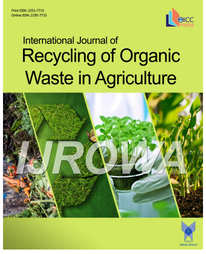

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Hydrolysates of fish waste as potential plant biostimulants

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Original Research

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Abstract:

Purpose: The development of plant biostimulants from fish industry waste will increase crop yields and reduce environmental pollution. The aim of our study was to develop an optimal method for the production of fish hydrolysates and to test their effectiveness as plant biostimulants for important crops such as corn and beans.

Method: Three different hydrolysates were used: I from guts only, II and III from guts and heads. I and III were hydrolyzed using fish guts own enzymes, while II was additionally stimulated with nitric acid.

Results: It was found that for beans, the treatment with a low concentration of fish hydrolysates (1%) was more optimal, while for corn, higher concentrations of hydrolysates (2% and 5%) were more effective. Treatment with all hydrolysates reduced the chlorophyll a content in maize leaves. For plants, hydrolysates from fish guts alone were more effective as biostimulants, especially for legumes that formed a root-nodule symbiosis.

Conclusion: According to all the studied indicators, hydrolysate I (only from viscera) at a concentration of 1% was the most effective in the treatment of beans and corn, increasing the index of seedling vigour, growth and total vegetative mass.

Keywords: Fish hydrolysates; Bean; Corn; Seed germination; Seedling vigour index; Chlorophylls

1. Introduction

Plant biostimulants are defined as products that stimulate plant nutrition processes regardless of the nutrient content of the product and stimulate resistance to abiotic stress (Bulgari et al., 2019; Drobek et al., 2019). Currently, biologically active preparations based on the active biomass of microorganisms and their metabolites, plant growth regulators, and animal waste are widely used (Polo and Mata, 2018; Madende and Hayes, 2020). At the same time, the production of plant biostimulants from fish waste is much less developed. In global practice, fish waste is traditionally processed mainly by composting to produce plant fertilizer (Kusuma et al., 2019). However, this process is lengthy and only allows the raw material to be degraded to proteins. The production of fish protein hydrolysates, which are dominated by low molecular weight protein fragments, is much faster (Ideia et al., 2019). On the other hand, fish industry waste (about 50 – 80% of the total weight of fish) often becomes a problem for the environment (Chalamaiah et al., 2012). Therefore, their timely targeted recycling is extremely important from an environmental point of view. In addition, imperfect crop cultivation technologies, including excessive use of inorganic fertilizers, have a major negative impact on the environment. This once again emphasises the importance of using biological products. The development of high-quality plant biostimulants will help increase crop yields, while reducing the use of inorganic fertilizers and efficient use of fishery waste will reduce environmental pollution.

According to the literature, fishery waste is hydrolyzed by adding exogenous enzymes (Vázquez et al., 2019; Henriques et al., 2021; Borges et al., 2023), high-pressure treatment, ultrasound, and heat treatment to increase the amount of low molecular weight peptides (Vázquez et al., 2020; Moya Moreira et al., 2023). However, most of these methods are costly, have small processing volumes of raw

materials and require complex equipment. New waste treatment technologies can reduce process losses and achieve better results by efficiently breaking down proteins.
Thus, the aim of our research was to develop an optimal method for the production of fish hydrolysates and test their effectiveness as plant biostimulants for important crops such as corn and beans.

