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Influence of Soil-application of Fish-protein Hydrolysate Liquid on Growth and Yield of Spinach (*Spinacia oleracea* L.)

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ABSTRACT

Background: Organic inputs, especially biostimulants, are gaining immense importance in enhancing crop yields. In the present study, the effect of soil-application of fish-protein hydrolysate (FPH) on the growth and yield of spinach was evaluated. The effect of various doses on the yield and correlation of root growth with yield was attempted to establish.

Methods: In the current experiment, spinach was grown in growing pots. Four different concentrations of the FPH liquid *viz.* 0.5 ml, 2 ml, 5 ml and 10 ml per plant were applied to each plant through soil application at the frequency of 8 days.

Result: The study revealed that amongst the various tested doses of 0.5 ml, 2 ml, 5 ml and 10 ml, the highest yield was observed with a 2 ml dose. Compared to untreated control plants, about a 40% increase in the yield was observed in the treatment with a 2 ml dose. The higher yield was associated with better root development. The higher doses of 5 ml and 10 ml did not result in a proportional increase in yields. On the contrary, these higher doses resulted in an adverse effect on the growth of roots and yield. So, the present study demonstrated the utility of FPH in increasing the yield of spinach in organic farming at the experimental level.

Key words: Amino acid, Biostimulant, Fish-protein hydrolysate, Spinach, Yield.

INTRODUCTION

Currently, we are in a race against the time with respect to healthy food availability to a growing population at a reasonable cost. The population of the world is growing fast while land under cultivation is decreasing rapidly owing to urbanization. In this crucial scenario, it is a necessity to obtain additional yields from the same existing land and water resources. This situation is putting tremendous pressure on growers to increase food production to feed to a rapidly growing population using available limited resources (Parant, 1990). To achieve this goal of increasing yield and protecting crops, chemical synthetic fertilizers, micronutrients and pesticides are being used excessively. Almost about a half-century ago, in the seminal work Rachel Carson narrated the first time the adverse effects of agrochemicals on the environment and human health in his book “Silent Spring” (Carson, 1962). Since then great concerns are being raised towards the fertilizer and pesticide residues that are found in soil, drinking water, food and even in mother’s milk. In this background, the last decade has seen a burgeoning interest in the use of natural organic residue-free inputs to increase yield and protect crops (Hamedani *et al*., 2020). To avoid the excessive use of chemical fertilizers, one of the successful smart strategies being adopted recently is the use of biological and botanical products such as biofertilizers and biostimulants. Various biofertilizers have been shown to benefit improving yield and quality in the case of crops such fenugreek (Godara *et al*., 2017), mung bean (*Vigna radiata* L) (Mondal *et al*., 2013; Bahadur and Tiwari, 2014), Pear (*Pyrus* spp.) (Kumar 2013), Chickpea (*Cicer arietinum* L.) (Anjali 2019) and Garden pea (*Pisum sativum* L.) (Kumar 2014). Yield and fruit quality enhancement were found in mango with the applications of organic amendments containing various biofertilizers (Ram *et al*., 2019). Along with biofertilizers, biostimulants are gaining tremendous popularity nowadays as organic inputs to substitute chemical fertilizers (Drobek *et al*., 2019). Biostimulant is a relatively new group of agro-inputs used for enhancing yield and quality of crops (Radkowski and Radkowska, 2013; Du Jardin, 2015; Hamedani *et al*., 2020). Biostimulants are broadly defined as organic materials when applied in small quantities to enhance plant growth and development such that the response cannot be attributed to the application of traditional plant nutrients (Yakhin *et al*., 2017).

Biostimulants mostly consist of marine-algae extracts (seaweed extracts), plant extracts (*Moringa olifera* extract), humic substances, vitamins, protein-hydrolysates (plant and animal-derived), chitin, chitosan, poly-and oligo-saccharides (Bulgari *et al*., 2019; Drobek *et al*., 2019; Zulfiqar *et al*., 2019; Rouphael and Colla, 2020). There are numerous examples of biostimulants demonstrating beneficial effects on crop yields and quality (Yakhin *et al*., 2017; Bulgari *et al*., 2019;

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Drobek *et al*., 2019; Parağiković *et al*., 2019). Recently, Balakumbahan and Kavitha (Balakumbahan and Kavitha, 2019) demonstrated the cost-benefit ratio of 1:2.45 in the case of annual moringa (*Moringa oleifera* Lam) Var. PKM - 1 with the application of a biostimulant mixture of humic acid, vermiwash and seaweed extract. Protein hydrolysate is one of the biostimulants having a positive effect on plant growth stimulation and enhancement of tolerance to biotic and abiotic stresses (Rouphael *et al*., 2020). Protein hydrolysates are a mixture of peptides and amino acids either animal or plant origins such as glutamate, glutamine, proline and glycine betaine and prepared by various methods like enzymatic, chemical, or thermal hydrolysis of a variety of animal and plant residues (Ertani *et al*., 2015; Grabowska *et al*., 2015; Halpern *et al*., 2015; Cristiano *et al*., 2018; Nurdiawati *et al*., 2019).

