



Efficacy of Organic Biostimulant (Fish Protein Hydrolyzate) on the Growth and Yield of Tomato (*Solanum lycopersicum*)

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ABSTRACT

Background: Organic agricultural inputs are getting immense importance for enhancing growth, yield and nutritional value of the crop. The aim of this experiment was to evaluate the effect of soil application of different doses of fish protein hydrolysates (FPH) on the growth parameters of the tomato (*Solanum lycopersicum*) plant.

Methods: FPH treatment were applied through soil application in three different doses such as 0.5 mL, 2.0 mL and 5.0 mL diluted into 2 liters of water and one control group was maintained that did not receive any FPH but only equal amount of water. The growth parameters in terms of fruit yield, shoot morphology and root morphology were studied. The 0.5 ml dose resulted in the 48% increase in the yield to tomatoes. The dose proportional linear increase in the tomato yield was not observed. All the doses of FPH showed increase in the fresh shoot weight as compared to control, however differentiation with different doses was not profound.

Result: The optimal dose of 0.5 ml showed significantly positive effect on the root morphology. However, the highest dose of 5.0 ml resulted in the negative effect on the root development. This research work demonstrated that optimal dose of the organic biostimulant FPH can help to increase the yield of tomato.

Key words: Biostimulant, Fish protein hydrolysate, Growth, Tomato, Yield.

INTRODUCTION

According to the World Population Prospects 2019 published by Department of Economic and Social Affairs of United Nations, the global population could increase to 850 crores by 2030. (Nations, 2019). Even though the population is growing, land and water resources that produce the food will remain same or shrink further because of urbanization and industrialization. Chemical fertilizers and pesticides have been traditionally employed to increase crop yields from constantly shrinking resources. However along with assisting to increase food production, this approach of chemical intervention has resulted in some deteriorating effects on environment and human health (Soppelsa *et al.*, 2019). The alternative sustainable approach that is being recently used and becoming popular is to use non-synthetic naturally derived organic agricultural inputs to increase growth and protect plants. Among the various organic inputs, use of organic biostimulants is considered to be the most innovative and sustainable approach to enhance crop yield and quality (Povero *et al.*, 2016). Biostimulants are natural substances, other than fertilizer and pesticides, which are able to promote not only growth and yield but also produce quality food when applied at lower quantities (Parađikoviæ *et al.*, 2019). Biostimulants include natural substances like humic acid, protein hydrolysates, seaweed extracts, beneficial microorganisms belonging to the genera *Azospirillum*, *Azotobacter* and *Rhizobium* spp. (Wozniak *et al.*, 2020) and plant growth promoting rhizobacteria (PGPR) including species of *Pseudomonas*, *Xanthomonas* *Bacillus* *etc* (Khalid *et al.*, 2004). One of the most important class of the biostimulants is the protein hydrolysates (PHs). PHs

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content polypeptides, oligopeptides and amino acids that are prepared by partial hydrolysis of animal or plant origin proteins (Colla *et al.*, 2014). Plant-origin PHs are mostly obtained from legume seeds, alfalfa hay, corn wet milling, vegetable by-products (Colla *et al.*, 2012). Animal-origin protein hydrolysates are mainly derived from leather by-products, blood meal, fish by-products, chicken feathers and casein (Casadesús *et al.*, 2019). Elaborative mechanisms of action and target metabolic pathways exhibited by the PHs as biostimulants is not completely understood. However, in several cases, it is shown that biostimulant activity of PHs mainly arises from the modulation of plant molecular and physiological processes. These modulations in turn trigger growth, increase yield and reduce the impact of abiotic stress on crops (Calvo *et al.*, 2014; Yakhin *et al.*, 2017). PHs show direct and indirect effects on the growth of plants. The direct effects include stimulations of carbon metabolism, nitrogen metabolism, regulation of nitrogen