2. Materials and methods

Fish hydrolysates were prepared using three slightly different approaches. In all cases, the waste (bones, heads) of rainbow trout (*Oncorhynchus mykiss*) was used as the starting material, and trout entrails served as a source of proteolytic enzymes. At the first stage, the bones, heads, and entrails were ground to homogenity in a blender. The samples No. II and III consist of homogenized bones and heads that were mixed with homogenized fish entrails at a ratio of 3:1 (w:w). The sample No. I consists of an equal mass of homogenized fish entrails. All samples were placed in a water bath at +37 °C. After 12 hours of incubation, the sample No. II was mixed with an equal amount of 2 M nitric acid, and the samples No. I and III were mixed with an equal amount of distilled water and left in a water bath (+37 °C) for the next 12 hours. All samples were then boiled for 1 hour, cooled to room temperature, and filtered with Whatman qualitative filter paper, Grade 1. The hydrolysate prepared using nitric acid was neutralized to pH 7.0 – 7.5 with 1 M NaOH. At the final step, all hydrolysates were sterilized by the tyndallization method.
Seeds of medium-ripening varieties of bean (*Phaseolus vulgaris* L.) variety Red Cap of bush type and corn (*Zea mays* L.) variety Golden Cob of Ukrainian production were used for the study. In order to improve selection and subsequent germination, the corn and bean seeds were soaked in water for 8 hours before planting. Soaking helps to soften the seed coat, which facilitates the penetration of water and oxygen, which are essential for germination. This helps the seeds to germinate faster and more simultaneously and also allows you to identify and eliminate seeds that are not viable. Seeds were selected that were visually well filled, with intact seed coats and approximately the same size. Seeds were not treated with fungicides to avoid possible interaction of different active ingredients.
The seeds were planted in Peatfield universal soil with a pH of 5.5 – 6.5 without any biological additives. In each experimental group, 10 bean or corn seeds were planted in triplicate. After planting, the seeds were watered once with 20 mL per well of 1%, 2% or 5% solutions of fish hydrolysates I, II and III. The control group was watered with water without hydrolysates. The plants were grown in greenhouse conditions with a daytime temperature of +25 °C and a nighttime temperature of +20 °C, which was close to the optimal conditions for planting seeds in the ground.
The effectiveness of hydrolysates I, II and III on the germination and growth of maize and beans was determined. Seed germination was recorded daily for 14 days after sowing. Seedling growth was measured every 3 days for two weeks after seed germination.
The percentage of seed germination was determined on the 14th day, when the last individual seeds germinated.

$$\text{Germination\%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds taken for germination}} \times 100 \quad (1)$$

To determine the seedling vigour index, the average value of seedling length at 21 days (when the latest seedlings grew) and the percentage of germination at 21 days (the value was the same as at 14 days) were taken. Seedling vigour index was calculated following Srivastava (2015).

$$\text{Seedling vigour index} = (\text{Germination\%} \times \text{Seedling length}) \quad (2)$$

Three weeks after sowing the seeds, the total vegetative mass of bean and maize plants, aboveground and underground vegetative mass were weighed. After that, leaf samples were immediately taken for spectrophotometric measurement of chlorophyll and carotenoid content and total flavonoid content.
The content of the substances was determined using a spectrophotometer SF-2000: chlorophylls and carotenoids were extracted from plant material with 80% acetone and determined at $\lambda = 663$, 646, and 470 nm in terms of wet weight (Lichtenthaller, 1987), total flavonoid content in terms of rutin and absolute dry weight was measured at $\lambda = 410$ nm and expressed as a percentage (Shraim et al., 2021).
Statistical analysis was performed using GraphPad Prism 8 software (GraphPad Company, San Diego, USA, 2014) by multivariate ANOVA with Tukey's correction.

3. Results and discussion

Single maize seeds began to germinate on day 4 in all groups. Mass shoots in most groups appeared on the 6th day after sowing and active germination lasted up to 9 days. Only a few seeds germinated 10 – 13 days after sowing. Seeds treated with hydrolysate I (2%) germinated most synchronously and quickly, so germination in this group on day 4 (43.3%) and day 6 (76.7%) was significantly higher than in other experimental groups and the control. In particular, in the control, seed germination on day 4 was 13.3% and on day 6 was 33.3%. After 9 days of germination, seed germination did not differ significantly between the experimental groups. At the same time, there was a tendency to later stimulation of seed germination by hydrolysates II (2%) and III (5%) – on day 14, germination was 90% and 86.7%, respectively, compared to 73.3% in the control group (Fig. 1).
The control group showed the best results of seed germination (70%). Similar data to the control group were obtained for seeds treated with hydrolysates I (2%) and III (1%) (Fig. 2). Inhibition of seed germination was observed when bean seeds were treated with hydrolysate I (5%) – 30% germination and III (5%), where no seeds sprout up. Thus, fish hydrolysates in high concentrations had either a neutral or negative effect on bean germination.
As shown in Table 1, a significant stimulation of maize seedling growth was observed on days 8 and 12 only after treatment with 2% solution I, compared to the control group. Further growth of maize in group I (2%) maintained the trend of the highest values of the indicator, but did not statistically differ from other groups. Also, on day 8,

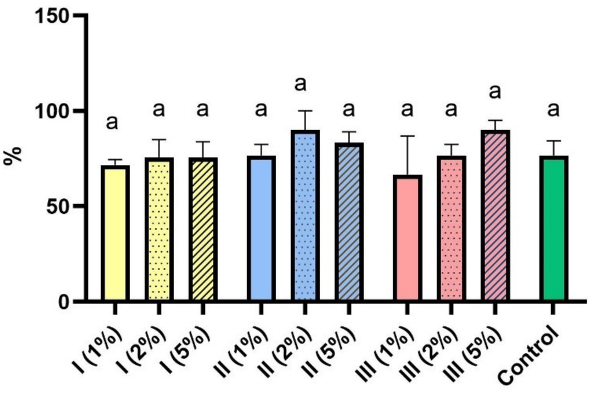

Figure 1. Germination of maize seeds on the 14th day after treatment with fish hydrolysates I, II, III (M ± m; N = 30) Bean seeds began to sprout up on day 4, most intensively on day 6-7, and after day 10 the seeds did not germinate.