Spinach (*Spinacia oleracea* L.) is one of highly nutritive, nutritional and biological value vegetable from the family Amaranthaceae (Roberts and Moreau, 2016). Also, it is extremely rich in antioxidants when eaten fresh steamed or quickly boiled (Cho *et al*., 2008). Moreover, spinach is a great source of vitamins (A, C, E, K, B2, B6, B9, folic acid) and minerals (Mn, Mg, Fe, K, Ca, Se) (Kunicki *et al*., 2010). There have been reports of beneficial effects of protein hydrolysate on the growth and yield of several plants such as gold cherry tomatoes (*Solanum lycopersicum* L.) (Polo and Mata, 2018), potted Snapdragon (*Antirrhinum majus* L.) (Cristiano *et al*., 2018), lettuce (*Lactuca sativa*) (Xu and Mou, 2017), maize (*Zea mays* L.) (Ertani *et al*., 2009), Lily (de Lucia and Vecchietti, 2012), Papaya (*Carica papaya*) (Morales-Payan and Stall, 2003), (Halpern *et al*., 2015) and perennial wall rocket [*Diplotaxis tenuifolia* (L.) DC.] (Caruso *et al*., 2019). The commercially available amino acid-based biostimulant, Aminoplant (Siapton®), was evaluated for effect on the yield of spinach by foliar application. However, in this case, the foliar application showed no effect on spinach yield (Kunicki *et al*., 2010). In the current research paper, the effect of fish protein hydrolysate (FPH) on the overall yield and root growth is reported.

MATERIALS AND METHODS

The study was conducted during the period of October-November 2017. The seeds of spinach, *Spinacia oleracea* L. were obtained from Golden Seeds Corporation, Ahmedabad. Good quality Red soil was procured from the farm in Nelamangala taluka of the Bangalore district of Karnataka State in India. The obtained soil was mixed well with organic manure, which was obtained from Sonkul Agro Industries, Nashik. The major constituents of organic manure are given in (Table 1). The ratio of Soil:Manure was maintained at 4:1. The planting pots of the size of 35cm*20cm*65cm (Legth*Width*Height) made up of high-density polyethylene (HDP) (Fig 4) were used for growing the spinach plants. FPH liquid was obtained from the company named as Janatha Fish Meal and Oil Products based in village Kota of Udipi district of Karnataka state in India.

FPH liquid was analyzed by HPLC for the amino acid profile.

Experimental design

A total of 20 planting pots were used for this experiment. Each pot was filled with 40 Kg of the above-mentioned manure-fortified soil. The study was initiated by seeding one spinach seed in each planting pot. The study period was about 60days. In the current experiment four different concentrations of the FPH liquid *viz*. 0.5 ml, 2 ml, 5 ml and 10 ml per plant at the frequency of 8 days were used. For each concentration four replicates were maintained and also four planting pots were kept as the control that did not receive any FPH dose. In every application, 2 liters of water were used and the designated dose. In the case of the set of four pots of control received only water. The same dose was repeated at an interval of every 8 days for 45 days till spinach reached its full maturity stage. A total of 5 times the dose was given during the entire period of 45 days. Each treatment was replicated four times and analyzed using DMRT (Duncan's Median Range Test) at a 5 percent significance level.

After completion of 45 days, each replicate treatment-set and control were uprooted and washed carefully with water to remove soil from roots. The yield as the total weight of spinach shoot and root weight were recorded. Growth parameters were measured fresh shoot length, plant height, plant height with the longest root, Root length, longest root length, dry root length, total plant weight, Fresh shoot weight, Fresh root weight, Number of leaves.

RESULTS AND DISCUSSION

The effect of different concentrations of fish-protein hydrolysate liquid on growth and yield of spinach is discussed here. The results of the plant growth parameters are depicted in Table 2. The fresh shoot weight was found to be the highest in T_3 (216.50 g/plant) followed by T_2 (197.95 g/plant) and both the treatments were significant differences from each other. Fan et. al. also reported 1.6 times increase in shoot weight of spinach as the effect of the application of an extract of brown macroalga (*Ascophyllum nodosum*) (Fan *et al*., 2013). Although T_4 (168.35 g/plant) and T_5 (156.78 g/plant) performed better than the control, they were not significantly different from each other. The lowest fresh shoot weight was observed in control, T_1 (154.00 g/plant).

