	9.0^{A}	11.2
Width of river downstream over a distance of 50 meters from dam [m]	3.7^{A}	4.0^{A}	3.8
River surface over a distance of 50 meters upstream from dam [m^2]	666.7^{A}	450.0^{A}	558.3
River surface over a distance of 50 meters downstream from dam [m^2]	183.3^{A}	200.0^{A}	191.7

The same capital letters of the alphabet indicate no statistically significant differences between beaver populations, while different letters indicate significant differences (Mann-Whitney U test, $\alpha = 0.05$)

Animals from the younger population foraged on 20 species of trees and shrubs, while those from the older population fed on 18 species (Table 3). Five species of trees and shrubs were mainly browsed or damaged by beavers from KPN: *Corylus avellana* L. (14.6%), *Alnus glutinosa* (L.) Gaertn. (15.2%), *Quercus robur* L. (17.4%), *Tilia cordata* Mill. (9.6%) and *Prunus padus* L. (13.4%). In terms of basal area, these were mainly: *Quercus robur* L. (41.5%), *Alnus glutinosa* (L.) Gaertn. (23.2%) and *Betula pendula* Roth. (11.0%). The beavers from older population, in terms of number of gnawed trees, foraged mainly on *Corylus avellana* L. (91.7%). In terms of basal area, foraging was spread out among three species: *Alnus glutinosa* (L.) Gaertn. (34.4%), *Corylus avellana* L. (21.2%) and *Tilia cordata* Mill. (10.6%). Beavers from both populations almost entirely used *Populus tremula* L. – from the older population, they fed on almost 100% of the trees of this species occurring near the rivers.

The analysis of the proportion of undamaged tree and shrub individuals (*U*) shows that the foraging base of the Wigry population is shrinking. Coniferous species – *Picea abies* (L.) H. Karst and *Pinus sylvestris* L. – dominate in the surroundings of the beaver's lodges. In terms of basal area, these species account for almost 45% of the share along the length of the entire transect and more than 30% of the share along the 0–10 meter section. It should be noted that cases of damaged individuals of these species were encountered in the area of the older population. The Kampinos

population existed in more favorable conditions. Among the undamaged trees, the species preferred by beavers were *Prunus padus* L. and *Alnus glutinosa* (L.) Gaertn. in terms of number, and *Quercus robur* L., *Alnus glutinosa* (L.) Gaertn. and *Betula pendula* Roth. in terms of basal area.

Table 3

Percentage of woody plant species by number and basal area divided into preferred by beavers (*S* + *D*) and undamaged trees and shrubs (*U*). Bold values indicate the three species with the highest share. Numbers in the anvias refer to the 0–10 m section. In the younger population area, species shares for the total and for the 0–10 m section were the same

Gatunek	Younger population (KNP)				Older population (WNP)			
	number		basal area		number		basal area	
	S + *D*	*U*	*S* + *D*	*U*	*S* + *D*	*U*	*S* + *D*	*U*
	%							
Corylus avellana L.	**14.6**	7.0	0.2	0.1	**91.7 (90.8)**	**38.2 (27.8)**	**21.2 (18.2)**	2.2 (1.2)
Alnus glutinosa (L.) *Gaertn.*	**15.2**	**9.3**	**23.2**	**30.3**	**1.9 (2.3)**	8.2 **(12.0)**	**34.4 (39.2)**	**35.8 (48.5)**
Quercus robur L.	**17.4**	5.7	**41.5**	**20.4**	0.1 (0.1)	0.6 (0.1)	0.5 (0.5)	2.1 (2.1)
Tilia cordata Mill.	9.6	3.4	5.4	4.3	**2.5 (3.0)**	5.4 (5.6)	**10.6 (11.5)**	7.1 (9.1)
Betula pendula Roth	5.0	2.8	**11.0**	**16.0**	0.6 (0.6)	0.4 (0.5)	7.8 (6.4)	1.0 (1.3)
Prunus padus L.	13.4	**26.6**	3.6	3.7	1.5 (1.8)	**9.1 (13.7)**	1.2 (0.9)	0.9 (1.2)
Populus tremula L.	5.9	0.1	5.0	1.4	0.1 (0.1)	0.01 (0.02)	1.7 (2.0)	0.01 (0.01)
Sorbus aucuparia L.	0.0	1.6	0.0	0.1	0.3 (0.2)	2.2 (1.6)	5.7 (6.4)	1.0 (0.9)
Picea abies (L.) *H. Karst*	1.2	0.0	0.1	0.0	0.3 (0.4)	**9.3** (8.2)	5.3 (5.5)	**24.2 (18.6)**
Fraxinus excelsior L.	0.3	0.3	0.4	0.0	0.2 (0.3)	1.3 (1.9)	3.9 (4.4)	1.1 (1.5)
Pinus sylvestris L.	1.9	1.5	1.6	10.9	0.1 (0.0)	1.8 (1.2)	1.7 (0.0)	**20.4 (11.3)**
Frangula alnus Mill.	0.3	**23.7**	0.4	0.6	0.0 (0.1)	3.7 (4.6)	0.0 (0.03)	0.2 (0.2)
Other*	15.2	18.0	7.6	12.2	0.7 (0.4)	19.8 (22.6)	6.0 (5.0)	4.0 (4.1)
All	100	100	100	100	100	100	100	100

*** Younger population (*S* + *D*):** *Acer platanoides* L., *Malus sylvestris* L., *Malus domestica* Borkh., *Ulmus minor Mill., Pyrus communis* L., *Acer negundo* L., *Salix caprea* L., *Prunus domestica* L., *Crataegus monogyna Jacq*. **Younger population (*U*):** *Euonymus verrucosus Scop., Euonymus europaeus* L., *Carpinus betulus* L., *Rhamnus cathartica* L., *Prunus myrobalana* (L.) *Loisel, Juglans regia* L., *Ribes nigrum* L., *Robinia pseudoacacia* L., *Salix cinerea* L., *Salix aurita* L., *Sambucus nigra* L., *Viburnum opulus* L., *Acer pseudoplatanus* L., *Larix decidua Mill., Padus serotina (Ehrh.) Borkh, Cornus sanguinea* L.

*** Older population (*S* + *D*)**: *Acer platanoides* L., *Euonymus verrucosus Scop., Carpinus betulus* L., *Acer negundo* L., *Rhamnus cathartica* L., *Ulmus laevis Pall., Lonicera xylosteum* L. **Older population (*U*):** *Salix caprea* L., *Crataegus monogyna Jacq., Ribes nigrum* L., *Daphne mezereum* L., *Sambucus nigra* L., *Viburnum opulus* L., *Acer pseudoplatanus* L., *Juniperus communis* L.

The dwindling food supply in the WNP may also be indicated by the foraging range and the diameter and number of trees gnawed (Table 4).

Table 4

Foraging ranges and the diameter and number of trees gnawed

Specification	Younger population (KNP)			Older population (WNP)		
	average	median	max	average	median	max
Beaver foraging ranges						
All rivers or canals	5.7^{B}	5.5	26	20.0^{A}	16.0	97
Rivers or canals without a beaver dam	6.0^{aB}	5.5	15	17.0^{bA}	15.2	75
Rivers or canals with a beaver dam	5.9^{aB}	5.6	25	26.6^{aA}	32.7	95
Beaver foraging ranges in relation to the dam						
Upstream	6.0^{Ba}	5.6	15	27.8^{Aab}	25.7	75
Dam	9.0^{Ba}	7.4	25	18.1^{Ab}	16.1	45
Downstream	5.0^{Ba}	4.7	6	35.1^{Aa}	28.4	97
Diameter and number of damaged and cut trees						
Number of gnawed trees and shrubs (S + D) per location	35.8^{B}	38	61	456.1^{A}	293	2330
Sum of diameters of gnawed trees and shrubs (S + D) per location	367.8^{A}	270	730	455.6^{A}	320	1270
U diameter	16.9^{bA}	16.6	40	13.5^{bB}	15.6	40
S diameter	15.1^{bA}	14.6	40	10.5^{bB}	8.7	40
D diameter	29.9^{aA}	29.5	40	28.0^{aA}	30	40

Explanations: U – undamaged trees and shrubs; S – stumps; D – damaged standing trees. The same uppercase letters mean no differences between columns, different letters mean statistically significant differences (Mann-Whitney U test, $\alpha = 0.05$). The same lowercase letters indicate no differences between row averages, different letters indicate statistically significant differences (Mann-Whitney U test, $\alpha = 0.05$ for differences between foraging range in plots with and without a dam, and Kruskal-Wallis ANOVA, Dunn-Bonferroni post-hoc test, $\alpha = 0.05$ for other analyses in the table)

Beavers from the older population foraged much farther from the axis of the watercourse to compare with beavers from the younger population. Beavers from the WNP made the farthest wanderings at a distance of almost 100 meters. With 43% of gnawed or damaged trees located within 10 meters of the river, more than 90% of gnawed trees or shrubs within 40 meters of the river. In the case of the younger population from the KNP, the maximum foraging range did not exceed 30 meters, and we recorded

92% of the gnawed trees or shrubs up to 10 meters from the channel. Beavers from the older population migrated significantly farther in plots where beaver dam were present – 26.6 m with dam and 17.0 m without dam. In the case of the younger population, beavers migrated similar distances in both plots with and without a dam (about 6.0 m). Within the plots with dams, beavers from the older population migrated significantly further on transects located behind dam (downstream – 35.1 m) compared to sites near dams (18.1 m) and before dam (upstream – 27.8 m). In the area inhabited by the younger population, beavers migrated similar distances regardless of the transect's location relative to the dam (5.0–9.0 m). In terms of the sum of diameters of gnawed or damaged trees and shrubs, both populations had similar conditions. The younger population gnawed an average of 367.8 cm in diameter per location, while the older population gnawed 455.6 cm in diameter per location. However, the older population had to acquire more than 10 times the number of trees and shrubs (456.1 per location) than the younger population (35.8 per location). The diameter of undamaged trees (*U*), damaged trees (*D*) and stumps (*S*) indicates that the younger population had a greater availability of thick trees. Interestingly, both populations damaged and left trees (*D*) with similar diameters. In WNP, beavers damaged mainly alder (63% of damaged trees) and pine (9% of damaged trees), while in KPN beavers damaged mainly oak (44%) and alder (17%). Within both parks, damaged trees tended to be thicker than trees that were cut down (stumps – *S*). Within each park, the diameters of stumps and undamaged trees did not differ significantly from each other.

Damaged trees (*D*) were mainly found in the immediate vicinity of the river. The younger population damaged and left trees up to 20 meters from the river or channel, while the older population damaged trees up to 40 meters from the river. The number of damaged trees was negatively correlated with the distance from the river bank (Table 5). The number and diameter of stumps from felled trees and shrubs decreased with increasing distance from the river. We showed significant correlations in this aspect for the number of stumps at the site of the younger population (r = -0.85) and for the diameter of stumps at the site of the older population (r =-0.45). The diameter of the stumps of undamaged trees did not change significantly in the WNP and was about 15 cm in diameter along the entire 100-meter transect. In the case of the KNP, the diameter of undamaged trees and shrubs successively increased from 15 to 20 cm on the 0–30 m section. This was a statistically significant increase (r = 0.42). Analysis of correlations between the number and diameter of gnawed trees and distance from the river or canal bank showed no significant

relationship for most species. The only correlation was shown for *Corylus avellana* (L.) in the WNP – as the distance from the river increased, the number and diameter of gnawed hazel trees decreased (r = -0.18 for diameter and r = -0.14 for number). Examining correlations between other traits, there were found that dam volume was positively correlated with gnawed wood diameter and negatively correlated with foraging range.

Table 5

Correlation between the number and diameter of trees and distance from the river or canal bank (Spearman's monotonic relationship, α = 0.05)

Feature	Population	Uundamaged (*U*)	Damaged (*D*)	Stumps (*S*)	All
Number	younger	-0.38	-0.48*	-0.85*	-0.37
	older	0.25	-0.53*	-0.24	0.11
Diameter	younger	0.42*	0.13	-0.22	0.39
	older	-0.05	-0.74*	-0.45*	-0.24

Due to that the beaver populations differed significantly in terms of foraging range, the comparison in terms of diversity was carried out on the whole material, i.e. the entire foraging range, and for the section located in the immediate vicinity of the river or channel (0–10 m). The KNP showed significantly higher species diversity of woody vegetation measured by the *R* and *H* indices and lower species dominance measured by the *C* index, both for the whole material and for the 0–10 m section (Table 6).

Table 6

Indicators of biological diversity of trees and shrubs by population

Wariant	Simpson's dominance index (*C*)	Shannon-Wiener diversity index (*H*)	Margalef diversity index (*R*)
All			
Younger population (KPN)	0.43^b	1.04^a	1.39^a
Older population (WPN)	0.58^a	0.73^b	1.05^b
First 10 metres of transect (section 0–10 m)			
Younger population (KPN)	0.46^b	1.03^a	1.39^a
Older population (WPN)	0.56^a	0.72^b	1.07^b

The same lowercase letters indicate no differences between the row, different letters indicate statistically significant differences (Mann-Whitney U test, α = 0.05)

The analysis of correlation between the foraging range and values of ecological indicators showed that in the younger population's area, the dominance index *C* decreased and diversity indices *H* and *R* increased as the distance from the river or channel was longer (Table 7). In the area of the older population, an inverse relationship was shown. However, the correlations for this population were statistically insignificant. The diameter of gnawed trees and shrubs was usually not correlated with the values of the indices. The exception was the index of dominance in the older population's area. This index decreased as the diameter of gnawed trees increased.

Table 7

Correlation between foraging range and diameter of gnawed trees and shrubs and biodiversity indicators

Feature	Population (Park)	Biological indicator		
		Simpson's dominance index (*C*)	Shannon-Wiener diversity index (*H*)	Margalef diversity index (*R*)
Beaver foraging range	older (WPN)	0.26	-0.35	-0.12
	younger (KPN)	-0.81*	0.80*	0.82*
Diameter of gnawed trees and shrubs	older (WPN)	-0.54*	0.10	0.08
	younger (KPN)	-0.05	-0.05	0.32

* Correlation significant at $\alpha = 0.05$ (Spearman's monotonic relationship)

In the case of the analyses for the area with the current beaver dam, it was shown that in the vicinity of the dam the tree and shrub layer is richer in species than the areas upstream and downstream of the dam (Table 8). In most cases, these differences were not statistically significant. We showed the only significant differences for the dominance index C in the area occupied by the younger population, and for the Margalef index *R* in the section of the first 10 meters in the area occupied by the older population.

Table 8

Values of indices of woody vegetation diversity depending on the location of the transect in relation to the dam in the variant for the entire transect and for the first 10 meters of the transect

Population (Park)	Variant	Simpson's dominance index (*C*)	Shannon-Wiener diversity index (*H*)	Margalef diversity index (*R*)
All				
Younger (KPN)	upstream	0.44^{a}	1.02^{a}	1.38^{a}
	dam	0.27^{b}	1.10^{a}	1.60^{a}
	downstream	0.45^{a}	1.03^{a}	1.43^{a}
Older (WPN)	upstream	0.62^{a}	0.65^{a}	0.98^{a}
	dam	0.59^{a}	0.70^{a}	1.25^{a}
	downstream	0.57^{a}	0.68^{a}	0.92^{a}
First 10 metres of transect (subplot 0–10 m)				
Younger (KPN)	upstream	0.42^{a}	1.05^{a}	1.40^{a}
	dam	0.35^{a}	1.19^{a}	1.58^{a}
	downstream	0.45^{a}	1.03^{a}	1.43^{a}
Older (WPN)	upstream	0.62^{a}	0.59^{a}	1.01^{b}
	dam	0.43^{a}	1.02^{a}	1.37^{a}
	downstream	0.51^{a}	0.62^{a}	0.94^{b}

Same lowercase letters indicate no differences between row, different letters indicate statistically significant differences (Kruskal-Wallis ANOVA, Dunn-Bonferroni post-hoc test, $\alpha = 0.05$)

Discussion

The results of the study indicate foraging behavior of European beaver according to the optimal foraging theory (OFT) for younger and older populations, thus confirming the studies of JENKINS 1980, BELOVSKY 1984, BUSHER 1996, HAARBERG and ROSELL 2006, RAFFELA et al. 2009. Both populations presented similarly feeding behaviour, despite differences in foraging range away from the river or channel. As the distance from the river or canal increased, the number and diameter of trees and shrubs felled decreased. Thus, the age of the population did not affect foraging strategy in this case. The younger population inhabited more fertile habitats (with a higher proportion of deciduous species) than the older one (lower proportion of deciduous species) hence it also did not confirm the theory of GALLANT et al. (2004) according to which in richer habitats beavers show selectivity consistent with the central foraging theory. The beavers did not move further away from the river to search for specific species

of trees or shrubs. Beavers of both populations gnawed almost 100% of aspen poplar, but this species was found in close proximity to rivers and canals. The species that were gnawed farthest from the river on the KNP were: pine, apple and oak, while on the WNP were: hornbeam, linden and hazel – the choice of species was not dictated by foraging preference, but rather reflected the local habitat layout. The decrease in diameter and number of stumps with distance from the river may also be due to the presence of predators and humans. A study by MYSŁAJEK et al. 2019 realised in the WNP showed that beaver make up almost 10% of the wolf's diet. In the KNP area, the wolf is less numerous, but it is one of the most populous parks in Poland.