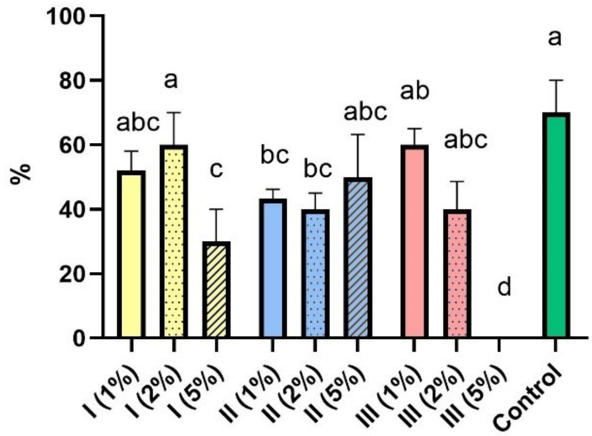

Figure 2. Germination of bean seeds on the 14th day after treatment with fish hydrolysates I, II, III (M ± m; N = 30).

more intensive stimulation of maize growth was observed in treatment I (1%) compared to treatment III (5%); and in treatment I (2%) compared to treatment II (2%), III (1%) and III (5%). On day 12, better growth was observed only after treatment I (2%) compared to treatment III (5%). Thus, as a result of treatment with hydrolysates immediately after planting seeds, plant growth was stimulated during the first two weeks, and during further cultivation the difference in height was only slightly maintained.
There was no significant difference in maize growth between groups with different concentrations of the same hydrolysate (Table 1). Hydrolysate I performed best at a concentration of 2%, and hydrolysates II and III performed best at a concentration of 1%. In general, the treatment with all hydrolysates in all concentrations slightly stimulated the growth of maize compared to the control, but the best treatment was performed by hydrolysates I (2%) and I (1%).
On the sixth day after planting bean seeds, the tallest plants were those treated with 1% hydrolysate II, this group significantly differed in growth from both the control group and the groups treated with I (1%) and II (5%) (Table 2).
On days 14, 17 and 20, the highest growth rates were shown by bean plants treated with 1% hydrolysate I: twice the height compared to the control group, as well as compared to plants of groups I (2%), I (5%), II (5%), III (1%) and III (2%). Also, good height indices were obtained after watering with 1% and 2% solutions of hydrolysate II. Group III (5%) is not shown in Table 2, because at this concentration of this hydrolysate no bean seeds germinated. It should also be noted that in high concentration (5%) hydrolysates I and II also showed the worst growth of bean seedlings, although they did not differ significantly from the control group.
The seedling vigour index is one of the most indicative parameters, as it reflects not only the number of germinated seeds, but also the quality of the seedlings themselves. This parameter, as well as the previous data, indicates a tendency to stimulate the germination and development of maize by hydrolysates I and II at a concentration of 2% and hydrolysate III at a concentration of 5% (Fig. 3). For beans, hydrolysate I at a concentration of 1% was the most

Table 1. Growth dynamics of maize seedlings after treatment with fish hydrolysates, cm (M ± SE, n = 20).

Group	5 days	8 days	12 days	15 days	20 days
Control	2.1±0.3[a]	6.1±0.7[bc]	14.5±1.2[b]	22.6±3.0[a]	32.6±2.0[a]
I (1%)	2.5±0.2[a]	8.8±0.4[ab]	17.9±0.6[ab]	24.6±0.7[a]	35.7±0.9[a]
I (2%)	2.8±0.2[a]	9.5±0.4[a]	18.9±0.5[a]	24.2±0.6[a]	36.2±0.8[a]
I (5%)	2.3±0.2[a]	7.5±0.5[abc]	15.7±0.9[ab]	21.3±1.1[a]	34.0±1.2[a]
II (1%)	2.2±0.2[a]	7.7±0.5[abc]	16.7±0.8[ab]	22.8±1.0[a]	35.3±1.2[a]
II (2%)	2.0±0.3[a]	6.7±0.7[bc]	15.4±1.2[ab]	22.0±1.5[a]	32.1±1.8[a]
II (5%)	1.9±0.2[a]	7.4±0.5[abc]	16.7±1.1[ab]	23.3±1.2[a]	34.7±1.8[a]
III (1%)	2.3±0.3[a]	6.9±0.6[bc]	15.4±1.1[ab]	22.4±1.1[a]	36.1±1.6[a]
III (2%)	2.1±0.5[a]	7.1±0.9[abc]	14.9±1.4[ab]	21.1±1.7[a]	33.1±2.0[a]
III (5%)	1.5±0.2[a]	5.5±0.6[c]	14.4±0.9[b]	20.3±1.1[a]	32.5±1.4[a]

The same letters indicate the absence of a significant difference between the groups at $P \leq 0.05$.