uptake and regulation of activity of three enzymes involved in the tricarboxylic acid cycle (citrate synthase, isocitrate dehydrogenase and malate dehydrogenase) (Colla *et al.*, 2015; Du Jardin, 2015; Nardi *et al.*, 2016). Also, due to presence of bioactive peptides PHs have also shown hormone like activities enhancing root and shoot growth and crop yield (Ertani *et al.*, 2009; Matsumiya and Kubo, 2011; Colla *et al.*, 2014; Lucini *et al.*, 2015). Indirect effect of foliar and root application of PHs show the improvement in the uptake and utilization of macro and micronutrients (Halpern *et al.*, 2015). The biostimulant action of PHs also depends upon their origin, species, cultivars, phenological stages, growing conditions, concentration, time and mode of application, solubility and leaf permeability (Colla *et al.*, 2016). Among the PHs derived from different sources of animal and plant proteins, FPHs are very effective biostimulants. FPH has shown positive effect on the seed vigour property, nutritional value, bioactive compounds and antioxidant activity of ricegrass (Rattanapon *et al.*, 2016) and on lettuce (*Lactuca sativa*) (Xu and Mou, 2017). In the Solanaceae family after potato (*Solanum tuberosum* L.), second most important fruit or vegetable ubiquitously consumed is the tomato (*Solanum lycopersicum* L.). In 2018, approximately 18.23 Crore tons of tomato fruits were produced on 47.62 lakh hectare globally (FAOSTAT, 2019). Tomato is rich in several phytochemicals that are involved in prevention of cancer. These phytochemicals include carotenoids, polyphenols and vitamins (Raiola *et al.*, 2014; Martí *et al.*, 2016; Li *et al.*, 2018). There are several scientific reports to enhance nutritional quality and yield of the tomato fruits using seaweed extracts (Hernández-Herrera *et al.*, 2014; Di Stasio *et al.*, 2018; Kavipriya and Boominathan, 2018), however, the potential of fish protein hydrolysates (FPHs) has been less explored towards the enhancement of growth and nutritional quality of the tomato fruit. In the current research work, we are evaluating the effect of soil-application of FPHs on the growth and yield on hybrid S-22 variety of tomato.

MATERIALS AND METHODS

The study plan was executed at VHD Institute of Home Science, Bangalore University and conducted at Peenya Industrial Area, Bangalore. The Study was conducted during the period of July 2019 to November 2019. The seeds of tomato (*Solanum lycopersicum*) hybrid variety S-22 were obtained from Golden Seeds Corporation, Ahmedabad. Good quality Red soil was procured from a farm in Nelamangala taluka of the Bangalore district of Karnataka, India. The obtained soil was mixed well with organic manure, which was procured from Sonkul Agro Industries, Nashik. The ratio of Soil:Manure was maintained at 4:1. The planting pots of the size of 35 cm × 20 cm × 65 cm (length × width × height) made up of high-density polyethylene (HDP) were used for growing the tomato plants. FPH liquid was obtained from the company named as Janatha Fish Meal and Oil

Products based in Kota village of Udipi district in Karnataka state, India. The used FPH contains 18 different amino acids amounting to total minimum concentration of 20% w/v.

Experimental design

Total 20 planting pots were used for this experiment. Each pot was filled with 40 kg of the above-mentioned manure-fortified soil. The manure contained 1.5% nitrogen, 25% moisture and was having particle size of 4 mm. The above-mentioned seeds were used to obtain the seedlings. The seeds were planted in polyethylene bags and allowed to grow with watering once in two days. When the plants had an average height between 10 and 15 cm, then these plants were transplanted from bags to HDP-pots as one plant per pot. Each HDP-pot contained the mixture of soil and manure in the ratio of 4:1. There were 4 different treatment groups. One group was the control group which did not receive any FPH and other 3 groups were treatment groups that received FPH in three different doses through soil application viz. 0.5 mL, 2.0 mL and 5.0 mL per plant at the interval of 8 days. The specified dose was diluted in 2 liters of water for soil application. The control group received equal amount of water without FPH. In each of the four groups, 5 replicates were maintained. The treatments were continued at the interval of 8 days for 80 days completing 10 applications. After 53 days ripened tomatoes were harvested every alternate 4 days till plants reach its full maturity. These tomatoes were counted, weighed and kept for room temperature. After completion of 80 days plants were uprooted carefully, washed with running water to remove soil from roots. The physical parameters such as plant height, root length, plant weight; root weight, total number of tomatoes and total weight of tomatoes were recorded.