The own study shows that the dominant factors modifying the foraging behavior of beavers and their foraging ranges in the analysed populations were: the presence of the dam, the availability of food and its spatial variation. The older population migrated farther in plots with a current dam – this may be due to the need to obtain more raw materials for dam construction. It should be noted that the volume of the dam was positively correlated with the diameter of the gnawed trees and negatively correlated with the foraging range. At the same time, within the plots with dams, beavers migrated to the shortest distances on transects located next to the dam - this in turn may indicate the selection of the species-richest area for dam construction. This was confirmed by biodiversity analyses – we tended to find higher richness (*H*, *R*) and lower dominance (*C*) of tree and shrub species on transects next to the dam. Beavers from the younger population moved similar distances in both plots with and without the dam. In the case of the younger population, we showed a little further range of migration for trees and higher biodiversity on transects located next to the dam. The lack of comparison plots makes analysis in this aspect difficult. It is difficult to find sites unaffected by beaver activity, especially in the WNP. In previously research in the Polesie National Park, was managed to find plots that beavers have not yet colonized (PIĘTKA and MISIUKIEWICZ 2022). On the beaver-inhabited rivers, it has been showed greater variation in tree layer than in areas that beavers have not yet colonized. Although the compositions of the tree stands in the beaver-occupied and comparison plots were very similar they were not identical – for example, in the beaver-occupied areas we showed a higher proportion of aspen – 6.8% in the beaver-occupied river area and 1.4% in the comparison plots, respectively. This already indicates that the greater diversity in beaver-inhabited areas may be both a result of beaver activity and the selection of more attractive areas for dam construction. Most studies in this area indicate that beavers increase the species richness of the areas they inhabit (GAYWOOD 2016).

There are also studies indicating that beavers are selective in terms of, and in the first instance, beavers populate areas that are most attractive to them (DVORNIKOVA 1987). In order to determine which factor plays a greater role, it is necessary to move away from comparative plots to cyclic surveys within these parks.

Analysis of damaged trees provided interesting results. Damage usually consisted of ringing the tree. It was noticed that beavers usually damaged very thick trees and, importantly, we encountered them mainly within 30 meters of the river or canal bank. We know from stump analysis that further away from the river thick trees were not cut down, and we also know that thick live trees were quite common further away from canals and rivers. It can be assumed that due to their thickness, the beavers did not complete the felling of these trees due to energy conservation, or the felling process is still ongoing. The thickest stumps were found just off the river bank. In our surveys, however, we encountered many trees damaged in an advanced stage of decay that were ultimately not cut down, mainly alder. One might be tempted to say that the goal was not to cut down a tree, but to create conditions for the renewal of a new generation of trees by allowing more light into the forest floor. Numerous studies and the results of our research show that beavers mainly harvest small-diameter trees and shrubs. Killing a tree creates a niche for smaller sized trees and shrubs such as hazel. At the same time, felling trees with large dimensions would involve a large energy expenditure. Thus, ringing was a way to expand the foraging base at the lowest possible cost. It should be noted that beavers have damaged species such as pine and spruce – species they do not prefer in their diet.

Conclusion

Beavers from the older population moved farther away from the water bank than beavers from the younger population. This could be the result of a shrinking foraging base or an overcrowded population in the Wigry National Park. The study did not confirm the theory of central foraging. According to this theory, beavers, in order to maximize the profit associated with the energy expended, should reach for thicker trees at distances further from the river. Both the younger and older populations foraged according to the theory of optimal foraging, according to which the distance from the available food, and the time spent to reach it, depends on its size and the distance to be traveled to reach it. Our study shows that the dominant factors modifying the foraging behavior of beavers and their

foraging ranges were the presence of a dam and the availability of preferred species. Greater diversity in the areas where the beaver is present may be the result of both its activities and the selection of more attractive areas for the construction of the dam.

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Appendix 1

Table 1.1

Location and brief description of survey plots

Number	Park (population)	Near river, canal, lake	Beaver dam presence	Coordinates WGS 84		Plant community*
				x	*y*	
1	WNP (older)	Wiatrołuża river	yes	54,15257405	23,08446543	1, 2
2	WNP (older)	Wiatrołuża river	no	54,14787708	23,08224792	1, 2
3	WNP (older)	Maniówka river	no	54,13080822	23,06159436	1, 3
4	WNP (older)	Samlanka river	yes	54,10953227	23,05589423	1, 4
5	WNP (older)	Kamionka river	no	54,10146956	23,02890188	1, 4
6	WNP (older)	Kamionka river	no	54,09678799	23,0580681	1, 4
7	WNP (older)	Suchar I lake	yes	54,08461992	23,01411128	1
8	WNP (older)	Czarna Hańcza river	no	54,0531611	23,0317406	1
9	WNP (older)	Gremzdówka river	no	54,04688593	23,21697512	1, 2
10	KNP (younger)	Kromnowski canal	no	52,37289914	20,34984958	5
11	KNP (younger)		yes	52,36899707	20,3188032	5, 6
12	KNP (younger)		no	52,36902899	20,38481193	5, 6
13	KNP (younger)	Wilcza Struga canal	yes	52,35149243	20,7482574	7
14	KNP (younger)	Ł9 canal	no	52,34615397	20,61470229	7
15	KNP (younger)	Łasica canal	no	52,33661303	20,65928257	7
16	KNP (younger)	Ł9 canal	no	52,33144887	20,52919234	7
17	KNP (younger)	Łasica canal	no	52,32081006	20,50320054	3, 7
18	KNP (younger)	Struga	yes	52,28501501	20,7949335	5

*plant community (MATUSZKIEWICZ and WOLSKI 2023): 1 – *Fraxino-Alnetum* (=*Circaeo-Alnetum*) – Lowland alder and ash-alder forest on the periodically swamped ground-water soils; 2 – *Tilio-Carpinetum* – Subcontinental lowland lime-oak-hornbeam forest; subboreal vicariant with spruce, eutrophic („rich") communities; 3 – *Tilio-Carpinetum* – Subcontinental lowland lime-oak-hornbeam forest; subboreal vicariant with spruce, mesotrophic („poor") communities; 4 – *Pino-Quercetum* (=*Querco-Pinetum* + *Serratulo-Pinetum*) – Continental mesotrophic oak-pine mixed forest; 5 – *Pino-Quercetum* (=*Querco-Pinetum* + *Serratulo-Pinetum*) – Continental mesotrophic oak-pine mixed forest; 6 – *Ficario-Ulmetum typicum* – Lowland ash-elm floodplain forest; occasionally flooded; 7 – *Carici elongatae-Alnetum* (=*Ribeso nigri-Alnetum* + *Sphagno squarrosi-Alnetum*) – Middle-European alder fen forest

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CHEMICAL PROFILING AND ANTIOXIDANT STUDIES ON THE LEAF OF *BREONADIA SALICINA* HEPPER AND J.R.I. WOOD (RUBIACEAE)

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Key words: *Breonadia salicina*, Rubiaceae, antioxidant, flavonoids, phenolic acids.

Abstract

This study evaluated the antioxidant properties of the leaves of *Breonardia salicina* and the profiling of its chemicals. The leaves of the plant are widely used ethnobotanically for the treatment of cancer, gastrointestinal diseases, fevers, headaches, arthritis, diabetes, inflamed wounds, and ulcers. The plant is an evergreen growing along riverbanks, streams, and river tributaries, belonging to the family Rubiaceae. The plant was collected and identified, samples and fractions of the samples were then evaluated for antioxidant activity using DPPH and ABTS and chemical profiling of the sample was performed using LC–ESI-MS/MS. Antioxidant equivalence of the leaf extracts/fractions of the plant at R^2 value of 0.9938 and standard equation ($y = 0.9891x - 1.996$) was found to be highest at 281.7 ±0.8 mg Trolox Equivalent and lowest at 118.7 ±2.7 mg Trolox Equivalent. The LC–ESI-MS/MS of the sample identified 22 compounds with their structures, belonging to different classes including flavonoids, glycosides, phenolic acids, triterpenoids, amides, and sulphonamides. The identified compounds are of medicinal importance, which is undoubtedly responsible for the antioxidant and anticancer properties of the plant. The good antioxidant values obtained revealed the possible use of the plant in the treatment of many diseases that are known to respond to antioxidation.

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Introduction

Phenolic compounds especially Phenolic acids and flavonoids are health enriching and found mostly in plants (CERVENKA et al. 2018). They provide plants with their antioxidant properties (TUNGMUNNITHUM et al. 2018) and prevent conditions triggered by free radicals (AKINOLA et al. 2014, ZAHID et al. 2018), due to their antioxidant ion on the free radicals (CARVALHO-SIVA et al. 2013).

Antioxidants are natural or synthetic central basics that intercept or mitigates damage to cells caused by the free radicals or unstable molecules that the body manufactures in response to environment or pressures (KASOTE et al. 2015). These free radicals, which are also referred to as reactive oxygen species (ROS) perhaps, are the major cause of degenerative diseases such as cancer and neurodegenerative diseases (LIU et al. 2018). They can be managed using a synthetic or natural approach. The synthetic antioxidants such as vianol and embanox have been useful in managing degenerative free radical complication. However, due to their associated side effect and attendant problems such as accumulation in tissues, people are forced to look for an alternative approach. This issue makes natural antioxidants more popular.

Natural antioxidants are metabolites that are mostly from plant origin (LOURENÇO et al. 2019), and are often phenolic and organic acids (CROZIER et al. 2007, EL-KASHAK et al. 2017). This study therefore was carried out to determine the Total Phenolic Contents, Total Flavonoid Contents and antioxidant capacity of the leaf extract and fraction of the plant.

Methods

Collection, identification, and preparation of the leaves of *Breonadia salicina*

Breonadia salicina was first identified on the field using its morphological features around Kudingi Village, Giwa Local Government Area, Kaduna state, Nigeria. Sample of the plant was then collected and transported to Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria for proper identification and authentication. The plant collected identified as *Breonadia salicina* and a voucher specimen number of ABU900383 given and deposited in the Herbarium of the Department. Sufficient quantities of the leaf obtained for further studies. The leaves were garbled and all foreign matters were removed. The material was air-dried in the shade, comminuted into a powder form using a pestle and mortar, and then stored in an airtight container.

Extraction of the leaves of *Breonadia salicina*

The dried powdered leaf sample of the *B. salicina* (300 g) each macerated with 1 L of 95% ethanol using mechanical shaker (Stuart Scientific Flask Shaker, Great Britain) at 25°C, 200 rpm for 6 hours. The extract obtained was filtered with a Whatman filter paper No 1, and then evaporated to dryness using rotary evaporator (Büchi Labortechnik) at 50°C and reduced pressure. The dried extract then weighed, and the percentage yield calculated. The extract transferred into an airtight container and kept properly in a dessicator for further use.

Fractionation of the aqueous ethanolic extract of the leaf of *B. salicina*

The extract of the leaf (2.5 g) suspended in 500 mL of water and sonicated at 20°C for 10 minutes. Thereafter, n-Hexane (300 mL) added and then shaken using the mechanical shaker (Stuart Scientific Flask Shaker, Great Britain) at 20°C, 200 rpm for 30 minutes. The mixture was transferred to a separating funnel, allowed to stand and the hexane fraction was collected, and then evaporated to dryness using rotary evaporator (Büchi Labortechnik) at 50°C. The aqueous portion then extracted with ethyl acetate (300 mL) as described above. The same procedure was repeated using *n*-butanol. The Ethyl acetate, *n*-butanol and aqueous fractions were concentrated over a water bath, transferred into sample bottles and kept for further use.

Determination of the antioxidant activities of *Breonadia salicina* leaf extract DPPH radical scavenging assay

DPPH radical scavenging activity of the leaf ethanolic extract (LEE), ethyl acetate leaf fraction (EAL), *n*-butanol leaf fraction (NBL) and the aqueous leaf fraction (AQL) were all determined using method described by (Blois 1958), modified by Chan et al. (2018). Absorbance of each solution measured at 517 nm using a microplate reader. Gallic acid used as the standard (positive control). The percentage of radical scavenging activity was calculated as follows:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}})] \cdot 100,$$

where:
A_{control} – the absorbance of the control
A_{sample} – the absorbance of the test extracts.

ABTS radical cation scavenging assay

The ABTS radical cation scavenging of the leaf ethanolic extract (LEE), ethyl acetate leaf fraction (EAL), *n*-butanol leaf fraction (NBL) and the aqueous leaf fraction (AQL) were all determined using method described by (Re et al. 1999). The ABTS (7 mM) and potassium persulfate solutions (2.45 mM) were prepared and mixed together, incubated for 8-hours in the dark. The stock solution was then diluted with methanol and its absorbance adjusted to 0.900 (±0.02) at 745 nm at 30°C. 300 µL (125–2000 µg/mL in methanol) for each of the sample mixed with the ABTS working solution and measured the absorbance. The percentage scavenging property of the samples and the standard calculated thus:

$$\text{Scavenging effect [\%]} = [(\text{control absorbance (ABTS)} - \text{sample absorbance} / (\text{control absorbance})] \cdot 100.$$

Liquid Chromatography–Mass Spectroscopy of the ethyl acetate leaf fraction (EAL) of *Breonadia salicina* (LC–MS/MS) analysis sample preparation

The ethyl acetate leaf fraction (EAL) of *B. salicina* leaf (1 mg) dissolved in 1 mL of LCMS grade methanol as the master stock (MS) and 10 µg/mL as the working stock (WS) in methanol before analysis. All samples were filtered with a 0.22 µm PTFE membrane filter and transferred to 2 ml vials for analysis.

Liquid Chromatography–Mass Spectroscopy procedure

The sample analysed using ultra-performance liquid chromatography with high-resolution mass spectrometry (LC–MS/LC–HRM) for the identification of compounds. The method used employed reversed-phase chromatography with a gradient range of solvent strengths. The online high-resolution accurate mass (HRAM) fragmentation library contains highly curated MS/MS and MSn spectra from different collision types and collision energies. Cloud Search was integrated into the compound discoverer along with other tools, such as predicted compositions based on high-resolution full MS and ChemSpider search, that helped partially identify the compounds. Built-in FISH scoring was used to verify hits from ChemSpider against the MS2 data.

Operating conditions

The results of the (LC–MS/LC-HRMS) subjected to the Thermo Scientific Compound Discoverer software version 3.1 for online compound database matching using cloud and ChemSpider.

Results

Extraction of the powdered leaf of *Breonadia salicina*

The 95% ethanol cold maceration of the dried powdered leaf yielded 14.14%.

Fractionation of ethanol extracts of the leaf of *B. salicina*

Four (4) fractions were obtained, after successive fractionation with three solvents, these are: Hexane (HX), ethyl acetate (EA), and *n*-butanol (NB). The fractions were HXL, EAL, NBL and AQL.

Free radical scavenging activities of the leaves of *Breonadia salicina* by DPPH

Free radical scavenging power (Antioxidant property) of the extracts/fractions (LEE, EAL, NBL and AQL) of the *B. salicina* were determined using DPPH method with Trolox as the standard antioxidant. Trolox antioxidant equivalence were calculated for each of the extract/fraction using a standard regression curve of Trolox with R^2 value of 0.9938 and standard equation ($y = 0.9891x - 1.996$).

Table 1

Antioxidant activities of the extracts by DPPH

mg Trolox Equivalent ±SD					
Con µg/ml	0	200	400	800	*P*-value*
LEE	0.0	55.7 ±2.5	101.7 ±2.1	205.4 ±0.6	<0.005
EAL	0.0	77.2 ±1.5	141.3 ±0.6	281.7 ±0.8	<0.005
NBL	0.0	40.5 ±2.5	85.4 ±1.5	176.3 ±2.8	<0.005
AQL	0.0	28.2 ±2.1	56.3 ±1.9	118.7 ±2.7	<0.005

Key: values are means ±SD of 3 replicates; LEE – 95% leaf ethanol extract; EAL – leaf ethyl acetate fraction; NBL – *n*-butanol leaf fraction; AQL – aqueous leaf fraction

* All concentrations of different extracts had significantly high mg Trolox/g Equivalence at $p < 0.05$ using one-way ANOVA post hoc (donnet test).