Table 2. Growth dynamics of beans after treatment with fish hydrolysates, cm (M ± SE, n = 15).

Group	6 days	10 days	14 days	17 days	20 days
Control	$3.5{\pm}1.9^{b}$	$12.8{\pm}4.4^{a}$	$19.3{\pm}7.1^{b}$	$25.8{\pm}4.7^{b}$	$29.6{\pm}2.8^{b}$
I (1%)	$3.2{\pm}0.8^{b}$	$13.8{\pm}0.8^{a}$	$30.2{\pm}2.1^{a}$	$37.4{\pm}2.4^{a}$	$24.2{\pm}3.3^{b}$
I (2%)	$5.0{\pm}0.5^{ab}$	$11.0{\pm}1.4^{a}$	$17.2{\pm}1.2^{b}$	$22.3{\pm}2.7^{b}$	$50.2{\pm}3.9^{a}$
I (5%)	$3.8{\pm}1.3^{ab}$	$10.5{\pm}2.1^{a}$	$17.3{\pm}1.8^{b}$	$21.1{\pm}2.4^{b}$	$23.3{\pm}2.6^{b}$
II (1%)	$7.9{\pm}0.9^{a}$	$15.3{\pm}0.2^{a}$	$25.5{\pm}2.1^{ab}$	$29.0{\pm}0.5^{ab}$	$39.0{\pm}5.8^{ab}$
II (2%)	$6.0{\pm}1.2^{ab}$	$15.3{\pm}0.6^{a}$	$25.8{\pm}3.4^{ab}$	$36.0{\pm}6.1^{ab}$	$45.5{\pm}10.1^{ab}$
II (5%)	$1.8{\pm}0.4^{b}$	$10.2{\pm}1.9^{a}$	$19.2{\pm}3.0^{b}$	$22.6{\pm}3.8^{b}$	$28.2{\pm}5.6^{b}$
III (1%)	$4.1{\pm}1.0^{ab}$	$12.6{\pm}2.4^{a}$	$19.2{\pm}1.5^{b}$	$22.7{\pm}2.6^{b}$	$27.5{\pm}3.8^{b}$
III (2%)	$2.7{\pm}0.8^{ab}$	$9.3{\pm}2.3^{a}$	$17.3{\pm}1.5^{b}$	$18.8{\pm}2.5^{b}$	$23.0{\pm}4.0^{b}$

The same letters indicate the absence of a significant difference between the groups at $P \leq 0.05$.

effective, while higher doses of this hydrolysate and other hydrolysates had a suppressive effect. The use of fish hydrolysates at a concentration of 5% caused a significant decrease in the vigour index of bean seedlings (Fig. 4). These data indicate a greater sensitivity of beans to these hydrolysates compared to corn (Figs. 3, 4).

At the same time, biostimulants can affect not only the rate of germination and growth in height, but also the overall development of vegetative organs. In particular, the aboveground mass index depends on the number and size of leaves, stem thickness, and the underground mass index depends on the number and size of roots. In general, at 21 days after sowing seeds, there was no significant difference in the aboveground and underground mass of maize after treatment with hydrolysates, both in comparison with the control group and between different hydrolysates and different concentrations of the same hydrolysate (Table 3). For each hydrolysate separately, a slightly better effect on the growth of maize vegetative mass was observed when using them at a dose of 5%.

For beans, as well as for maize, at 21 days after sowing seeds, no significant difference was found in the weight of aboveground vegetative organs and total plant weight after treatment with hydrolysates (Table 4). At the same time, the development of the root system was best affected by the treatment with hydrolysate I (1%), and the underground mass after treatment with hydrolysates II (1, 2 and 5%), I (5%) and III (2%) was significantly lower than in group I (1%) (Table 4). A general tendency of better influence of the studied fish hydrolysates on the development of vegetative mass of beans can be observed when used in low concentration (1%). Group III (5%) is not included in Table 4, since no bean seeds germinated at this concentration of this hydrolysate.