Statistical analysis

The results of the study were subjected to statistical analysis using the DMRT (Duncan's multiple range test) at 5 per cent significance level.

RESULTS AND DISCUSSION

Total yield

Total yield of tomato was enhanced by application of FPHs as depicted in (Table 1). As compared to the control set that did not receive any amount of FPHs, in all the treatment experiments, we observed increase in the overall yield. Similar growth enhancing effect was reported when legume-derived PH was used in greenhouse tomato (Rouphael *et al.*, 2017). Also in the case of Potted Kiwifruit (*Actinidia deliciosa*) plants, protein hydrolysates showed positive effect on the growth (Quartieri *et al.*, 2002). The application of chicken feather protein hydrolysate to the seedlings of wheat (*Triticum aestivum* L. 'Altindane' and 'Bezostaya'), resulted in the enhanced growth parameters (Genç and Atici, 2019). Additionally several type of protein hydrolysates have been recently reported to have similar enhancement in growth parameters of various plants such as perennial wall rocket

Table 1: Effect of different concentrations of fish-protein hydrolysate liquid on growth parameters of tomato.

Treatment	Fruit (Yield)					Shoot morphology					Root morphology				
	Total weight		Number of tomatoes		Average weight per tomato	Shoot height		Fresh shoot weight		Shoot dry mass	Root length		Fresh root weight		Root dry mass
	g/plant	% change	no./plant	% change	g/plant	cm	% change	g/plant	% change	g/plant	cm	% change	g/plant	% change	g/plant
Control (T1)	1307.6 ^c	NA	61.8 ^c	NA	21.4 ^d	42.8 ^b	NA	445.6 ^c	NA	119.4 ^b	27.8 ^b	NA	31.4 ^b	NA	5.2 ^b
0.5 mL (T2)	1928.6 ^a	47.5	75.0 ^{ab}	21.4	25.6 ^b	63.0 ^a	47.2	600.2 ^{ab}	34.7	153.6 ^a	38.6 ^a	38.8	59.2 ^a	88.5	12.4 ^a
2.0 mL (T3)	1820.4 ^{ab}	39.2	84.0 ^a	35.9	21.4 ^a	66.4 ^a	55.1	679.6 ^a	13.2	166.2 ^a	33.4 ^{ab}	20.1	38.4 ^b	22.3	10.4 ^a
5.0 mL (T4)	1554.2 ^{bc}	18.9	69.2 ^{bc}	12.0	22.2 ^c	62.0 ^a	44.9	530.6 ^{bc}	19.1	134.4 ^b	25.6 ^c	-7.9	28.8 ^c	-8.3	6.6 ^b
SEM±	52.22		1.50		0.90	1.57		15.88		2.45	1.09		1.40		0.49
CD @ 5%	350.12		10.40		6.04	106.51		16.44		16.44	7.36		9.42		3.29
CV	15.80		10.70		0.80	14.08		8.55		8.55	17.51		17.81		28.37

CD: Critical difference; CV: Coefficient of variation; SEM: Standard error of mean. Similar average followed by the same letter within columns is not significantly different.

[*Diplotaxis tenuifolia* (L.) DC.] (Caruso *et al.*, 2019), patchouli (*Pogostemon cablin* Benth) and mung bean (*Vigna radiata*) (Nurdiawati *et al.*, 2019) and spinach (Carillo *et al.*, 2019). The highest mean total yield (1928.6 g/plant) (Table 1) was noted in the treatment T2 (0.5 ml dose). In the cases of higher doses of 2.0 ml (T3) and 5.0 ml (T4) (2 ml and 5 ml dose) the total yields obtained were higher than the control but were lower than the 0.5 ml dose. This observation indicates that the increase in the doses did not linearly increase the yield. When treatments T2 and T3 are compared, the dose was increased 2.5 times however this increase did not result in growth enhancement. The percent increase in the total yield was calculated for each treatment, taking yield of the control group as a base. The formula used to calculate the percent increase in the total yield was:

Percent increase in yield with specific dose =

$$\frac{\text{Yield obtained with that dose} - \text{Yield obtained in the control set}}{\text{Yield obtained in the control set}} \times 100$$

With this formula we were able to evaluate the quantitative comparative impact of the application of FPHs on the total yield. In the terms of percentage increase the T2 treatment (0.5 ml) exhibited 47.5% increase over the control. This was followed by treatments T3 (2.0 ml) and T4 (5.0 ml) showing 39.2% and 18.9% increase, respectively.

Effect on total number of tomatoes

The positive effect of application of different biostimulants on number of tomatoes per plant has been reported in the literature. Zodape *et al.* (2011) studies the effect *Kappaphycus alvarezii* sap (seaweed) on the growth parameters of tomato plant. They attributed the increase in the yield of tomatoes to increase in number of tomatoes per plant (Zodape *et al.*, 2011). In the present experiment with application of FPHs, we noted that total numbers of tomatoes per plant were increased as compared to control in the case of 0.5 ml and 2.0 ml significantly however, in the case of dose 5.0 ml marginal increase was observed. The treatment T3 (2.0 ml dose) yielded the highest number of tomatoes per plant (84 numbers/plant) even though the total weight of obtained tomatoes was not the highest in this dose. This shows that numbers of tomatoes were more in this treatment T3 but the average weight and size of tomatoes may be less than the treatment T2 where we observed the highest yield. In the case of treatment T3 (2.0 ml) and T4 (5.0 ml) total number of average tomatoes per plant were 84.0 and 69.2 respectively. Thus, here we can attribute the increase in the yield observed not only to the enhanced fruit bearing per plant but also to the increased average weight of tomato obtained. In alignment with the observed yield, control group showed the lowest average number of tomatoes per plant (61.8 no./plant). When we compare the fruit bearing of highest yielding dose (0.5 ml; 75 tomatoes/plant) with control (T4), it can be concluded that the 0.5 ml dose has resulted in the nearly 21.4% increase in the fruit bearing. The findings

observed the similar positive effect of application of standardized extract of *Ascophyllum nodosum* on the fruit bearing in the case of eggplant (*Solanum melongena* L.) (Pohl *et al.*, 2019). Compared to the untreated group, in the case of 2.0 ml dose and 5.0 ml dose about 35.9% and 12.0% increase in the fruit bearing was observed respectively. The highest fruit bearing observed in the case treatment T3 did not result in increase in the highest yield, indicating that even though the fruit bearing was increased to greater extent, the weight per tomato was of lesser magnitude and hence has not resulted in the highest yield. We also calculated the average weight per tomato by dividing the total weight of tomatoes obtained by the total number of tomatoes obtained from that plant. We observed that among control, T3 and T4 there was no significant difference in the weight per tomato. However, in the case of high-yielding treatment T2, the weight per tomato was observed to be improved to the tune of 31.5% more than control. This interestingly indicates that treatment T2 not only enhanced the yield but also increased quality of the individual fruit in terms of weight per tomato.