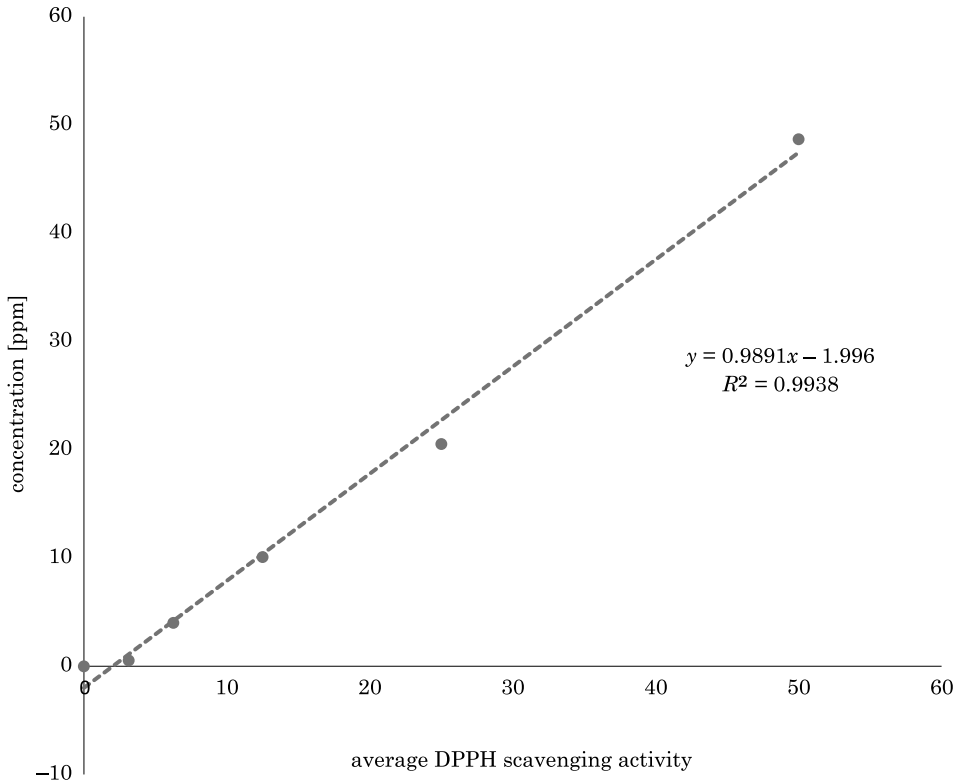


Fig. 1. Standard curve of Trolox for the determination of mg equivalence of scavenging activities of the extracts and fractions for the leaf of *Breonadia salicina*

Free radical scavenging activities for the leaves of *Breonadia salicina* by ABTS

Free radical scavenging power for the extracts/fractions (LEE, EAL, NBL and AQL) of the *B. salicina* were also determined using ABTS method with Trolox as the standard antioxidant. Trolox antioxidant equivalence were calculated for each of the extract/fraction using a standard regression curve of Trolox with R^2 value of 0.9996 and standard equation ($y = 0.8039x + 0.2045$) – Figure 2.

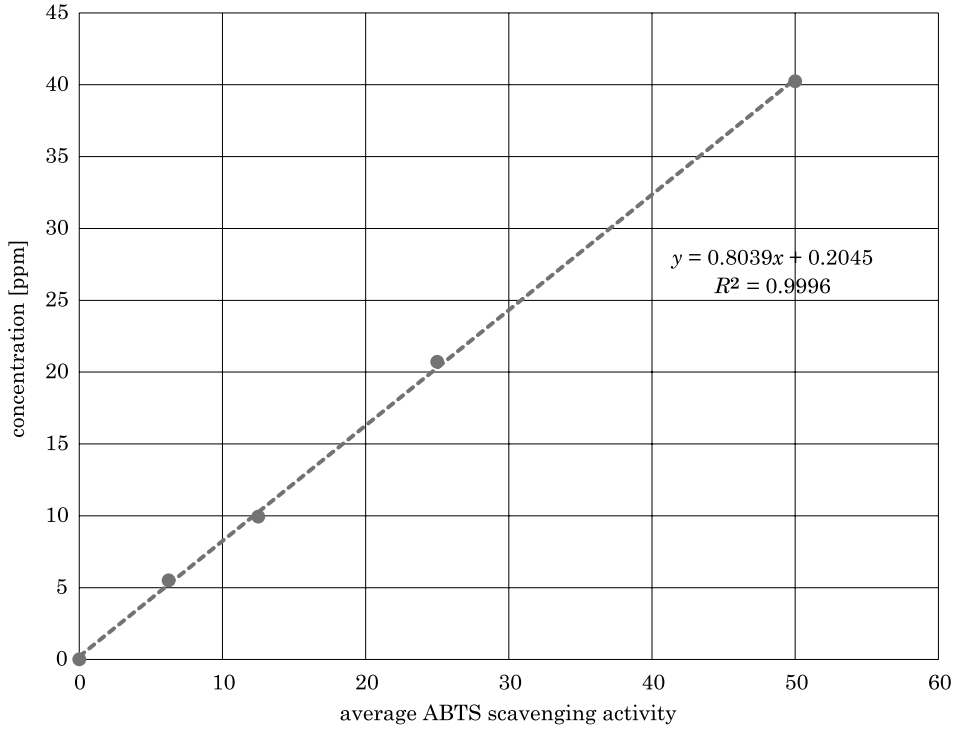


Fig. 2. Standard curve of Trolox for the determination of scavenging activities of the extracts and fractions of the leaf of the *B. salicina*

Table 2

Antioxidant of the extracts by ABTS

mg Trolox Equivalent ±SD					
Con µg/ml	0	200	400	800	*P*-value*
LEE	0.0	47.1 ±1.5	102.4 ±2.4	195.4 ±0.8	<0.005
EAL	0.0	69.7 ±3.5	132.0 ±0.9	275.8 ±0.6	<0.005
NBL	0.0	51.7 ±2.5	91.6 ±3.1	196.5 ±1.1	<0.005
AQL	0.0	32.3 ±1.6	73.5 ±1.9	143.1 ±1.8	<0.005

Key: values are means ±SD of 3 replicates; LEE – 95% leaf ethanol extract; EAL – leaf ethyl acetate Fraction; NBL – *n*-butanol leaf fraction and AQL – aqueous leaf fraction

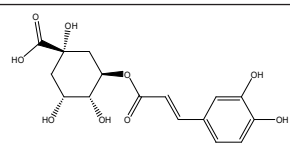
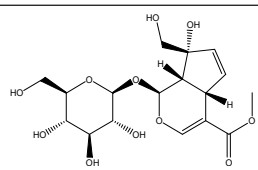
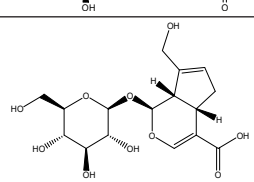
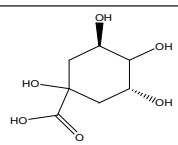
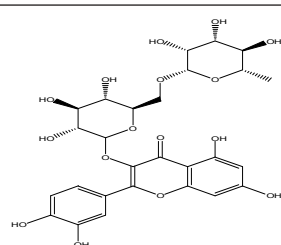
* All concentrations of different extracts had significantly high mg Trolox/g Equivalence at $p < 0.05$ using one-way ANOVA post hoc (Donnet test).

Isolation of chemical compounds from the ethyl acetate fraction of the *B. salicina* Leaf Using LC–ESI-MS/MS Analysis

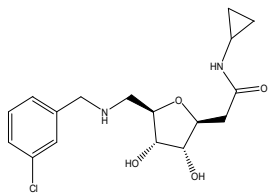
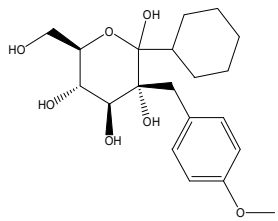
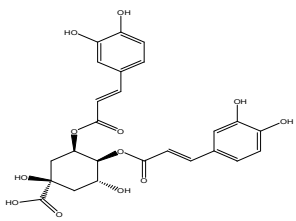
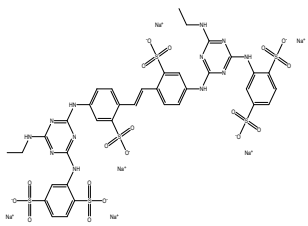
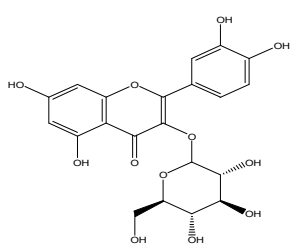
Twenty-two (22) chemical compounds were isolated using LC–MS/MS identified with the assistance of Thermo Scientific Compound Discoverer software version 3.1 for online compound database matching using cloud and ChemSpider. The twenty-two (22) compounds with their chemical structures, belonging to different classes of chemical compounds; includes flavonoids, glycosides, phenolic acids, triterpenoids, amides and sulphonamides as shown in Table 3.

Table 3

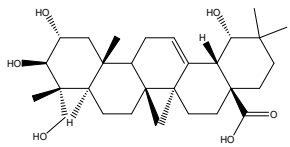
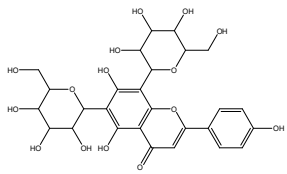
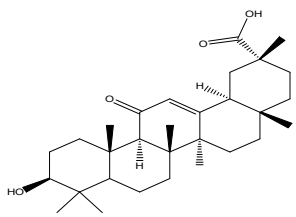
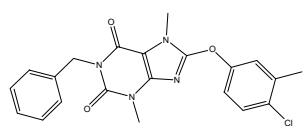
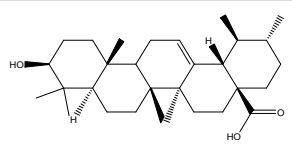
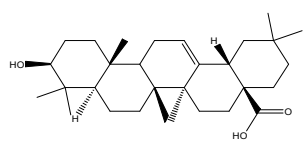
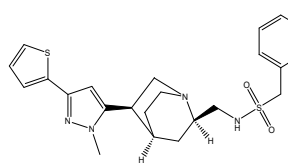
Chemical compounds of the Ethyl Acetate Leaf fraction (EAL) of *Breonadia salicina* using LC–ESI-MS/MS

S/N	Structure of the compound	Molecular Formula	Name of the compound	Molecular weight	RT [min.]
1	2	3	4	5	6
1.		$C_{16}H_{18}O_9$	chlorogenic acid	354.0944	4.09
2.		$C_{17}H_{24}O_{11}$	methyl1 (hexopyranosyloxy) 5-hydroxy-7- (hydroxymethyl) 1,4a,5,7 atetrahydrocyclopenta [c]pyran-4-carboxylate	404.1310	6.04
3.		$C_{16}H_{22}O_{10}$	geniposidic acid	374.1207	3.80
4.		$C_7H_{12}O_6$	D-(-)-Quinic acid	192.0621	4.08
5.		$C_{27}H_{30}O_{16}$	rutin	610.1532	9.50

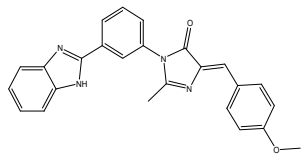
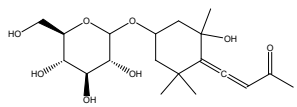
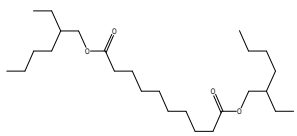
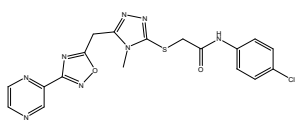
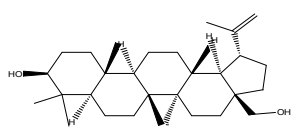
cont. Table 3

1	2	3	4	5	6
6.		$C_{17}H_{23}ClN_2O_4$	2-[(2S,3R,4S,5R)-5-{[(3-Chlorobenzyl)amino] methyl}-3,4-dihydroxytetrahydro-2-furanyl]-N-cyclopropylacetamide	390.1159	3.64
7.		$C_{19}H_{30}O_8$	3-Hydroxy-3,5,5-trimethyl--4-(3-oxo-1-buten-1-ylidene) cyclohexyl ß-D-glucopyranoside	386.1938	4.47
8.		$C_{25}H_{24}O_{12}$	4,5-Dicaffeoylquinic acid	516.1263	12.29
9.		$C_{36}H_{36}O_{18}$	2-(3,4-Dihydroxyphenyl)-5,-7-dihydroxy-4-oxo-4H-chromen-3-yl 6-deoxy-2-O-{6-O--[(2E)-3-(4-hydroxyphenyl)--2-propenoyl]-ß-D-glucopyranosyl}-α-L-glucopyranoside	756.1897	16.62
10.		$C_{21}H_{20}O_{12}$	quercetin-3ß-D-glucoside	464.0955	9.63

cont. Table 3

1	2	3	4	5	6
11.		$C_{30}H_{48}O_6$	arjungenin	504.3448	18.64
12.		$C_{27}H_{30}O_{15}$	5,7-Dihydroxy-2-(4-hydroxyphenyl)-6,8-bis[3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl]-4H--chromen-4-one	594.1584	10.35
13.		$C_{30}H_{46}O_4$	18-ß-Glycyrrhetinic acid	470.3392	22.44
14.		$C_{21}H_{19}ClN_4O_3$	1-Benzyl-8-(4-chloro-3-methylphenoxy)-3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione	410.1178	6.04
15.		$C_{30}H_{48}O_3$	ursolic acid	456.3595	25.49
16.		$C_{30}H_{48}O_3$	oleanolic acid	438.3490	25.43
17.		$C_{23}H_{28}N_4O_2S_2$	N-({(2R,4S,5R)-5-[1-Methyl-3-(2-thienyl)-1H-pyrazol--5-yl]-1-azabicyclo[2.2.2]oct-2-yl}methyl)-1-phenylmethanesulfonamide	456.1608	8.90

cont. Table 3

1	2	3	4	5	6
18.		$C_{25}H_{20}N_4O_2$	SSR161421	386.1727	19.07
19.		$C_{19}H_{30}O_8$	3-Hydroxy-3,5,5-trimethyl--4-(3-oxo-1-buten-1-ylidene) cyclohexyl ß-D-glucopyranoside	386.1938	4.47
20.		$C_{26}H_{50}O_4$	Bis(2-ethylhexyl) sebacate	426.3703	29.38
21.		$C_{18}H_{15}Cl-N_8O_2S$	N1-(4-Chlorophenyl)-2-({-4-methyl-5-[(3-pyrazin-2-yl--1,2,4-oxadiazol-5-yl) methyl]-4H-1,2,4-triazol--3-yl}thio)acetamide	884.1550	6.06
22.		$C_{30}H_{50}O_2$	betulin	442.3808	27.15

Discussion

Antioxidants are natural or synthetic central basics that intercept or mitigates damage to cells caused by free radicals or unstable molecules that the body manufactures in response to environment or pressures (KASOTE et al. 2015). These free radicals, which are also referred to as reactive oxygen species (ROS) perhaps, are the major cause of degenerative diseases such as cancer and neurodegenerative diseases (LIU et al. 2018). They can be managed using a synthetic or natural approach. The synthetic antioxidants such as vianol and embanox have been useful in managing degenerative free radical complication. However, due to their associated side effect and attendant problems such as accumulation in tissues, people forced to look for an alternative approach. This issue makes natural antioxidants more popular.

Natural antioxidants are metabolites that are mostly from plant origin (LOURENÇO et al. 2019), and are often phenolics and organic acids (CROZIER et al. 2007, EL-KASHAK et al. 2017). DPPH assay was based on the measurement of the scavenging capacity of antioxidants towards a stable free radical α,α-diphenyl-ß-picrylhydrazyl (DPPH). The odd electron of the nitrogen atom from antioxidants to the corresponding hydrazine (KEDARE and SIGH 2011). The DPPH and ABTS results presented as percent scavenging activity. Their scavenging activities increased with increased concentration (Table 1 and Table 2). SAWALE et al. (2017) observed the same trend of increased scavenging activity with an increase in concentration on the family. A better scavenging activity in the DPPH and ABTS antioxidant assays observed with the ethyl acetate leaf fraction. The DPPH radical scavenging activity ranged from; 43.4 mg Trolox Equivalence to 281.7 mg Trolox Equivalence for the leaf, stem-bark and the root extarcts and fractions. The ABTS antioxidant assay for the extracts and fractions of the leaf, stem-bark and the root of the plant gave radical scavenging effects ranging from 97.4 mg Trolox Equivalence to 275.80 mg Trolox Equivalence. The performance of the extracts and fractions of the plant using the two antioxidants assays shows strong positive correlation. This result is in agreement with that of MATUSZEWSKA et al. (2018). The LC–ESI-MS/MS of the EAL fraction of the *B. salicina* identified 22 compounds with their structures, belonging to different classes including flavonoids, glycosides, phenolic acids, triterpenoids, amides and sulphonamides as shown Table 3. Most of the compounds have been reported to have good antioxidant activities. The identified compounds are of medicinal importance, which are responsible for the antioxidant and anticancer properties of the plant.

Conclusion

The present study suggests that *Breonardia salicina* leaf possesses potent antioxidant activity, which could be due to the various phenolic compounds profiled. Thus, it shows that the leaf may be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing down the progress of oxidative stress related degenerative diseases.

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WATER MANAGEMENT OF MIKOŁAJKI CITY AND COMMUNE

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Abstract

Using the example of the city and commune of Mikołajki, the state of water and sewage management as well as the management of groundwater resources available in the commune has been characterized for a typical tourist town and commune located in Mrągowo County, Warmian-Masurian Voivodeship. The town and commune of Mikołajki, located in the Warmian-Masurian Voivodeship, has about 7 457 inhabitants. Over 83% of the commune's inhabitants are covered by the water supply network. The Mikołajki commune has four water intakes (one of which is out of use) and three water treatment plants. The water used by the inhabitants of the commune meets all sanitary requirements.