The treatment with hydrolysates had a negative effect on the content of chlorophyll a in maize leaves, with the least negative effect being shown by hydrolysate I (Fig. 5 (A)). The content of chlorophyll b increased only after treatment with 2% hydrolysate III, which may be an adaptive reaction to the destruction of chlorophyll a (Fig. 5 (B)). At the

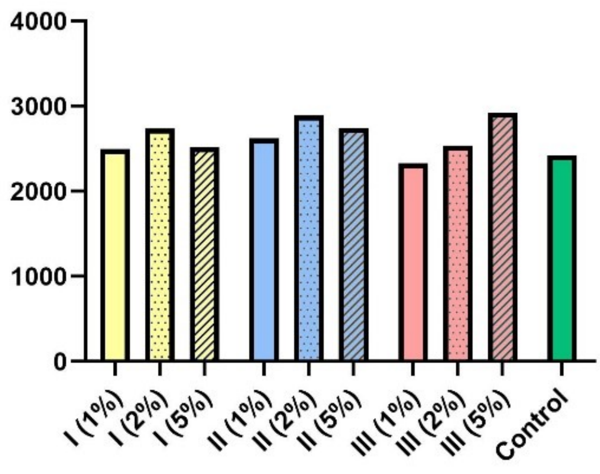

Figure 3. Vigour index of maize seedlings after treatment with hydrolysates I, II, III on the 21st day of germination.

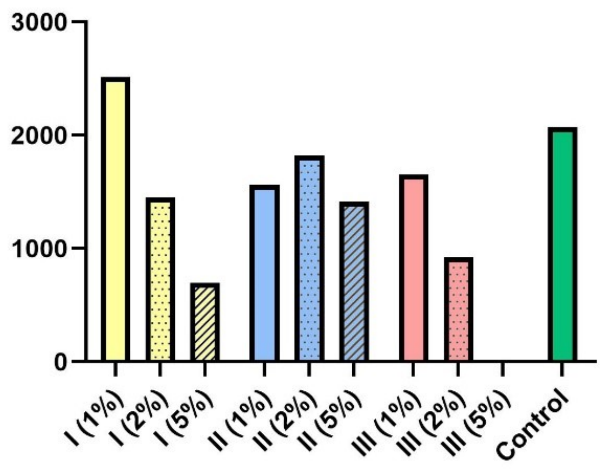

Figure 4. Vigour index of bean seedlings after treatment with hydrolysates I, II, III on the 21st day of germination.

Table 3. Maize weight after treatment with fish hydrolysates for 21 days, g (M ± m, n = 30).

Group	Above ground mass	Underground mass	Total weight
Control	2.54±1.27[a]	0.93±0.52[a]	3.47±1.61[a]
I (1%)	2.36±0.87[a]	0.73±0.33[a]	3.04±1.19[a]
I (2%)	2.48±0.46[a]	0.93±0.45[a]	3.41±0.83[a]
I (5%)	2.22±0.72[a]	1.03±0.38[a]	3.25±0.98[a]
II (1%)	2.42±0.87[a]	0.86±0.33[a]	3.28±0.81[a]
II (2%)	2.43±0.96[a]	0.87±0.37[a]	3.30±1.21[a]
II (5%)	2.68±1.13[a]	1.00±0.50[a]	3.68±1.62[a]
III (1%)	2.64±1.05[a]	0.82±0.35[a]	3.46±1.36[a]
III (2%)	2.58±1.19[a]	0.84±0.46[a]	3.42±1.57[a]
III (5%)	2.69±0.82[a]	0.87±0.26[a]	3.56±1.03[a]

The same letters indicate the absence of a significant difference between the groups at $P \leq 0.05$.

same time, a decrease in carotenoids, one of the functions of which is to protect the photosynthetic system, was observed in this group (Fig. 5 (C)). The content of flavonoids did not change significantly after treatment with hydrolysate III, but decreased under the influence of hydrolysate I 5% and increased under the influence of hydrolysate II 2% (Fig. 5 (D)).

All solutions at a concentration of 5% negatively affected the germination of beans, in group III 5% no seeds germinated at all, so biochemical studies in this group were not conducted. Hydrolysate III also had a destructive effect on chlorophyll at lower concentrations of 1 and 2% (Fig. 6 (A)). The content of chlorophyll *b* decreased only in the treatment with hydrolysate II 2% (Fig. 6 (B)), while the content of carotenoids tended to increase in all groups (Fig. 6 (C)). The content of flavonoids also did not differ from the control group, but a decrease in this indicator could be observed in group II 1% compared to group I 2% (Fig. 6 (D)).