The fresh root weight was found to be significantly highest in the treatment T_3 (24.18 g/plant) that were followed by T_2 (21.13 g/plant). Similar positive effects of the application of protein hydrolysates on root development were noted in tomatoes (Colla *et al*., 2014). There was a significant difference between the shoot weight in T3 (216.50g/plant) and T2 (197.95 g/plant) being highest in T_3 (216.50 g/plant) with a 2ml dose. It is also observed from the study that there was no significant difference in the root weight with the treatments T_1 (18.75 g/plant) and T_4 (16.53 g/plant). The

Table 1: Components of organic manure.

Primary nutrients	N.P.K.
Secondary nutrients	Mg, Ca, S
Micro nutrients	Fe, Zn, Mn, Bo

lowest fresh root weight was observed in the treatment of T_5 (15.00 g/plant).

The highest total fresh weight was observed T_3 (240.68 g/plant) followed by T_2 (218.88 g/plant). There was a significant difference in the sample with treatments T_1 (172.75 g/plant), T_4 (184.88 g/plant) and T_5 (171.78 g/plant). The lowest total fresh weight was recorded in the treatment of T_5 (15.00 g/plant).

The above observations signify that treatment T_3 performed superior over all other treatments. According to one of the reports, the maximum benefits were reported at very low dosages (Ertani *et al*., 2014). The study also reveals that plant growth parameters increasing with the concentration from 0.5 ml dosage to 2.0 ml and then further increase in the concentration decreases the plant growth parameters. In some parameters, it was observed that the highest dosage (10.0 ml dosage) underperformed as compared to control and it might have had a deleterious effect on the plant.

Effect on shoot weight (yield)

The average fresh shoot weight of the control group was 154g. When the control was compared with treatment groups (Fig 1) in all the cases there is a positive effect of the application of FPH on spinach yield except the highest dose of 10 ml per lit per plant. Dose proportionality, i.e. increase in the yield with an increase in the dose, was observed in the case of 0.5 ml and 2.0 ml doses. When the dose was enhanced from 2.0 ml to 5.0 ml, rather than increase, the decrease in the yield was observed, however, the yield (168.35 g) was more than the yield observed in the control-set. Recently many studies have reported the increase in yield with the use of organic inputs. One of such interesting study noted enhancement of yield and grain quality of winter wheat with applications of amino acid-based biostimulants (Popko *et al*., 2018). Another study showed an increase in the yield of greenhouse spinach by the application of plant and seaweed-based biostimulants (Rouphael *et al*., 2018). Also, protein hydrolysate-based biostimulant boosted the yield and nitrogen use efficiency in greenhouse spinach (Carillo *et al*., 2019). The increases in growth, leaf mineral composition, yield and fruit quality were reported in two greenhouse tomato cultivars as a result of foliar applications of legume-derived protein hydrolysate (Rouphael *et al*., 2017). In our study, a further increase in the dose to 10 ml resulted in bringing down the yield (156.78g) almost equal to the control group (154.00g). Thus, in the case of a 10 ml dose, the effect of the application of biostimulant FPH was not visible when compared to the control yield. Overall, it is concluded from these results that higher doses of 5 ml and 10 ml do not result in the dose-proportional increase in yield. The optimal dose at which the highest yield was obtained is 2 ml per lit. The percent increase in the yield (shoot weight) was calculated taking the yield of the control group (154.00g) as a base. The formula used to calculate the percent

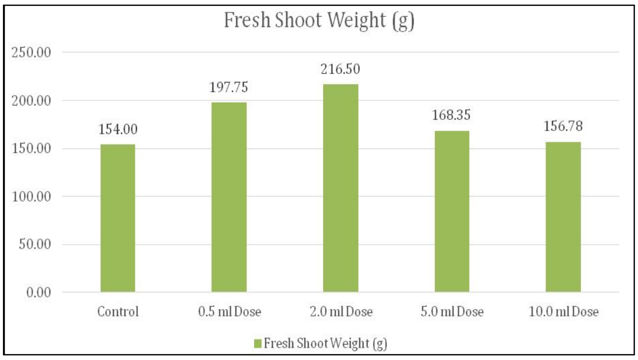

Fig 1: Effect of different concentrations of fish-protein hydrolysate liquid on fresh shoot weight of the spinach.