Introduction

Water plays a special role in the processes occurring in ecosystems, constituting an essential abiotic element of the environment for their functioning. It is a renewable raw material with resources that vary over time and fulfills many basic functions in the economy. These special functions make it necessary not only to protect it against pollution, but also to manage its resources rationally and economically. Qualitative and quantitative protection of water resources is an integral element of environmental protection. Many tourist destinations struggle with the problem of maintaining the appropriate condition of water and sewage management. The main reason is their sudden development and changes in the population in the commune, which varies depending on the season.

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The urban-rural commune of Mikołajki is located in the Warmian-Masurian Voivodeship, in the Mrągowo poviat. The area of the commune is 256. 41 km^2 and the headquaters is the city of Mikołajki, with the area of 8.85 km^2 (The Central Statistical Office. 2022). The entire commune occupies 24.07% of the area of the Mrągowo poviat. Mikołajki is located near Lakes Tałty and Mikołajskie, and the north-eastern part of the city borders with Łuknajno Lake.

Water and Sewage Company limited liability company (Zakład Wodociągów i Kanalizacji w Mikołajkach sp. z o.o. – later referred to as ZWiK Mikołajki) in Mikołajki conducts activities related to the collection, treatment and distribution of water for the needs of the residents and other recipients, collection of sanitary sewage to the sewage system and its treatment, renovation and investment works of water and sewage equipment, delivery of services to other business entities in the field of construction and operation of water intakes, sewage networks, water supply systems, sewage treatment plants, drilling works, preparation of documentation for investments and repairs in the field of water and sewage management, conduct of research in the field of water and sewage systems equipment efficiency, conduct of drilling works of deep wells and their renovation.

The paper characterizes the state of water management in the city and commune of Mikołajki and analyzes the operation of water intakes and water treatment plants to determine actions aimed at improving this condition.

Materials and Methods

This work was carried out based on the documentation from the Mikołajki Town and Commune Office, analysis of documents provided by the Water and Sewage Plant in Mikołajki, Polish legal acts, results of laboratory tests of water and sewage, data from the Central Statistical Office and the on-site inspections of the facilities.

Water supply

Water supply to the commune and the city of Mikołajki is provided by four water intakes and three water treatment plants belonging to ZWiK Mikołajki (Water and Sewage Company in Mikołajki). They are located in Mikołajki, Tałty and Cudnochy. ZWiK also owns the water intake in Prawdów, however it is out of use. Additional Hotel Gołębiewski water

intake is a separate intake providing water only for the hotel needs (The City Council in Mikołajki. 2020).

Water intakes

Figure 1 shows the localisation of intakes belonging to ZWiK in Mikołajki.

Fig. 1. Water intakes belonging to Water and Sewage Company sp. z. o.o. in Mikołajki
Source: Geoportal.gov.pl

Water intake – Mikołajki

The intake in 'Mikołajki' has three active wells: No. 1A, No. 2A and No. 4, which draw from the Quaternary aquifer. The intake is located approx. 250 m north-east of Lake Mikołajskie, in the southern part of the city. The Quaternary aquifer drawn by the wells of the 'Mikołajki' intake does not belong to the main groundwater reservoir. The towns included in

the service of this intake include: Mikołajki, Stawek, Kolonia Mikołajki, Woźnice, Lelek, Olszewo, Górkło, Grabówek and Grabówka, Grabnik, Pszczółki.

All wells have Lange-type casings and consist of a pressure pipeline, a check valve, a manometer, a shut-off damper, a depression pipe, a tap and a water meter. Water is extracted by means of three submersible pumps with a capacity of 75 m^3/h, which are suspended at a depth of 23 m in well No. 1A, 21 m in well No. 2A and 27 m in well No. 4 respectively. Basic data concerning the well are presented in the table below (Table 1).

Table 1

Basic data of active wells for 'Mikołajki' water intake [3]

Parameter	Well No. 1A	Well No. 2A	Well No. 4
State and function of well	active	active	active
Year of creation	1972	1972	2007
Terrain elevation [m] a.s.l. (above sea level)	128.3	131.0	130.0
Depth of the hole [m]	38.0	45.5	60.0
Operational efficiency – Qe [m^3/h]	15.45	63.0	135.0
Operationa depression – Se [m] (at Qe)	3.15	3.1	6.5
Unit capacity – q [m^3/h/1mS] (m^3/h/1m depression)	1.53	20.32	20.77
Filtrartion coefficient – *k* [m/d]	15.64	34.99	22.98
Static water level from the period of well construction – depth [m b.g.l.] (below ground level) – ordinate [m a.s.l.] (above sea level)	 10.6 117.7	 14.54 116.46	 13.4 116.6
Active part of the filter: – length of the active part of the filter [m] – diameter [mm] – foundation depth [m b.g.l.] (below ground level)	 5 200 38	 8.8 230 44.8	 20 250 59.5

The characteristics of water consumption at the 'Mikołajki' intake presented below was based on data on monthly intakes from 2015–2021. A detailed summary of the amount of monthly consumption is presented in Table 2.

Table 2

Total water intake [m^3] in wells of the 'Mikołajki' intake in years 2015–2020 (Water and Sewage Company in Mikołajki. 2022)

Month/year	2015	2016	2017	2018	2019	2020
I	23 198	25 222	28 301	27 941	35 876	37 971
II	17 187	21 748	24 595	25 421	35 779	30 939
III	19 636	24 968	26 425	29 438	42 109	31 252
IV	22 317	26 981	28 471	32 086	38 629	37 955
V	26 041	36 052	36 966	39 357	43 584	41 379
VI	30 523	33 194	42 090	39 760	49 208	50 899
VII	42 995	43 528	50 608	52 574	60 383	61 430
VIII	46 229	47 591	49 938	55 715	68 186	74 806
IX	30 081	37 027	32 541	38 133	44 307	49 179
X	25 544	33 374	28 955	34 235	38 966	40 591
XI	23 896	31 538	26 059	32 341	36 952	35 994
XII	24 303	28 478	28 900	34 240	40 351	37 631

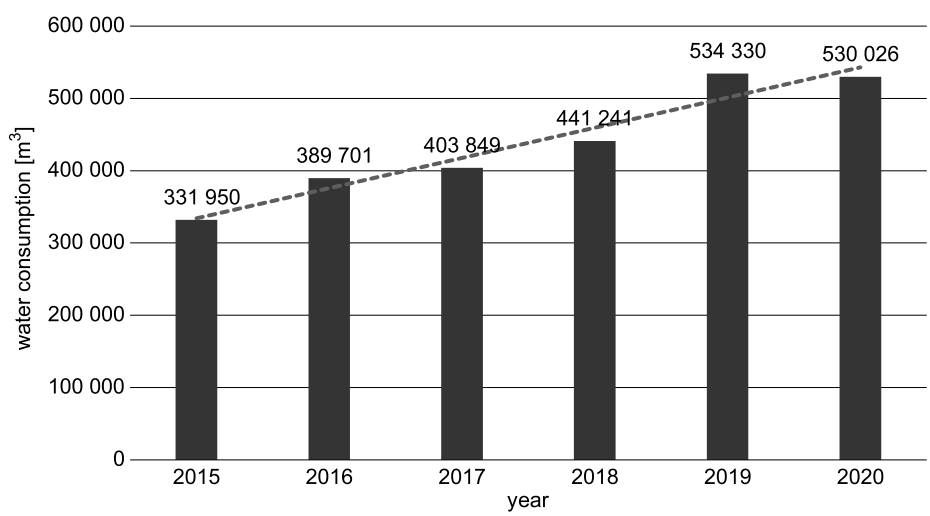

Fig. 2. The variability of the total annual water consumption (in m^3) at the 'Mikołajki' intake in 2015–2020 (Water and Sewage Company in Mikołajki. 2022)

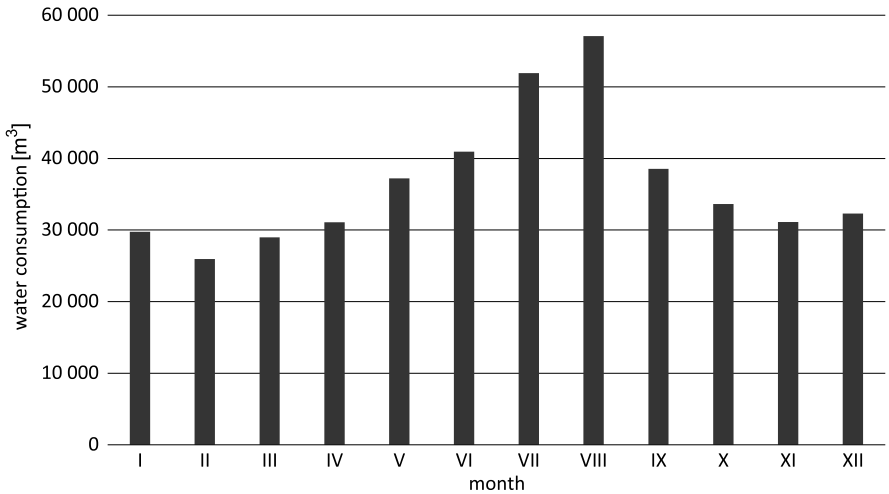

Fig. 3 Average monthly water consumption at the 'Mikołajki' intake in 2015–2020 (Water and Sewage Company in Mikołajki. 2022)

According to the data presented in Table 2 and the graphs above, the following were observed:

– current, total water consumption from the intake in 2020 with a value of 530 026 m^3 (i.e. on average 1 452.1 m^3/day), which is approx. 33.2% of the permissible value set in the water law permit;
– an increase in water consumption at the 'Mikołajki' intake in 2015–2019;
– the highest water consumption in the summer months – this is related to the tourist character of the area of Mikołajki;
– lower water consumption in 2020 compared to 2019 – the reason was a smaller number of tourists visiting the city of Mikołajki due to the pandemic situation caused by COVID-19 (Water and Sewage Company in Mikołajki. 2022).

Water intake – Tałty

The intake consists of two wells: No. 1 and No. 2, which draw from the Quaternary aquifer. Currently, only one well (No. 2) is in operation. The intake is in the southern part of the village of Tałty, about 350 m east of the shore of Lake Tałty and about 4 km north of the center of Mikołajki. The towns included in this intake are: Tałty, Kolonia Tałty, Kolonia Mikołajki. Wells No. 1 and No. 2 are enclosed with reinforced concrete rings with a diameter of 1 500 mm placed on a concrete bottom slab. Inside the housing there is a depression pipe, a power cable supplying the pump, a latch, a water meter, a discharge pipe, a check valve and a tap for water intake. Well No. 1 is intended for closing, as it has not been used for many years. Basic data on wells are presented in the table below (Table 3).

Table 3

Basic data of wells for 'Tałty' intake
(Water and Sewage Company in Mikołajki. 2022)

Parameter	Well No. 1	Well No. 2
State and function of well	inactive	active
Year of creation	1962	1973
Terrain elevation [m] a.s.l. (above sea level)	128.3	126.0
Depth of the hole [m]	38.0	42.0
Operational efficiency – Qe [m^3/h]	15.5	60.0
Operationa depression – Se [m] (at Qe)	3.1	12.0
Unit capacity – q [m^3/h/1 mS] (m^3/h/1 m depression)	4.9	5.0
Filtrartion coefficient – *k* [m/d]	15.6	6.0
Static water level from the period of well construction – depth – [m b.g.l.] (below ground level) – ordinate – [m a.s.l.] (above sea level)	 10.6 117.7	 10.2 115.8
Active part of the filter: – length of the active part of the filter [m] – diameter [mm] – foundation depth [m b.g.l.] (below ground level)	 5 8 38	 7.5 $11\frac{3}{4}$ 42

Table 4

Total water intake (in m^3) in wells of the 'Tałty' intake in years 2015–2020
(Water and Sewage Company in Mikołajki. 2022)

Month/year	2015	2016	2017	2018	2019	2020
I	840	834	1 333	992	1 177	1 088
II	821	974	1 169	997	1 062	900
III	955	759	1 800	1 101	1 231	1 121
IV	1 030	1 008	1 834	1 252	1 408	1 419
V	1 120	1 512	1 968	1 977	1 549	1 418
VI	1 250	1 693	2 563	2 202	2 099	1 943
VII	2 060	2 794	3 035	2 599	2 860	3 291
VIII	2 250	2 426	2 925	2 676	2 826	2 995
IX	1 425	1 331	1 395	1 398	1 565	1 631
X	1 520	950	962	1 257	1 212	1 124
XI	1 213	789	1 090	1 107	925	983
XII	1 450	1 015	1 058	1 177	1 079	995

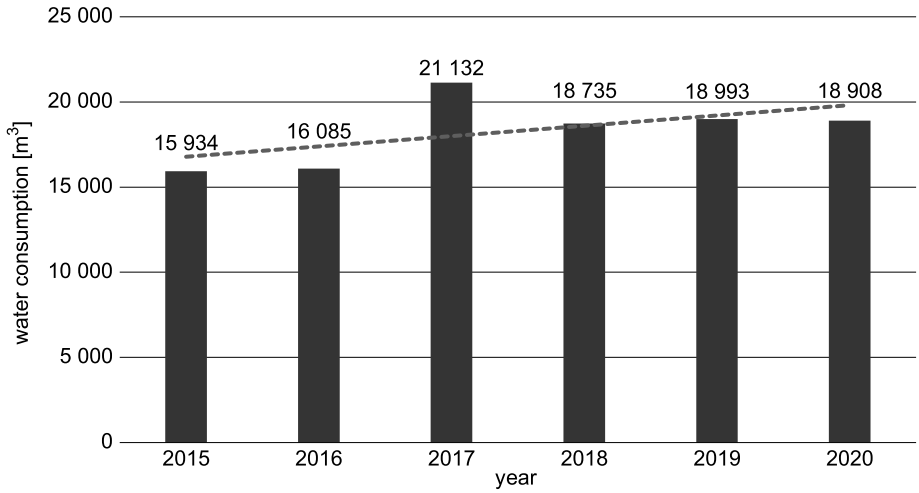

Fig. 4. The variability of the total annual water consumption (in m^3) at the 'Tałty' intake in years 2015–2020 (Water and Sewage Company in Mikołajki. 2022)

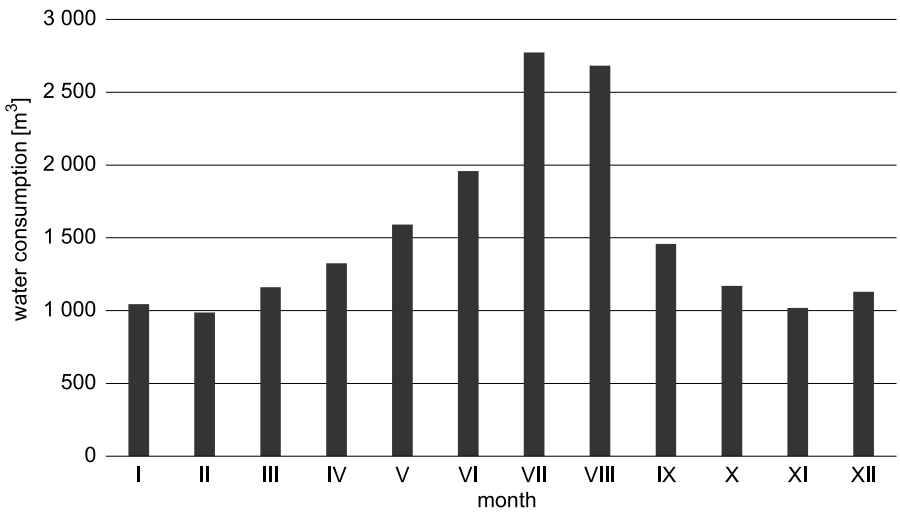

Fig. 5. Average monthly water consumption at the 'Tałty' intake in years 2017–2020 (Water and Sewage Company in Mikołajki. 2022)

According to the data presented in Table 4 and the charts, the following were observed:

– current, total water consumption from the intake in 2020 with a value of 18 908 m^3 (i.e. an average of 51.8 m^3/day), which is approx. 26.4% of the permissible value set in the water law permit;
– annual water consumption ranging from 2015 – 15 934 m^3 (i.e. 43.7 m^3/day) to 2017 – 21 132 m^3 (i.e. 57.9 m^3/day);

– in the last three years, the amount of water intake at a constant level;
– the highest water intake in the summer months from June to August – this may be due to the agricultural character of the intake area (Water and Sewage Company in Mikołajki. 2022).

Water intake – Cudnochy

The intake consists of two wells: one active, drawing from the Quaternary aquifer (well No. 1) and one not exploited (well No. 2). The intake is located in the central part of the village of Cudnochy, at the intersection of the road Faszcze – Jora Wielka with a local country road. The towns included in the service of this intake are: Cudnochy, Baranowo, Faszcze, Jora, Inulec, Śmietki, Nowe Sady, Stare Sady, Zełwągi, Lubiewo. The wells are enclosed with reinforced concrete rings with an internal diameter of 1 200 mm and two reinforced concrete slabs – bottom and ceiling. A PVC drainage pipe ø 160 mm is built into the bottom of the casing.