Effect on shoot morphology

Moringa Leaf Extract has shown positive effect on the shoot morphology of the tomato plant (Yasmeen *et al.*, 2014). Also, Ronga *et al.* reported the increase in vegetative vigor of tomato plant with application of different biostimulants in their study (Ronga *et al.*, 2019). When the effect of different treatments of FPH was observed on the plant height in our experiment, it was noted that FPH exerted growth-enhancing effect on the height of tomato plants but in the case of all treatments plant height was almost similar to each other. The FPHs treatments have resulted in increase in height to the extent of 44.9 to 55.1% increase as compared to control, but there was no significant co-relation among different doses and increase in height. To study the shoot morphology further, we recorded the weight of the total shoot after removing root part. In this case of shoot weight, it was seen that different treatments resulted in increase in shoot weight ranging from 19.1% to 34.7%. The treatment T3 exhibited the highest fresh shoot weight (679.6 g/plant), followed by treatment T2 (600.2 g/plant) and then treatment T4 (530.6 g/plant). All the doses exhibited increase in shoot weight as compared to control indicating that application of FPH has helped in vegetative growth. Similar shoot growth and biomass increments were reported by application of protein hydrolysate in the case of potted Kiwifruit by (Quartieri *et al.* 2002). From comparison of the results of treatments T2 and T3 it can be concluded that highest vegetative growth did not turned to be highest yield of tomatoes. So optimal dosing of FPHs seems to be crucial to drive the growth towards highest fruit yield. To evaluate the dry mass of the shoot, we dried the shoots to the constant weight. The dry mass recordings showed that the trend in increase was similar to the trend seen for the fresh shoot weight. Treatment T3 showed the highest shoot dry mass (166.2 g/plant), followed

by treatment T2 (153.6 g/plant) and T4 (134.4 g/plant). The control group (T1) showed the lowest shoot dry mass (119.4g/plant). The overall results indicate that application of FPHs needs to be optimized to drive the results towards maximizing the yield *i.e.* quantity of tomatoes and better vegetative growth may not be the sole indication to obtain better yields.

Effect on root Morphology

The effect of application of FPH on the root morphology was studied in the terms of root length; fresh root weight and root dry mass. The positive effect on the root development with the application of enzyme hydrolyzed animal protein and seaweed extract in the case of gold cherry tomatoes is reported (Polo and Mata 2018). Also alfalfa hydrolysate and meat-flour hydrolyzate have shown the growth enhancing effect on the roots in the case of corn (*Zea mays*) seedlings (Ertani *et al.*, 2009). In the current experiment, the maximum average root length was observed in the case of treatment T2 (38.6 cm) followed by T3 (33.4 cm), T1 (27.8cm) and T4 (25.6 cm). The treatment T2, (0.5 ml dose) and T3 (2.0 ml dose), resulted in the increase in the root length to the magnitude of 38.8% and 20.1% respectively. However, the highest dose, T4 (5.0 mL dose) caused negative effect on the length of the roots (-7.9%) as compared to the control. The effect of application of FPH on the volume of the roots in terms of the fresh root weight revealed that treatment T2 resulted in the highest fresh root weight (59.2g) followed by treatment T3 (38.4 g), T1 (31.4g) and the minimum weight in the case of highest dose treatment T4 (28.8 g). In the terms of percent increase as compared to control the dose 0.5 ml showed very profound effect on the root growth in terms fresh weight. The 88.5 % increase in the fresh weight was observed as compared to control. From this observation it seems that root development has direct correlation with increase in production. The root part was allowed to dry to constant weight and root dry mass was recorded. It was found the dry biomass found to be in congruence with the fresh root weight. The highest root dry mass (12.4 g) was seen in the case of treatment T2, followed by T3 (10.4 g), T4 (6.6 g) and control group (5.2 g). In terms of the percent increase in the biomass treatment T2 showed 138.5% increase in the root dry mass followed by 100% in the case of treatment T3. These observations overall reveal that highest dose had adverse effect on the root development and was not beneficial. Moreover, the dose 0.5 ml was helpful to obtain the best root development.

CONCLUSION

This research work has clearly demonstrated that soil application of the optimal dose of FPH is beneficial to increase the yield of tomato in organic manner. The maximum increase in the yield obtained with 0.5 ml dose can be correlated to the profound effect on the root development. The vegetative growth was found to be

increased in all the treatment groups as compared to control however the magnitude of increase with respect to different doses was not differentiable. These results should encourage the larger field trials and ultimately the use of FPH as organic input to increase the tomato yield. Also, further experiments would be undertaken to evaluate the effect of application of FPH on nutritional parameters of the tomato.

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Authors contribution

Sheetal P. Dewang has conducted the experiments under the guidance and supervision of Dr. Usha Devi C.

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