The results showed that the treatment with a low concentration of fish hydrolysates was more optimal for beans, while concentrations of 2 and 5% suppressed or tended to suppress most of the measured parameters. When treated with hydrolysate III at a concentration of 5%, not a single bean seed germinated, indicating a very potent effect of this hydrolysate. In lower doses (1 and 2%), hydrolysate III also had a negative effect, in particular, seed germination decreased, chlorophyll a content decreased, and as a result, a decrease in the seedling vigour index and a tendency to reduce the aboveground and underground vegetative mass of beans almost twice when treated with 2% hydrolysate III solution. Solution III of 1% had the least negative effect on beans.

The treatment with hydrolysate II had a negative effect on the germination of bean seeds, chlorophyll b content, and a tendency to a dose-dependent decrease in the total bean weight was observed. The best effect on beans was obtained after treatment with hydrolysate I at a concentration of 1%, namely the highest values of the seedling vigour index, growth and total vegetative mass of beans. At the same time, the concentration of 5% for this hydrolysate was

Table 4. Weight of beans after treatment with fish hydrolysates for 21 days, g (M ± m, n = 15).

Group	Above ground mass	Underground mass	Total weight
Control	3.94±1.35[a]	0.30±0.11[ab]	4.24±1.41[a]
I (1%)	4.56±1.31[a]	0.48±0.31[a]	5.04±1.61[a]
I (2%)	3.08±1.64[a]	0.31±0.11[ab]	3.39±1.68[a]
I (5%)	3.83±0.84[a]	0.11±0.05[ab]	3.95±0.88[a]
II (1%)	3.97±1.19[a]	0.14±0.04[b]	4.11±1.17[a]
II (2%)	3.82±1.33[a]	0.15±0.10[b]	3.97±1.39[a]
II (5%)	3.04±1.82[a]	0.18±0.14[b]	3.23±1.93[a]
III (1%)	3.69±1.58[a]	0.24±0.17[ab]	3.93±1.74[a]
III (2%)	2.16±1.41[a]	0.11±0.04[b]	2.26±1.43[a]

The same letters indicate the absence of a significant difference between the groups at $P \leq 0.05$.

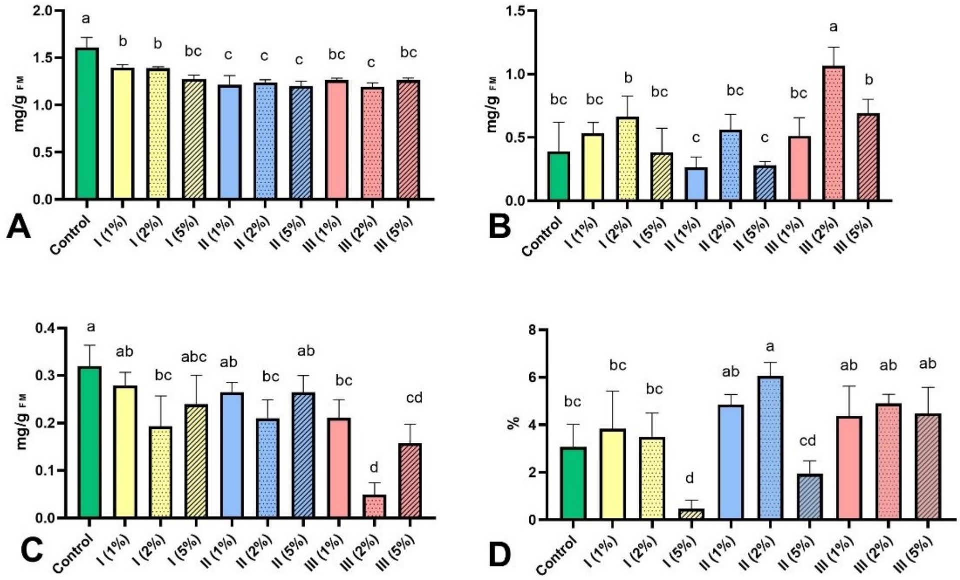

Figure 5. Pigment content in maize leaves after treatment with fish hydrolysates: (A) chlorophyll a; (B) chlorophyll b; (C) carotenoids; (D) total flavonoids (M ± m; N = 30).
The same letters indicate the absence of a significant difference between groups at $P \leq 0.05$

also too high for beans.
Treatment of maize seeds with hydrolysate I (2%) accelerated and synchronized the germination of viable seeds, while hydrolysates II (2%) and III (5%) stimulated the germination of seeds with low viability. Faster germination of seeds after treatment with hydrolysate I (2%) resulted in higher corn seedling length at the early stages of seed germination in this group, while after two weeks the groups did not differ significantly in this parameter. In general, hydrolysates had a lesser effect on the growth of maize seedlings, aboveground and underground plant mass, compared to the effect on beans. It was also found that higher