Water intake process is carried out by a GC.2.05 submersible pump with a capacity of Q = 15–35 m^3/h, lift of H = 11.8–6.5 bar, engine power of 11 kW, installed at a depth of 12 m below the head. Basic data on wells are presented in the table below (Table 5).

Table 5

Basic data of wells for 'Cudnochy' intake (Water and Sewage Company in Mikołajki. 2022)

Parameter	Well No. 1	Well No. 2
State and function of well	active	inactive
Year of creation	1995	2010
Terrain elevation [m] a.s.l. (above sea level)	123.15	122.85
Depth of the hole [m]	40.0	38.0
Operational efficiency – Qe [m^3/h]	40.0	68.0
Operationa depression Se [m] (at Qe)	2.4	–
Unit capacity – q [m^3/h/1 mS] (m^3/h/1 m depression)	16.67	15.1
Filtrartion coefficient – k [m/d]	23.15	17.07
Static water level from the period of well construction – depth [m b.g.l.] (below ground level) – ordinate [m a.s.l.] (above sea level)	 +1.73 124.88	 +2.1 124.95
Active part of the filter: – length of the active part of the filter [m] – diameter [mm] – foundation depth [m b.g.l.] (below ground level)	 15.5 273.05 40.0	 19.0 250/280 37.0

Table 6

Total water intake (in m^3) in wells of the 'Cudnochy' intake in years 2015–2020 (Water and Sewage Company in Mikołajki. 2022)

Month/year	2015	2016	2017	2018	2019	2020
I	4 723	5 832	9 272	6 142	5 862	5 528
II	4 017	5 397	11 407	4 228	5 604	5 779
III	4 567	6 394	9 685	5256	5 453	5 232
IV	5 307	8 020	9 268	6 147	7 685	5 900
V	5 479	7 764	12 090	8 653	7 456	6 021
VI	7 237	8 508	13 969	10 187	8 931	8 201
VII	11 010	11 272	13 457	11 250	11 535	11 603
VIII	12 844	10 140	13 246	10 970	12 757	13 862
IX	7 436	6 827	6 729	6 979	7 362	8 656
X	5 031	5 268	5 496	6 150	5 400	5 916
XI	4 447	5 147	4 765	4 717	4 681	4 892
XII	5 080	7 495	4 609	5 587	5 085	5 820

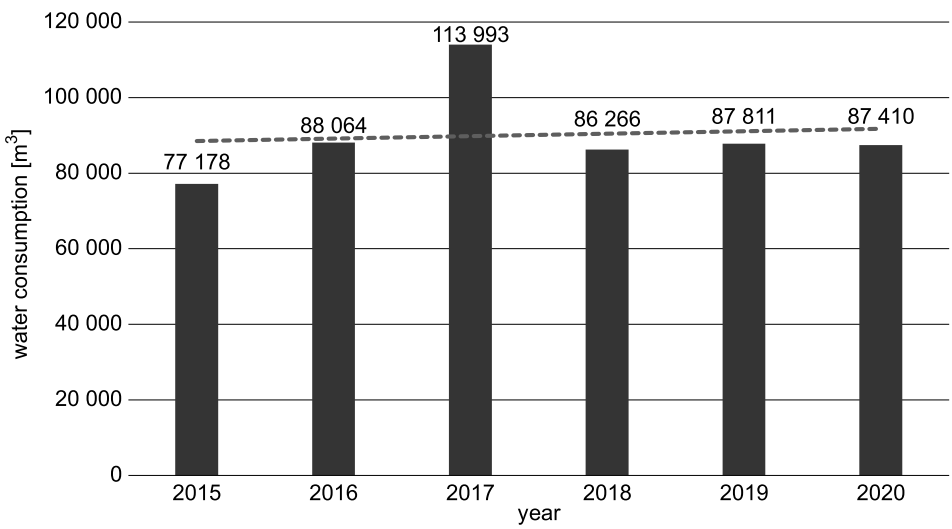

Fig. 6. The variability of the total annual water consumption (in m^3) at the 'Cudnochy' intake in years 2015–2020 (Water and Sewage Company in Mikołajki. 2022)

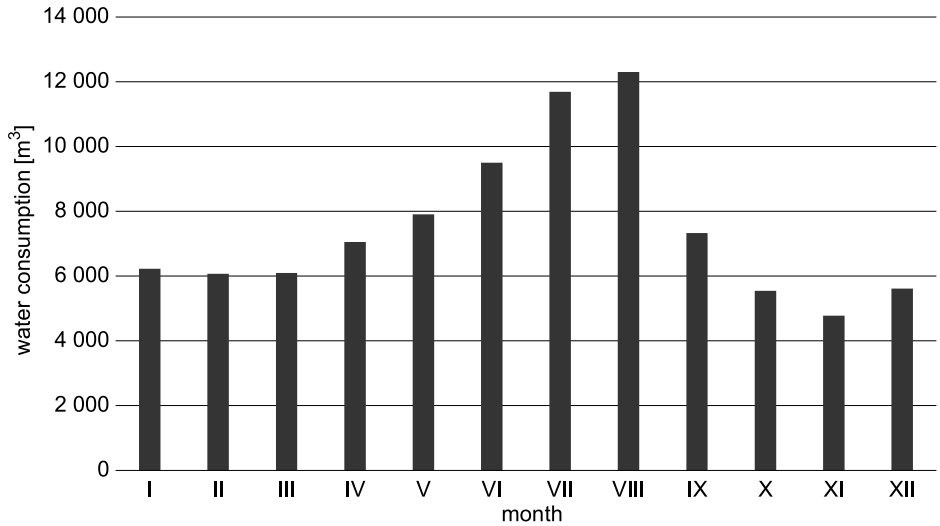

Fig. 7. Average monthly water consumption at the 'Cudnochy' intake in years 2017–2020 (Water and Sewage Company in Mikołajki. 2022)

According to the data presented in Table 6 and in the charts, the following were observed:

– current, total water consumption from the intake in 2020 with the value of 87 410 m^3 (i.e. on average 239.5 m^3/day), which is approx. 41.6% of the permissible value set in the water law permit;
– annual water consumption ranging from 2015 – 77 178 m^3 (i.e., 211.4 m^3/day) to 2017 – 113 993 m^3 (i.e., 312 3 m^3/day);
– the highest water consumption in the summer months and the lowest in the winter. In August, the consumption exceeds 13 000 m^3 (i.e., 420 m^3/day) – this is the value allowed in the water law permit (480 m^3/d); in November, water intake drops to as little as 5 000 m^3 – in this month the lowest value of water consumption is read (Water and Sewage Company in Mikołajki 2022).

Water treatment

In 2019, a total of 508 412 m^3 of treated water was fed into the network. In 2020, the consumption of treated water was lower by 7 582 m^3 and amounted to 500 830 m^3. For 2021, a value of 506 596 m^3 was recorded. Water treated in those years came from three intakes. Tables 7, 8 and 9 show the monthly water production for three water treatment plants (WTP).

Table 7

Water production in 2019 for each WTP in the Mikołajki commune
(Water and Sewage Company in Mikołajki. 2022)

Water production – 2019										
Specification	WTP Mikołajki				WTP Cudnochy			WTP Tałty		
	raw water [m^3]	treated water [m^3]	washings [m^3]	ZH Woźnice [m^3]	raw water [m^3]	treated water [m^3]	washings [m^3]	raw water [m^3]	treated water [m^3]	washings [m^3]
January	35 876	24 518	3 140	4 702	5 862	5 444	282	1 177	899	279
February	35 779	21 380	2 910	–	5 604	5 402	285	1 062	881	181
March	42 109	26 799	3 180	–	5453	4960	249	1 231	944	287
April	38 629	31 670	3 020	–	7 685	7 625	261	1 408	1213	195
May	43 584	33 881	3 171	6 137	7 456	7 207	249	1 549	1 305	235
Juni	49 208	42 220	281	7 350	8 931	8 650	281	2 099	1 754	345
Juli	60 383	53 655	3 130	7 624	11 353	11 072	281	2 860	2 582	287
August	68 186	56 719	3190	7 860	12 757	12 466	291	2 826	2 575	251
September	44 307	34 335	2 980	6 206	7 362	7 093	269	1 565	1 317	248
October	38 966	29 196	3 130	6 270	5 400	5 122	294	1 212	733	419
November	36 952	26 170	3 070	4 075	4 681	4 412	269	925	612	313
December	40 351	28 100	3 100	7 427	5 085	4 790	295	1 079	711	368
Total	534 330	408 643	34 302	57 651	87 629	84 243	3 306	18 993	15 526	3 408

Table 8

Water production in 2020 for each WTP in the Mikołajki commune
(Water and Sewage Company in Mikołajki. 2022)

Water production – 2020										
Specification	WTP Mikołajki				WTP Cudnochy			WTP Tałty		
	raw water [m^3]	treated water [m^3]	washings [m^3]	ZH Woźnice [m^3]	raw water [m^3]	treated water [m^3]	washings [m^3]	raw water [m^3]	treated water [m^3]	washings [m^3]
January	37 971	25 974	3 100	5 663	5 528	5 234	294	1 088	825	263
February	30 939	24 808	3 000	5 110	5 779	5 764	261	900	673	227
March	31 252	25713	3080	5 788	5232	4917	315	1 121	878	243
April	37 955	28 987	3 020	6 549	5 900	5 831	318	1 419	1120	299
May	41 379	31 244	3 060	5 699	6 021	5 895	315	1 418	1 085	333
Juni	50 899	37897	2 960	7 866	8 201	7 900	301	1 943	1 714	229
Juli	61 430	52 820	3 090	8 273	11 603	11 306	297	3 291	3 236	55
August	74 806	57 326	3040	8 581	13 862	13 589	273	2 995	2 964	31
September	49 179	36 790	3 010	6 645	8 656	8 037	280	1 631	1 561	70
October	40 591	28 465	3 070	5 983	5 916	5 646	270	1 124	998	126
November	35 994	24 509	3 070	6 053	4 892	4 740	272	983	947	36
December	37 631	25 171	2 960	6 000	5 820	5 307	284	995	959	36
Total	530 026	399 704	36 460	78 210	87 410	84 166	3 480	18 908	16 960	1 948

Table 9

Water production in 2021 for each WTP in the Mikołajki commune (Water and Sewage Company in Mikołajki. 2022)

Specification	Water production – 2021									
	WTP Mikołajki				WTP Cudnochy			WTP Tałty		
	raw water [m^3]	treated water [m^3]	washings [m^3]	ZH Woźnice [m^3]	raw water [m^3]	treated water [m^3]	washings [m^3]	raw water [m^3]	treated water [m^3]	washings [m^3]
January	34 076	24 304	3 110	5 815	5 486	5 212	274	988	984	4
February	34 148	24 300	2 750	5 815	5 068	4 825	243	904	890	14
March	37 350	26 696	2 970	0	6 103	5 569	325	1 122	1 043	79
April	26 989	25 838	1 970	6 048	5 497	5 324	277	5 497	5 324	277
May	33 356	33 356	2 030	6 689	7 038	6 727	311	1 290	1 235	55
Juni	47 958	47048	1 900	0	8 584	8 328	256	2 097	2 072	25
Juli	57 819	56 499	1 910	0	10 284	10 266	267	2 498	2 484	14
August	54 961	54 733	2020	6 738	8 554	8 242	278	2 325	2 247	78
September	39 707	36 930	1 880	7 574	5 314	4 990	256	1 125	1 049	76
October	33 798	31 507	1 970	0	3 831	3 233	272	853	653	200
November	32 432	28 655	1 850	7 027	2 955	2 591	271	749	600	149
December	32 537	29 472	1 950	59 993	3 792	2 785	277	792	585	207
Total	465 131	419 338	26 310	105 699	72 506	68 092	3 307	20 240	19 166	1 178

Water treatment plant – Mikołajki

The „Mikołajki" intake has a Water Treatment Plant (WTP) localized at Wawrzyńca Prusa Street. Water taken from the well is pumped through an aerator to 5 filters arranged in parallel and responsible for manganese and iron removal. The treated water goes to a reinforced concrete tank with a capacity of 175 m^3. It is placed on top of the water tower. Then, the water is gravitationally fed into the municipal water supply system.

The filters are rinsed every day at night, each rinsing lasts 1.5 hours. The amount of water used to rinse the filters is 100 m^3. Washing waters are discharged to the rainwater sewage settling tanks, and then through the connection to the sewage system. From the system, they go to the Municipal Wastewater Treatment Plant in Mikołajki (Water and Sewage Company in Mikołajki. 2022).

The water intake in Mikołajki has a permit for the abstraction of underground water in the amount of Q_{dav} = 2400 m^3/day and maximum hourly Qh_{max} = 182 m^3/h and supplies the town of Mikołajki with water (The City Council in Mikołajki. 2020).

The expansion and modernization of the water treatment system in Mikołajki was aimed at ensuring good quality drinking water for residents and increasing the efficiency of the intake to Qh_{max} = 200 m^3/h.

Based on the analysis of raw water, on-site inspection and information on the treatment processes used so far, a technology was designed to improve the quality of water, consisting of two-stage filtration through a multi-layer bed and water disinfection with the use of sodium hypochlorite and UV rays.

The works included the construction of water tanks with inter-facility networks, construction of a new technological building; selection, production and assembly of new technological devices, e.g., pressure aerators, filters, hydrophore set, rinsing pump, blower or compressors, and new stainless-steel piping for the plant. The operation of all devices is managed by a new PLC controller, which implements a prepared individual control algorithm, thanks to which the water treatment plant is fully automatic. All implementation works were completed in 2011. Since then, the quality of water supplied by the water treatment plant to the residents meets all the standards required by law, which confirms the effectiveness of the treatment technologies offered by Instalcompact.

Water treatment plant – Tałty

The "Tałty" intake has a Water Treatment Plant (WTP) located on the same plot as the intake wells. Water taken from the well is pumped to two raw water tanks with a capacity of 4.0 m^3 each. These tanks are equipped with compressors to aerate the water, which oxidizes the iron and manganese compounds before filtration. Then the water is directed to the iron and manganese removal filter with a diameter of 1400 mm. It is responsible for the removal of iron and manganese compounds and for the reduction of the turbidity of water. The next step in water treatment is its disinfection with sodium hypochlorite.

The treated water goes to the clean water tank, and then to the water supply system (Water and Sewage Company in Mikołajki. 2022).

The water intake in Tałty has a decision to withdraw underground water in the amount of Q_{dav} = 140 m^3/day and the maximum hourly Qh_{max} = 20 m^3/h and supplies the town of Tałty with water (The City Council in Mikołajki. 2020).

Water treatment plant – Cudnochy

The "Cudnochy" intake has a Water Treatment Plant (WTP) located on the same plot as the intake wells. Water drawn from the well is pumped

to aerators. To remove the excess of manganese and iron compounds from it, it is passed through the iron and manganese remover. The treated water is directed to two clean water tanks, and then to the hydrophore with a capacity of 6.3 m^3. From there it is fed into the water supply system.

The filters are rinsed twice a week. Washing waters are discharged to the pond through PVC pipes with a diameter of 160 mm through 6 settling tanks, where sludge from washings is collected (Water and Sewage Company in Mikołajki. 2022).

The water intake in Cudnochy has a decision for the intake of underground water in the amount of Q_{dav} = 480 m^3/day and maximum hourly Qh_{max} = 24 m^3/h and supplies the towns of Cudnochy, Śmietki, Stare Sady, Baranowo, Jora Wielka and Inulec with water (THE CITY COUNCIL IN MIKOŁAJKI. 2020).

Table 10 presents compare of the test results of treated water from the Mikołajki, Tałty and Cudnochy water treatment plants.

Table 10

Compare of the test results of treated water from the Mikołajki, Tałty and Cudnochy water treatment plants

Parameter	Unit	Maximum allowable parameter values	Test result		
			Mikołajki	Tałty	Cudnochy
Colour	mg Pt/l	15 mg Pt/l; acceptable by consumers and without incorrect changes	<5	<5	<5
Turbidity	NTU	1.0; acceptable by consumers and without incorrect changes	0.21	0.22	0.22
Smell	–	acceptable by consumers and without incorrect changes	<1	<1	<1
pH	–	6.5–9.5	7	7.2	7.1
General hardness	mg $CaCO_3$/l	60–500	389	379	332
Specific electrical conductivity (SEC) in temp. 25°C	µS/cm	2500	600	600	610
Manganese (Mn)	µg/l	50	15	18	13.8
Iron (Fe)	µg/l	200	82	102	< 60
Ammonium Ion	mg/l	0.5	0.05	0.05	0.1
Nitrates $(NO_3)^-$	mg/l	50	0.75	0.95	1.2
Nitrites $(NO_2)^-$	mg/l	0.5	<0.03	<0.03	<0.03
Fluorides	mg/l	1.5	0.22	0.22	0.23
Oxidisability with $KMnO_4$ (Permanganate index)	mg/l	5	0.56	0.66	0.73

The results of laboratory tests presented above meet the sanitary requirements for water intended for human consumption and do not exceed the permissible values of indicators (Regulation of the Minister of Health. 2017). Therefore, the State Poviat Sanitary Inspector in Mrągowo approved the suitability of water in Mikołajki for consumption.