Table 2: Effect of different concentrations of fish-protein hydrolysate liquid on growth parameters of Spinach.

Treatment details	Plant growth parameters		
	Fresh shoot weight (g/plant)	Fresh root weight (g/plant)	Total fresh weight (g/plant)
T_1-Control	154.00[b]	18.75[bc]	172.75[b]
T_2-0.5 ml Dosage	197.95[a]	21.13[ab]	218.88[a]
T_3-2.0 ml Dosage	216.50[a]	24.18[a]	240.68[a]
T_4-5.0 ml Dosage	168.35[b]	16.53[bc]	184.88[b]
T_5-10.0 ml Dosage	156.78[b]	15.00[c]	171.78[b]
SE.m±	4.23	0.78	4.33
CD @ 5%	25.51	4.71	26.12

Table 3: Effect of different concentrations of fish-protein hydrolysate liquid on plant growth parameters.

Dose	Fresh shoot weight (g)	Increase in shoot weight (g)	Increase in % shoot weight	Fresh root weight (g)	Increase in root weight (g)	% Increase in root weight
Control	154.00	NA	NA	18.75	NA	NA
0.5 ml Dose	197.75	43.75	28.4	21.13	2.38	12.7
2.0 ml Dose	216.50	62.50	40.6	24.18	5.43	28.9
5.0 ml Dose	168.35	14.35	9.3	16.53	-2.23	11.9
10.0 ml Dose	156.78	2.78	1.8	15.00	-3.75	20.0

increase in the yield was: Percent increase in yield with specific dose = [(Yield obtained with respective dose - Yield obtained in the control set)/ Yield obtained in the control set] *100. The percent increase has been tabulated in the Table 3. It was observed that the application of 0.5 ml dose resulted in 28.4% and in the case of 2 ml dose the yield increased to the tune of 40.6%. This increase in yield of 40.6% was the highest among all concentrations tested. In the case of a 5.0 ml dose, the percent increase in yield observed was 9.3%. This implies that when the dose was increased 2.5 times from 2ml to 5 ml, it did not result in increase in yield but contrarily resulted in drop in the yield. When compared to 2 ml dose, the yield obtained from 5 ml dose was about 77% less. In the case of the highest dose of 10 ml the yield obtained was almost similar to what was observed for the control. There was no significant increase in the yield. It can be concluded from the yields obtained from the two higher doses of 5 ml and 10 ml that use of optimal quantity of FPH is very crucial to obtain desired results. The excess use of FPH results in an adverse effect on growth. This can be further looked into details when we correlate the growth of roots with different doses (Fig 3).

Effect on the root growth and correlation with yield

Healthy roots are very important for the healthy growth of the plants. There are several reports available highlighting the role of protein hydrolysate in enhancing shoot and root biomass and productivity (Lisiecka *et al*., 2011; Paradiković *et al*., 2011; Colla *et al*., 2014; Nardi *et al*., 2016; Ertani *et al*., 2018). In this study, we uprooted the plants very carefully so that roots will not be cut or lost during the uprooting process. Then separated roots (Fig 5) from the shoot and noted down the weight and depth (length, not reported here) of the fresh-roots. The weights of the fresh-roots ranged from 15g to 24.18g (Fig 2). In the control group, a fresh-root weight of 18.75g was observed and it increased in the case of application of 0.5ml and 2.0 ml dose respectively to 21.13g and 24.18g. However, the doses of 5 ml and 10 ml did not impact the growth of roots positively. In both cases of 5 ml and 10 ml resulted in a slight decrease in the fresh-root weights respectively to 16.53g and 15.00gm compared to the control group (18.75 g). To correlate the effect of root growth on the overall yield of spinach. The percent change in root weight with respect to control using a formula similar to that used for calculating percent change in yield was calculated.

To correlate root weight and yield, Fig 3 depicted both the percent change in root weight and yield. This correlation (Fig 3) revealed that the highest percent increase in the yield (2.0ml dose; 40.6% increase) was observed where the

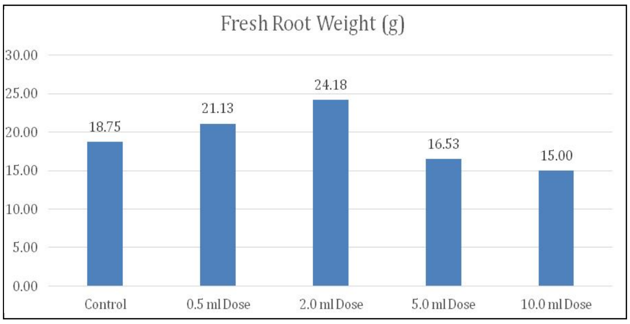

Fig 2: Effect of different concentrations of fish-protein hydrolysate liquid on fresh weight of the spinach roots.