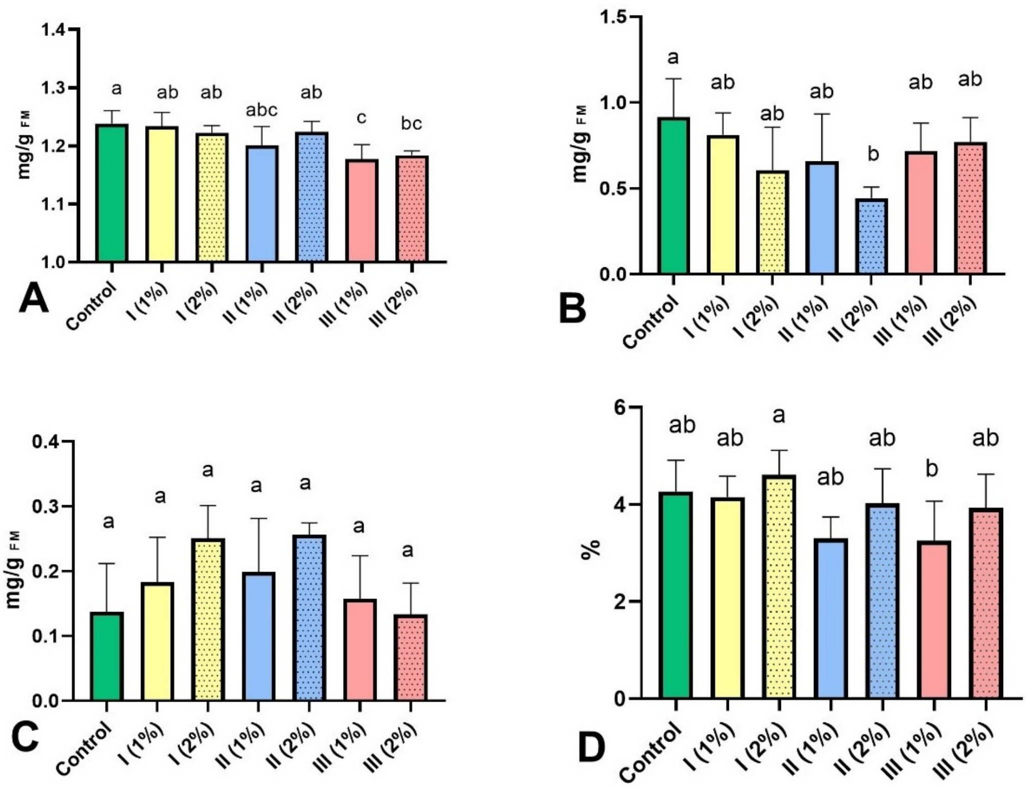

Figure 6. Pigment content in bean leaves after treatment with fish hydrolysates: (A) chlorophyll a; (B) chlorophyll b; (C) carotenoids; (D) flavonoids (M ± m; N = 30).
The same letters indicate the absence of a significant difference between the groups at $P \leq 0.05$

concentrations of hydrolysates (2% and 5%) were more effective for maize, while for beans, the treatment with hydrolysates at a concentration of 1% was optimal. This may be due to the thinner seed coat of beans and the presence of symbiotic interaction with nitrogen-fixing bacteria, which makes beans more susceptible to various external influences. It can also be noted that hydrolysate I had the mildest effect on the photosynthetic system of maize, while hydrolysates II and III had a greater negative impact. Similar results were also obtained for beans.

In general, for both cultures, hydrolysate I was the best. The raw material for this hydrolysate was only fish guts, compared to hydrolysates II and III, where the raw material was a mixture of guts:heads in a ratio of 1:3. Since the main factor of hydrolysis was endogenous enzymes contained in fish guts (proteases, chitinase, lipases, alkaline phosphatase, hyaluronidase, etc. (Venugopal, 2016)), it is obvious that the amount of these enzymes was much higher in the substrate containing only guts without heads. Hence, this could lead to more efficient breakdown of proteins into low molecular weight peptides, which, accordingly, facilitated their absorption by plants. This assumption is also confirmed by other researchers, namely, the average molecular weights of peptides were 600 Da in fish hydrolysates obtained from entrails and 947 Da in hydrolysates obtained from heads (Vázquez et al., 2020). It is also known from the literature that antioxidant activity was higher in the hydrolysis of fish entrails than heads, which may have a potential positive effect in stressful plant growing conditions (Vázquez et al., 2020). According to the literature, fish hydrolysates improve plant nutrient uptake, which is accompanied by better growth and development of roots and aboveground mass (Madende and Hayes, 2020; Sarkar et al., 2023). We observed an increase in above- and below-ground weight of beans only with a 1% solution of fish hydrolysate I, which is made from viscera only, while the increase in maize weight was not affected by the hydrolysates we obtained. This may be due to the positive effect of hydrolysates on nitrogen-fixing microbiota that forms a symbiosis with legumes. According to Caruso et al. (2020), fish hydrolysates can stimulate the growth and activity of beneficial microbes. At the same time, when using high doses of all hydrolysates, we observed inhibition of germination and growth of beans. This intensity of the hydrolysates' effect shows that they are more effective in lower concentrations for the treatment of pulses, which is also more cost-effective.