Water Supply Network

The water supply network of the Mikołajki commune belongs to the Water and Sewage Department in Mikołajki. The length of the water supply network is 135.3 km of the distribution network, 30 km of which are in urban areas and 105.3 km in rural areas. The number of water supply connections at the end of 2020 was 1753. As part of maintenance and repair works, 11 failures were removed, which in relation to 16 failures in 2019 means progress resulting from the replacement of the most worn sections (Water and Sewage Company in Mikołajki. 2022).

Conclusions

The paper presents the state of water management in the city and commune of Mikołajki, located in the Warmian-Masurian Voivodship, in the Mrągowo poviat. The commune belongs to the Land of the Great Masurian Lakes, has significant landscape, natural and tourist values.

In 2022, the commune was inhabited by 7457 people. There are four water intakes in the commune, including three belonging to ZWiK in Mikołajki. They are in Mikołajki, Tałty and Cudnochy. There is also a water treatment plant in each of aforementioned towns. The results of laboratory tests of the treated water in each of the plants meet the requirements for water intended for human consumption and do not exceed the permissible values of indicators. The water is fit for consumption by the inhabitants of the commune and tourists.

From the information provided in the work, the largest water intake and the largest amount of sewage were recorded in the summer seasons. The COVID-19 epidemic, including the closure of the economy and a smaller number of tourists, resulted in lower water consumption and a decrease in the amount of sewage supplied to the treatment plant in 2020.

The closing of hotels and boarding houses, which are the main "producer" of sewage in Mikołajki, also played a decisive role.

The increasing number of hotels, motels and bed places causes a large irregularity of hours, to which the existing system was not adapted.

Unfortunately, the main problem in the commune and the whole country are septic tanks, which largely pollute the environment. If society does not accept the fact, that the tanks pose an enormous threat to the environment as soon as possible the entire natural environment will suffer.

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EFFECT OF ACUTE AND SUBLETHAL CONCENTRATION OF *THEVETIA NERIIFOLIA* LEAVES ON THE GROWTH, SURVIVAL AND BLOOD PROFILES OF *CLARIAS GARIEPINUS* JUVENILES

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Key words: *Clarias gariepinus, Thevetia neriifolia*, haematology, histology, growth rate.

Abstract

The presence of predatory and unwanted fishes in cultured ponds is a serious problem for culturing edible freshwater fishes. The effects of acute and sublethal concentrations of *Thevetia neriifolia* leaves extract (TnLE) on the growth, survival and some haematological indices of *Clarias gariepinus* juveniles were investigated. The acute lethal toxicity (LC_{50}) of *T. neriifolia* leaves extract for 72-hour exposure of *C. gariepinus* was determined at 0.6 ml/L. *Clarias gariepinus* with mean weight of 15.65 ±0.02 g were exposed to TnLE at 0.00, 0.04, 0.08, 0.15, 0.30 and 0.60 ml/L concentrations for 4 weeks. The control and exposed groups were stocked with 20 fish per replicate and replicated three times. Data were analyzed using descriptive statistics and ANOVA at $p = 0.05$. This study revealed an increase in the weight of the fish exposed to TnLE, but the values decreased as the concentration of *T. neriifolia* increased. Significant ($p < 0.05$) increases were observed in the packed cell volume, haemoglobin, neutrophils and albumin of *C. gariepinus* juveniles exposed to *T. neriifolia* leaves extract compared to the control. The histology of the intestine revealed that no visible structural changes among the exposed groups while the skin, gills, and liver shows slightly abnormal structural changes in the exposed groups. This study can be concluded that the *T. neriifolia* leaves extract could serve as a biopiscicide in fish farming without compromising growth.

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Introduction

Aquaculture is a fast-growing sector in Nigeria, which contributes less than 5% of the total fish supply but has a growth rate of about 2% per year (MOSES 2006). Catfish farming and indeed aquaculture, offer strong potential for growth to meet the natural fish demand, thereby, reducing importation, providing employment, alleviating poverty and helping to meet the millennium development goals (WILLIAMS et al. 2007). Among the culturable fishes in Nigeria includes the African catfish (*Clarias gariepinus*), which is the most popularly cultured fish in Nigeria and indeed in Africa, and third in the world (GARIBALDI 1996) because of its hardiness, omnivores, ability to withstand adverse condition, and high economic value (HECHT et al. 1996).

Botanical piscicides are used by fishermen and fish farmers for catching or stupefying fish. They are applied during fishing or eradication of unwanted, predatory, and exotic fish species (CAGAUAN et al. 2004). The control of aquatic ecosystems with synthetic organic compounds reportedly caused health hazard (DALELA et al. 1978). Botanical ichthyotoxins are less expensive, biodegradable, readily available, easy to handle and safe for human and the environment (SINGH et al. 1996). Plant poisons are extracted from flowers, pulp, seeds, bark, fruits, root, leaves and even the entire plant (TYLER 1986). Some chemicals found in these plants will stun fish when passing through their gills or when ingested. The fish then float to the surface of the water for easy capture. The active ingredients in these plants are released by mashing and grinding the appropriate plants or plant parts, which are then introduced to the aquatic environment. Exposure of fish to these biocides may cause stress in fish without necessarily leading to death. Phytochemical analyses of piscicidal plants showed that they contain diverse toxic substances such as rotenones, saponins, flavonoids, alkaloids, glycosides, tannins, oxalic acids, solanine, selenium, nicotine, pyrethrum, and resin (AL ASHAAL et al. 2010). These substances are toxic to fish and other aquatic organisms at high concentrations and fade out within a short time (ADEWUNMI 1990).

Depending on the species of fish targeted, different plant species used as piscicides have different effects on fish (VAN ANDEL 2002). Piscicidal plants like *Blighia sapida, Kigelia africana, Tetrapleura tetraptera, Raphia vinifera, Parkia biglobosa* and *Tephtosia vogelli* are commonly used by fisher folks to catch fishes because they are highly potent against fishes (FAFIOYE et al. 2004). The active principles in the plant part used (leaves, seeds, kernel and bark) have varying potencies and modes of action depending on forms of extracts, aqueous or alcohol used (SAMBA-

SIVAM et al. 2003). Lethal and sublethal concentrations of plant poisons are known to have effects on fish behaviour, growth, reproduction, feeding, respiration and generally other physiological processes of exposed organisms, which are visible also in haematological and histological studies (OLUSOLA et al. 2021). Several factors influence the response of organisms to toxicity tests which include age, disease, spawning time, condition, and water quality (OMONIYI et al. 2013).

Thevetia neriifolia (Juss) is an evergreen tropical, perennial shrub which belongs to the family Apocynaceae and is commonly known as "yellow oleander", but also called "milk bush", "trumpet flower" or "lucky nut". Utilization of this plant as a natural piscicide has not been well elucidated. In the present study, the piscicidal activity of aqueous extracts of the leaves of *T. neriifolia* plant was evaluated on *Clarias gariepinus* juveniles under controlled conditions.

Materials and Methods

Plant collection and identification

Mature leaves of *T. neriifolia* were obtained from the school premises of Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The plant was identified at the Department of Biological Sciences (Botany Programme) of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria.

Preparation of aqueous leaves extract

Two hundred and fifty grams (250 g) of fresh *T. neriifolia* leaves were washed with distilled water, macerated and squeezed in 30 litres of distilled water to obtain the aqueous leaves extract, and concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 ml/L of *T. neriifolia* ($TnLE_{a–e,}$ respectively) were used for 72-hour range test.

Experimental fish

Four hundred and eighty *Clarias gariepinus* juveniles of average weight 15.64 ±0.02 g were obtained from a fish farm in Okitipupa, Nigeria. They were transported to the Fisheries and Aquaculture Technology Laboratory, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria in an adapted plastic jerry can containing 35 litres water from the farm. The fish were acclimatized for 7 days and fed twice a day at 3% body weight with 2 mm feed (Blue Crown) containing 40% crude protein.

Range test (72-hour LC_{50}) and sublethal toxicity test

After acclimation, the extract of the *T. neriifolia* leaves ($TnLE_{a–e}$ – 0.2, 0.4, 0.6, 0.8 and 1.0 ml/L, respectively) was introduced into 6 experimental bowls (40 cm × 20 cm × 20 cm) containing clean, dechlorinated and well aerated water. Fish were randomly selected and stocked at 8 juveniles per experimental bowl. Twenty-four hours prior to the test commencement, feeding of the fish was stopped. Each of the test solutions was introduced directly into the experimental tank in a single dose and replicate twice per treatment. The behaviour and mortality of the test fishes in each tank was monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours and every four hours for the rest of the 72 hours' period. The 72 hours LC_{50} value was recorded and tested by probit analysis as described by FINNEY (1971).

The value 0.6 ml/L of LC 50 (concentration where mortality reached 50%) was obtained after 72 hours range test. For sublethal toxicity test, twenty fish per treatment were exposed to sublethal concentrations for a period of 28 days. Five groups of fish were exposed to different concentrations of *Thevetia neriifolia* leaves extract including 0.04, 0.08, 0.15, 0.30 and 0.60 ml/L ($TnLE_{1–6,}$ respectively), while one group, which served as the control group for *Clarias gariepinus* did not receive the extract. Each treatment group was replicated three times and water in each tank was replaced every three days throughout the period of the experiment using static water renewal methods to maintain relatively uniform physiochemical parameters and also to prevent fouling that may result from food residues. Fish were fed twice daily at 3% body weight (08:00 and 18:00 hrs) and they were weighed at the beginning of the study and weekly. Measurement of the weight change was performed weekly using sensitive weigh balance (SF – 400, 1000 g x 0.2 g, China) and the food amount adjusted weekly according to the new body weight

Histopathological examination

The skin, gills, intestine and liver of fish from the control and exposed groups were studied and compared for histopathological changes. Fishes (n = 2) were obtained from each treatment, dissected and immediately fixed in 10% formalin solution for 24 hours (KHOSHNOOD et al. 2014). At the end of 24 hours, tissue samples were washed in running water to remove traces of formalin. Specimens were dehydrated by passing through graded series of alcohol (30%, 50%, 70%, 95% and absolute ethanol) for two hours each. Specimens were later passed through xylene (clearing agent) to remove the alcohol, and molten paraffin wax was used to impregnate

the organs and tissues in a vacuum oven at 56°C and allowed to solidify following the procedures of LUNA (1968). Organs and tissues blocks were cut by trimming and attaching them to wood blocks in preparation for sectioning. Sectioning and staining of organs and tissues were done according to OMITOYIN et al. (1999). A light photomicroscope attached to a 35 mm camera was used to examine the organs sections.

Haematological and bio-chemical assessment

Prior to the collection of blood samples, the fish were anaesthetized with 2 – phenoxy ethanol and the blood samples (2 ml) were collected by the caudal ablation method from both the control and exposed fishes before the experiment, at the end of the experiment (28 days) and 14 days after post exposure (to assess recovery of fish). The blood samples were dispensed into tubes containing ethylene diamine tetraacetate (EDTA) anticoagulant and transported in ice-packed bags to the Microbiological Laboratory unit of Ondo State Specialist Hospital, Okitipupa for haematological analysis. Red blood cells (RBC) and white blood cells (WBC) were counted under a light microscope by improved Neubauer hemocytometer using Hayem's and Turk's solution as diluting fluids, respectively. Haemoglobin (Hb) was estimated by Cyanomethemoglobin method as described by BLAXHALL and DAISLEY (1993). Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated respectively using standard formula described by BLAXHALL and DAISLEY (1993).

Serum biochemistry

Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum biochemical parameters. Serum from the centrifuge blood was carefully siphoned out and the concentration of glucose, total proteins, albumin, globulin and Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined by the method described by OLUSOLA and NWOKIKE (2018). Glucose was measured in the laboratory using an electronic blood-glucose meter. A relatively small drop of blood from each sample was placed on a disposable test strip which interfaces with a digital meter. Within several seconds, the level of blood glucose was shown on the digital display.

Biological evaluation

Mean weight gain [g] = final body weight – initial body weight.

$$\text{Relative weight gain [\%]} = \frac{(\text{final body weight} - \text{initial body weight})}{\text{initial body weight}} \cdot 100 .$$

$$\text{Specific growth rate (SGR) [\%]} = \frac{(\log_e \text{final body weight} - \log_e \text{initial body weight})}{\text{experimental days}} \cdot 100 .$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{dry weight of the feed}}{\text{fish weight gained}}.$$

$$\text{Production performance index} = \frac{\text{final number of fish stocked} \cdot \text{weight gained}}{\text{experimental days}}.$$

$$\text{Survival rate (SR) [\%]} = \frac{\text{initial number of fish stocked} - \text{final number of dead fish}}{\text{initial number of fish stocked}} \cdot 100.$$

$$\text{Nitrogen metabolism [g]} = \frac{(0.549)\,(a + b)\,h}{2}.$$

where:
0.549 = constant value
a – initial mean weight of fish
b – final mean weight of fish
h – experimental periods in days (Bello et al. 2012).

Statistical Analysis

Growth performance and nutrient utilization, haematology and biochemical analysis resulting from the experiment was subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 20.0). Duncan multiple range test was used to compare differences among individual means.

Results

Behavioural responses of C. gariepinus juveniles exposed to different concentrations of *T. neriifolia* leaves extract

The results obtained in this study show that the behavioural changes of *C. gariepinus* juveniles exposed to the aqueous leaves extract of *T. neriifolia* increased with the concentration and the time of exposure. The fishes exhibited erratic swimming, air gulping, loss of reflexes, restlessness, and vertical positioning (Table 1).

Table 1

Behavioural changes and biological responses in *Clarias gariepinus* exposed to different concentrations of *T. neriifolia*; n = 8 specimens per treatment

Exp/Beh	24 hours						48 hours						72 hours					
CONC [ml/L]	0.00	0.20	0.40	0.60	0.80	1.00	0.00	0.20	0.40	0.60	0.80	1.00	0.00	0.20	0.40	0.60	0.80	1.00
A.G	−	−	−	−	+	+	−	−	−	+	+	N/A	−	−	+	+	N/A	N/A
E.S	−	−	+	+	+	+	−	−	+	+	+	N/A	−	+	+	+	N/A	N/A
L.R	−	−	−	−	+	+	−	−	−	−	+	N/A	−	−	−	+	N/A	N/A
LTG	−	−	−	−	+	+	−	−	−	+	+	N/A	−	−	−	+	N/A	N/A
M.S	−	−	+	+	+	+	−	+	+	+	+	N/A	−	+	+	+	N/A	N/A
V.P	−	−	−	−	+	+	−	−	−	+	+	N/A	−	−	+	+	N/A	N/A
L.B	−	−	−	−	+	+	−	−	−	−	+	N/A	−	−	−	+	N/A	N/A

Explanations: − = no change in behaviour found; + = change in behaviour found; A.G = air gulping; E.S = erratic swimming; L.R = loss of reflex; LTG = lethargy; M.S = mucus secretion; V.P = vertical positioning; L.B = loss of balance; N/A = not applicable (dead)

Growth performance and nutrient utilization of *Clarias gariepinus* exposed to *T. neriifolia* leaves extract

The growth performance and nutrient utilization of *C. gariepinus* exposed to aqueous leaves extract of *T. neriifolia* for 28 days revealed the highest body weight gain in $TnLE_2$ (0.04 ml/L) and lowest in $TnLE_6$ (0.60 ml/L) as 22.06 ±0.08 g and 15.93 ±1.58 g respectively. Generally, $TnLE_2$ showed better performance in all the parameters except survival rate, which was optimum in control (0.00 ml/L), $TnLE_3$ (0.08 ml/L), $TnLE_5$ (0.30 ml/L), and $TnLE_6$ (0.60 ml/L) each having 100.00 ±0.00%, and production performance index, which is optimum in $TnLE_3$ (0.08 ml/L) of 75.63 ±0.27. However, there were no significant differences ($P > 0.05$) in the mean final body weight, body weight gain, relative body weight gain, specific growth rate, feed conversion ratio, nitrogen metabolism and production performance index among the control and exposed groups (Figures 1–8).