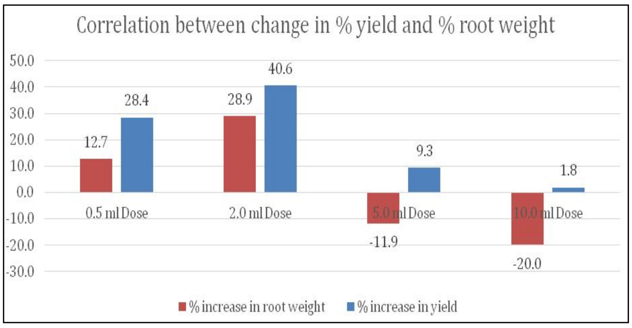

Fig 3: Co-relation between root weight and shoot weight of Spinach.

Fig 4: Pictures of shoots.

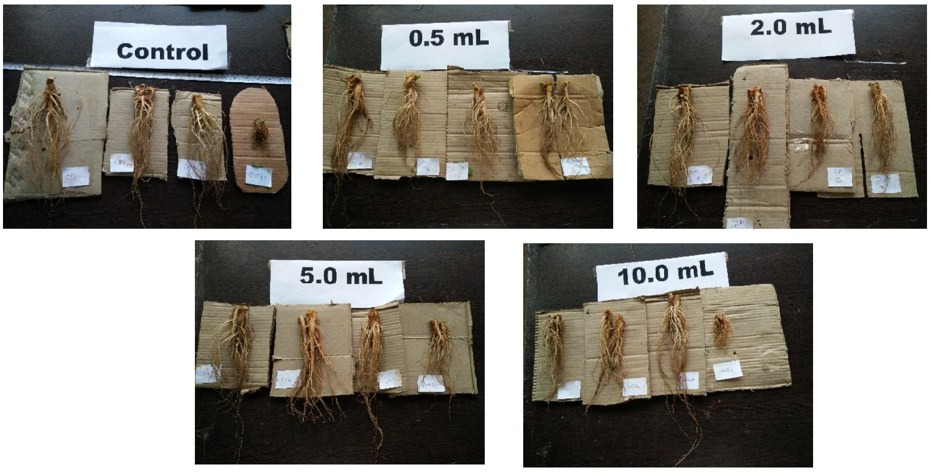

Fig 5: Pictures of roots.

root-weight was highest (24.18g). Also, an increase of 28.4% yield in the case of 0.5 ml dose was associated with a 12.7% increase in root-weight as compared to control. Furthermore, this study of correlating root growth with yield revealed that one of the reasons for the decrease in yield with the increase of the doses to 5 ml and 10 ml was because of the adverse effect of these higher doses on the root growth. In both cases of 5 ml and 10 ml root weights observed, respectively 16.53g and 15.00 g, were less than the root weight of control (18.75g). When viewed as a percent change in root weights, 5.0 ml and 10 ml dose exhibited respectively 11.9% and 20% lower weights with respect to control. Despite the lowering of root weight, the dose of 5.0 ml was able to maintain an increase in the yield to the tune of 9.3%. However, in the case of the highest dose,10 ml, the beneficial effect of FPH was not observed on the yield and the adverse effect on root growth was further profound. Similar phytotoxic and growth retarding effects were also reported in the case of repeated application of animal-derived protein hydrolysate (Cerdán *et al*., 2009; Lisiecka *et al*., 2011). Additionally, leaf chlorosis in basil was observed by foliar spray of animal-derived protein-hydrolysate at several trials carried out at the University of Tuscia-Italy (Colla *et al*., 2014). This reveals that optimal dose is important to attain full benefits of the application of biostimulants. The excessive dose may lead to retarded growth of roots and thus will not benefit plants for maintaining healthy growth. Visual observations in the research also revealed that the leaf size of the plants in 0.5 ml and 2 ml dose were bigger than that of the other doses.

CONCLUSION

It can be concluded that with the proper dosage of FPH the yield of the spinach can be increased up to 40%. Also, the overdose of the biostimulant FPH is not helpful so the farmers should not use the excess of doses of the biostimulants. The 40 percent increase in growth is very significant and will add significant value to the national goal of doubling farmers' income. FPH being derived from the fish is one of the environment-friendly inputs and will not harm soil health. Animal-derived PHs can be used in conventional and organic farming without posing harm to human health and the environment.

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