Hydrolysate III was less effective than hydrolysate I in growing corn and beans, on the one hand, it probably contained a smaller amount of low molecular weight peptides that are more easily absorbed by plants (Vázquez et al., 2020), on the other hand, this hydrolysate contains a large amount of calcium, as 3/4 of the bones were used as raw material (Kim and Mendis, 2006). Excess of calcium can cause chlorosis. It is known from the literature that both high and low levels of calcium inhibit chlorophyll formation in plants, with the effect being much more pronounced in the case of chlorophyll *a* than chlorophyll *b*. Calcium levels are believed to affect chlorophyll formation through control of the uptake of minerals required for chlorophyll biosynthesis and the control of membrane and cytoplasmic hydration (Pal and Laloraya, 1972). Our data confirmed a decrease in the amount of chlorophylls and carotenoids in both cultures after treatment with this hydrolysate, as well as a more destructive effect of fish hydrolysates on chlorophyll a rather than chlorophyll b. In addition, the literature reports a negative effect of low and high calcium concentrations on the uptake, distribution and use of nutrients (C, N, P, K) in plants (Weng et al., 2022). On the other hand, fluctuations in calcium ions have been found to be one of the main links in symbiotic signalling that occurs in the root hairs of legumes that form a root-nodule symbiosis, within the "nitrogen-fixing" clade (Lévy et al., 2004; Granqvist et al., 2015). Therefore, beans were more sensitive to high doses of hydrolysates II and especially III compared to maize. At high concentrations (5%), hydrolysate III inhibited the germination of beans altogether. According to the results of Xu and Mou (2017), there is an increase in the amount of chlorophylls in plants after treatment with fish hydrolysates. At the same time, the given composition of these hydrolysates contains a very small amount of calcium (Xu and Mou, 2017), which is more consistent with the composition of hydrolysate I studied by us.

Hydrolysate II had the greatest destructive effect on the pigments of the photosynthetic system when treated with both corn and beans. This is probably due to the fact that the substrate for its production was entrails and heads, which, as in the case of hydrolysate III, implies increased concentrations of calcium in the hydrolysate. On the other hand, nitric acid was added to stimulate peptide cleavage at the second stage of hydrolysate II production.

According to the literature, even the use of diluted HNO_3 to acidify the substrate did not have a negative effect on plant growth and development (Fornes and Belda, 2017), and nitrogen is absorbed by the plant more than any other element (Sharma et al., 2022), therefore, this acid was chosen for hydrolysis. The addition of acid probably caused a more efficient cleavage of peptides into low molecular weight fractions. Therefore, initially, the treatment with hydrolysate II stimulated the growth of beans, but then the growth slowed down because it also had a negative effect on the photosynthetic system of plants. Plant biostimulants obtained by hydrolysis of heads and guts were less effective than a biostimulant from guts alone due to the presence of excess phosphorus and calcium and lower concentration of nitrogen and low molecular weight compounds per unit volume.

The use of hydrolysate, which contains only entrails, allows additional use of bone waste from fish production in the form of bone meal, which can be used separately in larger dilutions.

4. Conclusion

The hydrolysis of fish waste without the additional introduction of exogenous enzymes is more cost-effective. The study of such hydrolysates showed that hydrolysates only from fish guts are more effective for plants as biostimulants than hydrolysates from entrails and bones. The effect of various fish hydrolysates on beans was significantly more pronounced than on corn. This is probably due to the effect of the hydrolysates on the microbiota that forms a root-nodule symbiosis with legumes.

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Authors contributions

Study conception and design: O. Tonkha; data collection: N. Nuzhyna, N. Raksha, T. Maievska; analysis and interpretation of results: O. Savchuk; draft manuscript preparation: N. Nuzhyna. Author. All authors reviewed the results and approved the final version of the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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