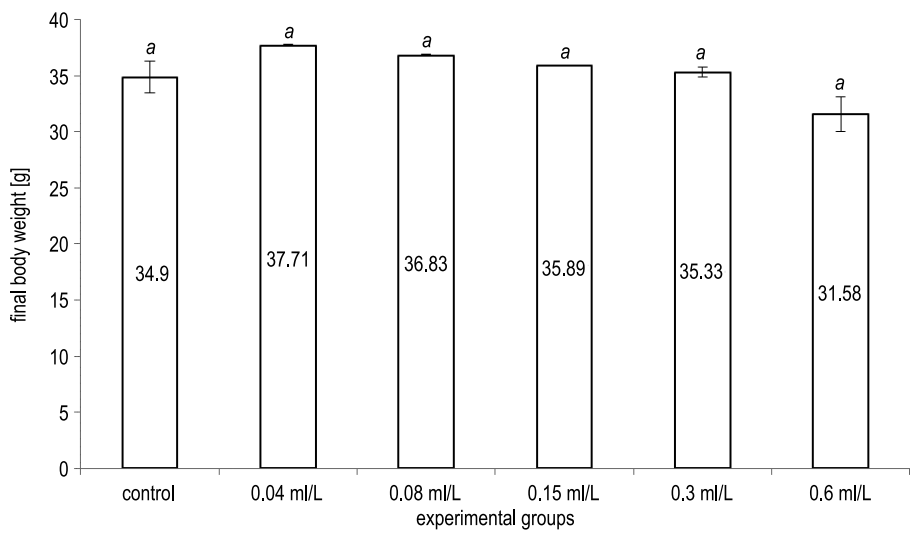

Fig. 1. Final body weight [g] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

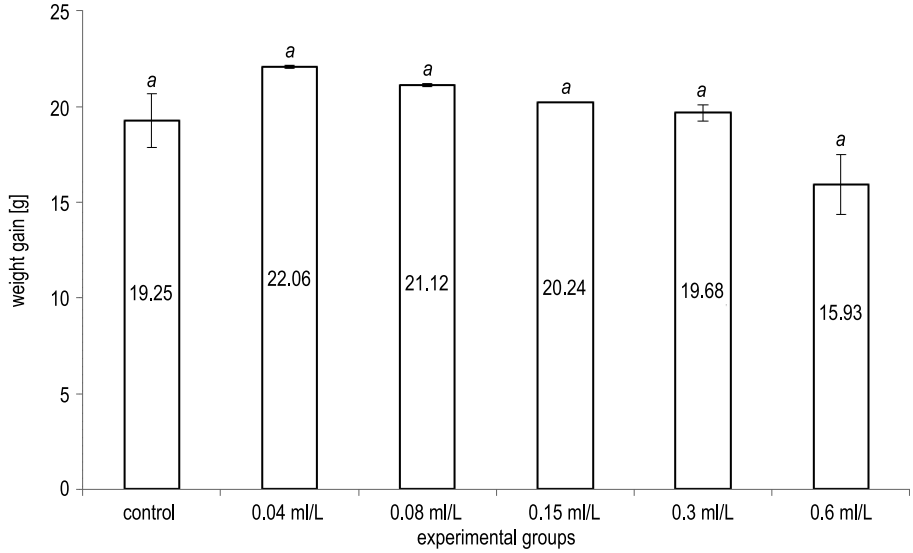

Fig. 2. Weight gain [g] of *C. gariepinus* juveniles 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

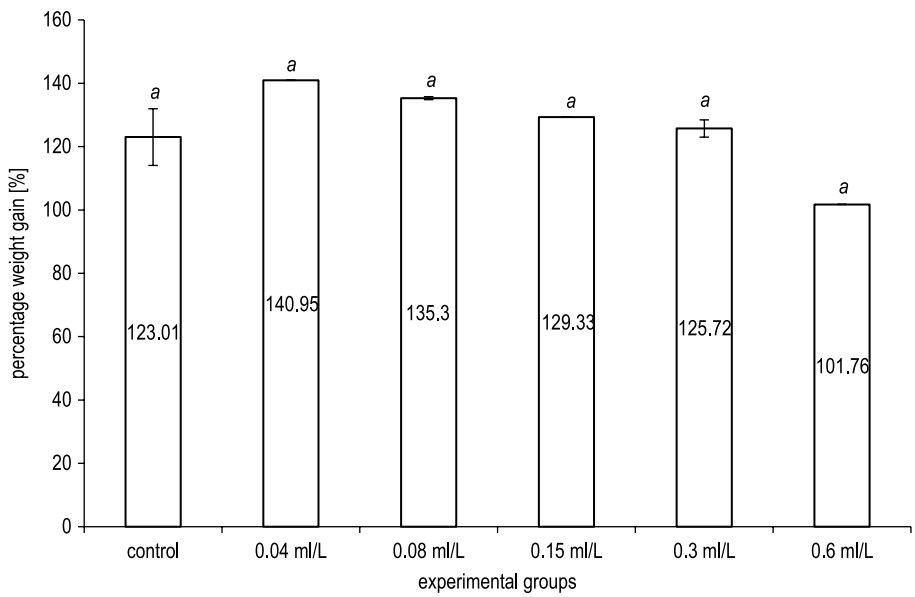

Fig. 3. Weight gain [%] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

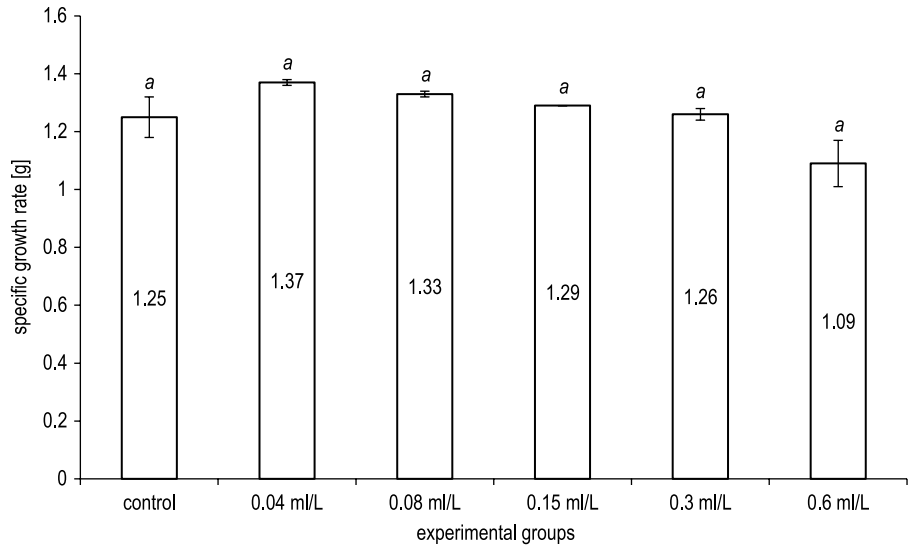

Fig. 4. Specific growth rate [%] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract

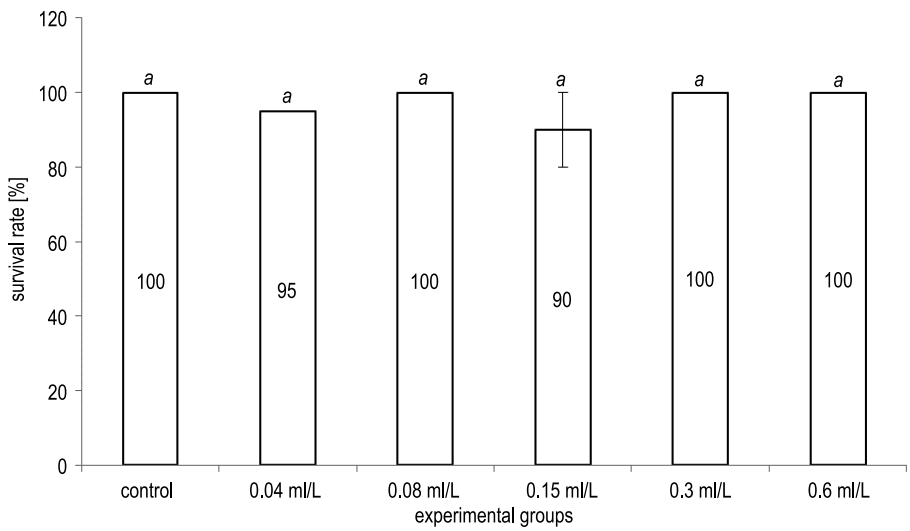

Fig. 5. Survival rate [%] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract
n = 20 specimens per treatment; a – means with the same letter are not significantly different from each other ($p > 0.05$)

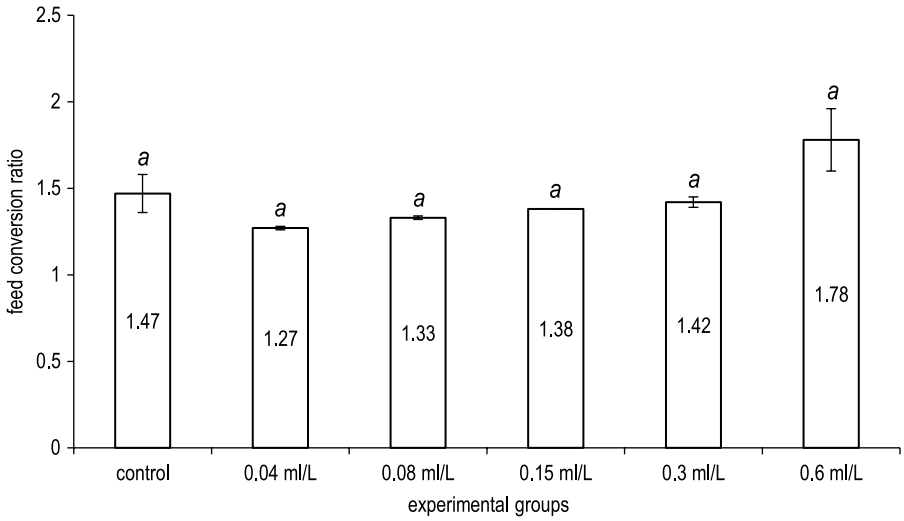

Fig. 6. Feed conversion ratio (FCR) of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; a – means with the same letter are not significantly different from each other ($p > 0.05$)

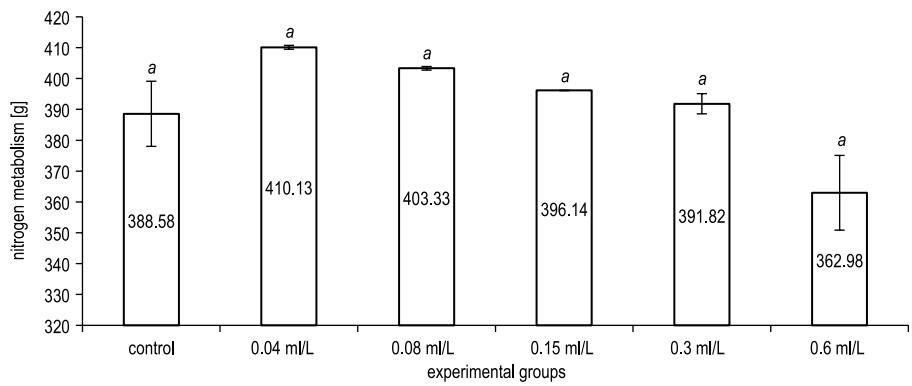

Fig. 7. Nitrogen metabolism of *C. gariepinus* [g] after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; a – means with the same letter are not significantly different from each other ($p > 0.05$)

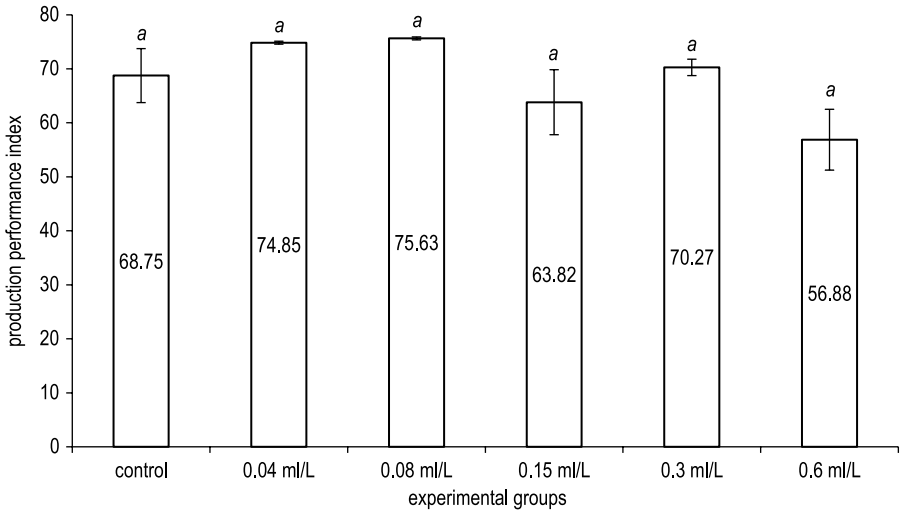

Fig. 8. Production performance index of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

Mean haematological parameters of *Clarias gariepinus* exposed to sublethal concentrations of *T. neriifolia* leaves extract

The highest values of packed cell volume (PCV), haemoglobin (Hb) and white blood cells (WBC) were recorded in $TnLE_3$ (0.08 ml/L) as 46.00 ±2.00%, 15.30 ±0.20 g/dl and 12.20 ±0.20 · 10a/l, respectively. There were no significant differences ($p > 0.05$) in red blood cell (RBC), mean corpuscular haemoglobin concentration (MCHC) and monocytes. The values of PCV, Hb and platelets obtained after 14 days' post exposure were recorded to be decreasing compared to those obtained on exposure to TnLE (Table 2 and Table 3).

Mean plasma biochemistry and blood serum parameters of *C. gariepinus* exposed to sublethal concentrations of *T. neriifolia* leaves extract

The results of plasma biochemistry obtained in this study show that the values of albumin generally increased in the exposed groups when compared to the values obtained in the control. It was also revealed that the values obtained after 14 days' post exposure decreased when compared to those obtained before the experiment and during exposure to *T. neriifolia*. The result of the 14 days' post exposure shows a general decrease in ALT and AST when compared to those obtained before the experiment and during exposure to *T. neriifolia* (Table 4–5).

Table 2

Mean haematological parameters of *Clarias gariepinus* juveniles before and after exposure to different concentrations of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 [ml/L]	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
PCV%	39.00 ±2.00^a	41.00 ±2.00^{ab}	39.00 ±2.00^a	46.00 ±2.00^b	41.00 ±2.00^{ab}	43.00 ±2.00^{ab}	43.00 ±2.00^{ab}
HB [g/dl]	13.00 ±0.20^a	13.70 ±0.20^{ab}	13.00 ±0.20^a	15.30 ±0.20^c	13.70 ±0.20^{ab}	14.30 ±0.20^b	14.30 ±0.20^b
RBC · $10^{12/l}$	4.50 ±0.20^a	5.10 ±0.20^a	4.90 ±0.20^a	4.90 ±0.20^a	4.60 ±0.20^a	5.00 ±0.20^a	4.50 ±0.20^a
WBC · 10 a/l	12.70 ±0.20^c	10.80 ±0.20^a	10.70 ±0.20^a	12.20 ±0.20^{bc}	10.90 ±0.20^a	10.60 ±0.20^a	11.80 ±0.20^b
Platelets [m/µl]	20.50 ±0.20^a	28.70 ±0.20^b	29.90 ±0.20^c	31.70 ±0.20^e	29.80 ±0.20^c	30.60 ±0.20^d	33.00 ±0.20^f
MCV [F1]	86.70 ±0.20^a	80.40 ±0.20^c	79.60 ±0.20^b	93.90 ±0.20^f	89.10 ±0.20^e	86.00 ±0.20^d	95.60 ±0.20^g
MCH [pg/cal]	28.90 ±0.20^b	26.90 ±0.20^a	26.50 ±0.20^a	31.20 ±0.20^d	29.80 ±0.20^c	28.60 ±0.20^b	31.80 ±0.20^d
MCHC [g/dl]	33.30 ±0.20^a	33.40 ±0.20^a	33.30 ±0.20^a	33.30 ±0.20^a	33.40 ±0.20^a	33.30 ±0.20^a	33.30 ±0.20^a
NEU [%]	64.00 ±2.00^{ab}	64.00 ±2.00^{ab}	65.00 ±2.00^{ab}	69.00 ±2.00^b	60.00 ±2.00^a	70.00 ±2.00^b	70.00 ±2.00^b
LYMP [%]	31.00 ±2.00^a	33.00 ±2.00^a	33.00 ±2.00^a	29.00 ±2.00^a	40.00 ±2.00^b	30.00 ±2.00^a	29.00 ±2.00^a
MONO [%]	4.00 ±2.00^a	2.00 ±2.00^a	2.00 ±2.00^a	1.00 ±0.20^a	0.00 ±0.00^a	0.00 ±0.00^a	1.00 ±0.20^a
EOS [%]	1.00 ±0.20^b	1.00 ±0.20^b	0.00 ±0.00^a	1.00 ±0.20^b	0.00 ±0.00^a	0.00 ±0.00^a	0.00 ±0.00^a

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; PCV = Packed Cell Volume; HB = Haemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; NEU = Neutrophils; LYMP = Lymphocytes; MONO = Monocytes; EOS = Eosinophil. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different ($p > 0.05$)

Table 3

Mean haematological parameters of *Clarias gariepinus* juveniles after 14 days' post exposure to different concentrations of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
PCV [%]	39.00 ± 2.00^{a}	44.00 ± 2.00^{a}	42.00 ± 2.00^{a}	40.00 ± 2.00^{a}	38.00 ± 2.00^{a}	41.00 ± 2.00^{a}	39.00 ± 2.00^{a}
HB [g/dl]	13.00 ± 0.20^{a}	14.70 ± 0.20^{d}	14.00 ± 0.20^{c}	13.30 ± 0.20^{abc}	12.70 ± 0.20^{a}	13.70 ± 0.20^{bc}	13.00 ± 0.20^{ab}
RBC · $10^{12/l}$	4.50 ± 0.20^{a}	4.70 ± 0.20^{a}	4.70 ± 0.20^{a}	5.00 ± 0.20^{a}	4.30 ± 0.20^{a}	4.60 ± 0.20^{a}	4.80 ± 0.20^{a}
WBC · 10 a/l	12.70 ± 0.20^{c}	11.40 ± 0.20^{bc}	11.70 ± 0.20^{c}	11.20 ± 0.20^{bc}	8.90 ± 0.20^{a}	10.80 ± 0.20^{b}	11.00 ± 0.20^{bc}
Platelets [m/µl]	20.50 ± 0.20^{a}	28.80 ± 0.20^{b}	30.10 ± 0.20^{c}	28.50 ± 0.20^{ab}	28.20 ± 0.20^{ab}	27.90 ± 0.20^{a}	28.40 ± 0.20^{ab}
MCV [F1]	86.70 ± 0.20^{a}	93.60 ± 0.20^{e}	89.40 ± 0.20^{d}	80.00 ± 0.20^{a}	82.20 ± 0.20^{b}	89.10 ± 0.20^{d}	86.70 ± 0.20^{c}
MCH [pg/cal]	28.90 ± 0.20^{b}	31.30 ± 0.20^{e}	29.80 ± 0.20^{d}	26.60 ± 0.20^{a}	27.30 ± 0.20^{b}	29.80 ± 0.20^{d}	28.90 ± 0.20^{c}
MCHC [g/dl]	33.30 ± 0.20^{a}	33.40 ± 0.20^{a}	33.30 ± 0.20^{a}	33.30 ± 0.20^{a}	33.20 ± 0.20^{a}	33.40 ± 0.20^{a}	33.30 ± 0.20^{a}
NEU [%]	64.00 ± 2.00^{ab}	70.00 ± 2.00^{a}	70.00 ± 2.00^{a}	69.00 ± 2.00^{a}	70.00 ± 2.00^{a}	65.00 ± 2.00^{a}	68.00 ± 2.00^{a}
LYMP [%]	31.00 ± 2.00^{a}	28.00 ± 2.00^{a}	30.00 ± 2.00^{a}	29.00 ± 2.00^{a}	28.00 ± 2.00^{a}	30.00 ± 2.00^{a}	29.00 ± 2.00^{a}
MONO [%]	4.00 ± 2.00^{a}	2.00 ± 2.00^{a}	0.00 ± 0.00^{a}	2.00 ± 2.00^{a}	1.00 ± 0.20^{a}	3.00 ± 2.00^{a}	2.00 ± 2.00^{a}
EOS [%]	1.00 ± 0.20^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.00 ± 0.20^{b}	2.00 ± 0.20^{c}	1.00 ± 0.20^{b}

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; PCV = Packed Cell Volume; HB = Haemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; NEU = Neutrophils; LYMP = Lymphocytes; MONO = Monocytes; EOS = Eosinophil. Mean of duplicate data; mean value in each row with similar superscripts are not significantly different ($p > 0.05$)

Table 4

Mean Plasma Biochemistry parameters of *C. gariepinus* juveniles before and after exposure to different concentration of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
TP (g/dl)	76.00 ±2.00^a	72.00 ±2.00^a	72.00 ±2.00^a	70.00 ±2.00^a	76.00 ±2.00^a	76.00 ±2.00^a	72.00 ±2.00^a
ALB (g/dl)	43.00 ±2.00^b	34.00 ±2.00^a	38.00 ±2.00^{ab}	40.00 ±2.00^{ab}	39.00 ±2.00^{ab}	35.00 ±2.00^a	37.00 ±2.00^{ab}
GLO (g/dl)	33.00 ±2.00^{ab}	38.00 ±2.00^{bc}	34.00 ±2.00^{abc}	30.00 ±2.00^a	37.00 ±2.00^{abc}	41.00 ±2.00^c	35.00 ±2.00^{abc}
ALB/GLO (ratio)	1.30 ±0.20^a	0.90 ±0.20^a	1.10 ±0.20^a	1.30 ±0.20^a	1.10 ±0.20^a	0.90 ±0.20^a	1.10 ±0.20^a
ALT (U/I)	13.00 ±2.00^a	12.00 ±2.00^a	13.00 ±2.00^a	11.00 ±2.00^a	9.00 ±2.00^a	12.00 ±2.00^a	11.00 ±2.00^a
AST (U/I)	12.00 ±2.00^a	9.00±2.00^a	10.00±2.00^a	13.00±2.00^a	8.00±2.00^a	10.00 ±2.00^a	10.00 ±2.00^{ab}
GLUCO (mol/l)	7.8.00 ±0.20^e	5.70±0.20^d	6.00±0.20^d	4.30±0.20^{ab}	5.00±0.20^c	4.90 ±0.20b^c	4.10 ±0.20^a

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; TP = Total Protein; ALB = Albumin; GLO = Globulin; ALB/GLO = Albumin-Globulin ratio. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different ($p > 0.05$)

Table 5

Mean plasma biochemistry parameters of *C. gariepinus* juveniles before exposure and after 14 days' post exposure of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
TP [g/dl]	76.00 ±2.00^a	76.00 ±2.00^a	74.00 ±2.00^a	72.00 ±2.00^a	69.00 ±2.00^a	70.00 ±2.00^a	75.00 ±2.00^a
ALB [g/dl]	43.00 ±2.00^b	40.00 ±2.00^a	38.00 ±2.00^a	37.00 ±2.00^a	40.00 ±2.00^a	40.00 ±2.00^a	38.00 ±2.00^a
GLB [g/dl]	33.00 ±2.00^{ab}	36.00 ±2.00^{ab}	36.00 ±2.00^{ab}	35.00 ±2.00^{ab}	29.00 ±2.00^a	30.00 ±2.00^{ab}	37.00 ±2.00^b
ALB/GLO (ratio)	1.30 ±0.20^a	1.10 ±0.20^a	1.10 ±0.20^a	1.10 ±0.20^a	1.40 ±0.20^a	1.30 ±0.20^a	1.10 ±0.20^a
ALT [U/I]	13.00 ±2.00^a	12.00 ±2.00^a	10.00 ±2.00^a	9.00 ±2.00^a	13.00 ±2.00^a	12.00 ±2.00^a	10.00 ±2.00^a
AST [U/I]	12.00 ±2.00^a	11.00 ±2.00^a	9.00 ±2.00^a	9.00 ±2.00^a	9.00 ±2.00^a	11.00 ±2.00^a	8.00 ±2.00^a
GLUCO [mol/l]	7.8.00 ±0.20^e	4.70 ±0.20^a	6.00 ±0.20^{bc}	5.20 ±0.20^a	5.40 ±0.20^{ab}	7.30 ±0.20b^d	6.20 ±0.20^c

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; TP = Total Protein; ALB = Albumin; GLO = Globulin; ALB/GLO = Albumin-Globulin ratio. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different ($P > 0.05$), fish ($n = 2$) per treatment

Histological examination of *C. gariepinus* juveniles exposed to sublethal concentrations of *T. neriifolia* leaves extract

There were slight observable changes in the gills and liver among all the exposed groups except the control. The control group recorded no visible abnormal structural changes also in the skin and intestine (Table 6).

Table 6

Histological changes observed different organs of *C. gariepinus* juveniles exposed to sub-lethal concentrations of *T. neriifolia* leaves extract ($TnLE_{1–6}$)

Organ	Histological changes	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
Skin	moderate hyperplasia of keratinocytes	–	–	–	–	½	–
	atrophy of the epidermis and loss of keratinocytes	–	–	–	–	–	½
Gills	atrophy of secondary lamellae	–	–	½	–	–	–
	hyperplasia of secondary lamellae	–	–	–	½	½	½
Liver	moderate diffuse vacuolation of hepatocytes	–	½	–	½	½	–
	hepatocellular degeneration	–	–	½	–	–	–
	moderate diffuse atrophy of hepatocytes	–	–	–	–	–	½
Intestine	no visible structural changes	–	–	–	–	–	–

Explanations: ½ = present but mild; "–" = no lesion or morphological changes in tissue

Discussion

Aquatic species demonstrate alterations in their behavior in response to chemical stress, which can occur either as acute toxicity or sublethal toxicity (GRILLITSCH et al. 1999). The behavioural alterations exhibited by the fish subjected to *Thevetia neriifolia* were detected across varying levels of concentration. During the exposure period, the control group exhibited typical swimming behavior and maintained their natural coloration. However, as the concentration of the toxicant increased, the fish displayed an escalating frequency of anomalous behaviours. The observed behavioural alterations encompass a decline in equilibrium, ingestion of air and irreg-

ular swimming patterns. The fishes in the exposed groups exhibited heightened mucus secretion, potentially as an adaptation mechanism to mitigate the irritating effects of *T. neriifolia* on their body surface and mucus membrane.

The findings of this experiment indicated a notable overall increase in the weight of both the unexposed and exposed groups. The biggest increase in weight was observed in the $TnLE_2$ treatment, and weight growth reduced as the concentration of the extract increased. The $TnLE_2$ group exhibited superior performance across all biological indicators compared to the other exposed groups. However, statistical analysis revealed no significant differences ($p > 0.05$) among the exposed groups (Figure 2) There were notable disparities in the packed cell volume (PCV) between the control and exposed groups of fish, with statistical significance observed ($p < 0.05$). The recorded values exhibited an increase in comparison to the control treatment, apart from $TnLE_2$, where the result demonstrated a reduction (Table 2). The observed rise in red blood cell count (Table 2) may be attributed to several factors, such as an elevated number of red blood cells resulting from acute stress and spleen discharge, swelling of erythrocytes due to reduced blood pH and respiratory acidosis, or a decrease in circulating blood volume caused by the acidification of muscle tissue following exposure to stress or intense physical activity (GOMUŁKA et al. 2014). According to ETIM et al. (1999), haemoglobin is a significant indicator of fish survivability due to its direct correlation with the blood's capacity to bind and transport oxygen. This investigation revealed statistically significant variations ($p < 0.05$) in the levels of haemoglobin (Hb). The observed values exhibited an increase in comparison to the control treatment, except for $TnLE_2$ (Table 2).

The results indicate a marginal decline in the levels of red blood cells in the exposed groups as compared to the control group. However, statistical analysis revealed no significant differences ($p > 0.05$) between the control and exposed groups. A decrease in the white blood cell (WBC) values was observed in both the control and exposed groups, relative to the starting values recorded prior to the trial. Nevertheless, the experimental groups had greater values compared to the control group, except for $TnLE_2$ and $TnLE_5$, which showed somewhat lower values (Table 3). The observed elevation in the number of white blood cells in certain subjected fish can be related to modifications in their defensive mechanisms against the toxic effects of the extract (ZAGHLOUL 2001, ZAGHLOUL et al. 2005).

An elevation in mean cell volume (MCV) was observed in the groups that were exposed, apart from $TnLE_2$, where a reduction was noted, in comparison to the control group (Table 2). The elevation of mean corpuscu-

lar volume (MCV) is ascribed to the enlargement of erythrocytes due to hypoxia (insufficient oxygen levels) or disrupted water equilibrium (osmotic stress), leading to the development of macrocytic anemia in fish exposed to toxic substances (LARSSON et al. 1985). A decrease in lymphocyte count was observed in all treatment groups, apart from $TnLE_4$, which exhibited an increase (Table 2). The decrease in variable may be attributed to an immunological response, wherein the body produces antibodies to manage the stress caused by the hazardous substance. An observed elevation in the overall neutrophil count was documented (Table 2), either indicating the presence of a bacterial infection or arising from the influence of stress. The mean corpuscular hemoglobin concentration (MCHC) values exhibited a modest elevation compared to the control group, demonstrating continuous uniformity across the samples. However, it is worth noting that $TnLE_4$ displayed an identical numerical value to that of the control group (Table 2). Nevertheless, the study did not find any statistically significant differences ($p > 0.05$) between the control group and the groups who were exposed to the experimental conditions. This finding suggests that the exposure to the aqueous leaves extract of *T. neriifolia* did not have an impact on MCHC. The mean corpuscular hemoglobin (MCH) exhibited a statistically significant increase ($p < 0.05$) in numerical values when compared to the control group, except for $TnLE_2$ (Table 2).

Upon completion of the post-exposure period, the haematological analysis revealed a notable decline in the levels of PCV, Hb, MCV, and MCH within the exposed groups in comparison to the control group. Furthermore, there were no statistically significant differences observed in the values of PCV, RBC, MCHC, neutrophils, lymphocytes, and monocytes (Table 3). The findings additionally indicated a rise in the levels of white blood cells (WBC) in comparison to the values seen during exposure to the extract of *T. neriifolia* leaves. Nevertheless, the observed reversible hematological parameters after a 14-day post-exposure period (Table 3) provide evidence of the non-bioaccumulative action of the *T. neriifolia* extracts. The exposed groups exhibited minor variations in total protein (TP) and albumin/globulin (A/G) levels in comparison to the control group (Table 3). The globulin values of the exposed groups exhibited a substantial drop ($p < 0.05$) in comparison to the control group, except for $TnLE_5$. However, the exposed groups exhibited a modest yet statistically significant rise ($p < 0.05$) in albumin levels (Table 3).

Upon the conclusion of the post exposure period, it was observed that the levels of albumin (ALB) and the albumin-globulin ratio (A/B) in the exposed groups were comparable to those of their respective control groups. The observed data pertaining to the levels of total protein (TP) within the

groups that were exposed indicate a reduction in comparison to the control group. Nevertheless, the control and exposed groups did not exhibit any statistically significant differences ($p > 0.05$) in terms of TP, ALB, and A/B (Table 4). Substantial variations in the globulin (GLB) levels were observed within the exposed groups during post-exposure, in contrast to both the control group and the values recorded during exposure to *T. neriifolia* leaf extract. A notable decline in the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was observed in comparison to the measurements recorded prior to and after the administration of *T. neriifolia* leaf extract. No statistically significant differences ($p > 0.05$) were seen in the values of ALT between the control and exposed groups. However, a notable reduction was detected in the glucose levels, except for $TnLE_2$ (Table 4). At the conclusion of the post-exposure period, the study observed a statistically significant rise ($p < 0.05$) in glucose levels compared to those observed during exposure to *T. neriifolia*. The values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) exhibited an increase in comparison to the initial values acquired before to the commencement of the trial. No statistically significant differences ($p > 0.05$) were seen in the levels of ALT and AST between the control group and the exposed group (Table 5).

All fish organs in the study's control group were oriented similarly to those of other healthy finfish, with no tissue deformation or modification visible. However, as the concentration of the extract grew, there was clear evidence of changes in the structural formations of the gill tissues of the fish among the groups that were exposed to the extract. According to REDDY and WASKALE (2013), fish's gills are crucial in maintaining biological homeostasis in aquatic environments. Due to their proximity to the external environment and the large surface area of respiratory epithelium, the gills are susceptible to the negative impacts of contaminants and pollutants in the water. The gills of the fish subjected to a dose of 0.08 ml/L of *T. neriifolia* extract exhibited atrophy of the secondary lamellae. Additionally, hyperplasia of the secondary lamellae was detected at concentrations of 0.15 ml/L, 0.30 ml/L, and 0.60 ml/L. Similarly, the livers of the groups exposed to different amounts of the *T. neriifolia* extract exhibited varying degrees of structural deformations. The liver exhibited significant diffuse vacuolation of hepatocytes at a concentration of 0.04 ml/L. At a concentration of 0.08 ml/L, random hepatocellular degeneration was observed. Random swelling (vacuolation) of hepatocytes was observed at 0.15 ml/L and 0.3 ml/L concentrations. Likewise, moderate diffuse atrophy of hepatocytes was identified at a dose of 0.6 ml/L. The fish's skin exposed to an extract concentration of 0.3ml/L showed a modest increase in keratino-

cytes, indicating hyperplasia. Conversely, the skin exposed to a concentration of 0.6 ml/L had a reduction in the thickness of the outermost layer of skin (epidermis) and a decrease in the number of keratinocytes, indicating atrophy. No discernible alterations in the intestinal structure were identified between the control and exposed groups (Table 6).

Conclusion

The extract of *T. neriifolia* exhibited sublethal toxicity on the fish specimens, resulting in significant changes in the blood profiles of *C. gariepinus*. The observed variations in the fundamental characteristics of fish resulting from their exposure to the extract provide evidence of its mechanism of action as a piscicide. Therefore, it is plausible that this substance generated from plants could serve as a possible piscicide for the purpose of capturing fish or eliminating undesired fish populations in nursery and rearing ponds prior to stocking. This work has the potential to offer valuable insights for the potential future utilization of *T. neriifolia* in aquaculture applications. Further investigation is warranted to explore the effects of *T. neriifolia* leaf extracts on additional fish species, with the aim of comprehensively understanding their physiological and behavioral responses to these plant extracts.

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