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Growth promotion effects of humic acids isolated from co-compost prepared using fish processing waste and sugarcane bagasse on seeds of Fabaceae family

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ABSTRACT

Humic acids act as growth promoting agents to enhance plant growth and are considered as vital organic input to increase agricultural productivity. The present study was undertaken to evaluate the growth promotion effects of humic acids isolated from fish processing waste and sugarcane bagasse co-compost on seeds belonging to Fabaceae family. Various concentrations of humic acids ranging from 0.02-0.1% was treated to the seeds and growth parameters such as root length, shoot length and seed vigour index were recorded. Among the four seeds tested, maximum root length was observed in *V.radiata* (11±1.61 cm) and *V.mungo* (10.3 ± 0.35 cm) treated with 0.02 % of humic acids. Similarly, maximum shoot length was observed in *V. radiata* (14.1 ± 2.02 cm) at 0.02 % concentration of humic acids. High concentration of humic acids resulted reduction in the growth of the seedlings. Maximum shoot length (9 ± 0.79 cm) was recorded in *Vigna mungo* treated with high concentration (0.1%) of humic acids. Highest SVI (2510) was identified in *V.radiata* seeds treated with 0.02% concentration of humic acids. The preliminary study results identified that treatment of humic acids promoted the growth of *V.radiata* and *V.mungo* seedlings whereas less growth response was observed in *Vigna unguiculata* and *Cicer arietinum* seedlings. Based on the findings of the study, it was concluded that humic acids derived from co-compost produced using biological waste such as fish processing waste and sugarcane bagasse could be used as valuable organic growth enhancing agent to promote plant growth and crop productivity.

Key words : *Humic acids, Co-compost, Seed germination, Growth parameters, Vigna radiata*

Introduction

Humic acids are vital organic substances with multifunctional role in assisting soil chemistry, fertility and plant metabolic activities. The beneficial activity of humic acids is of great importance in agriculture to improve soil fertility, plant growth and crop yield. Humic acids are involved in the nutrient uptake in plants and most importantly for the transport and availability of micronutrients (Bohme and Thi Lua, 1997; Haghighi *et al.,* 2014). They also serve as a main source of macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) (Panuccio *et al.,* 2001). They are reported to enhance seed germination, crop yields, physico-chemical characteristics and directly or indirectly stimulate absorption of nutrients by roots (Nardi *et al.*, 2002; Nikbakht *et al.* 2008). Furthermore, humic acids enhances the absorption of micro-and macro-elements in plants (Varanini and Pinton, 1995). They act as biostimulants to increase the bioavailability of nutrients via reformation of the soil environment at the roots (Chen *et al.,* 2004). Beside, humic acids assist physiological process of plants by stimulating cell growth and nutrient uptake by forming soluble complexes with ions (Pinton *et al.,* 1999). Also, they acts as inducer of plasma membrane H^+-ATPase synthesis for stimulating cell energy (Canellas *et al.,* 2002). The main physiological effect of humic acids in plants is stimulation of root growth (Zandonadi *et al.,* 2007). Notably, humic acids stimulates lateral root emergence and root hair initiation that are involved in the nutrient uptake of plants (Puglisi *et al.,* 2013; Canellas and Olivares., 2014).

The plant growth promotion effects of humic acids could be postulated by two ways of action (i) direct action by its hormonal effects on plant growth (ii) indirect action through metabolism of microorganisms and promoting uptake of soil nutrients, and effects on seed germination, seedling and root growth and shoot development (Cacco and Dell'Agnolla, 1984; Chen and Aviad, 1990; Piccolo *et al.,* 1992). Several research findings have reported that humic acids indirectly assist several soil functions such as aeration, soil aggregation, microbial growth, mineralization of organic matter, water holding capacity and transport of macro and micronutrients (Sharif *et al.,* 2002; Saruhan *et al.,* 2011) and directly promotes plant cellular functions such as photosynthesis and respiration rates (Nardi *et al.,* 2002). Application of humic acids to sandy soils helps to improve water retention for improving root growth and prevents leaching of vital plant nutrients (Khaled and Fawy, 2011). Humic acid stimulates plant enzymes/hormones and improve soil fertility in an environmentally benign manner (Sarir *et al.,* 2005). Especially, the biostimulation activity of humic acids is credited to its functional activities related to auxins (Chen and Aviad., 1990) and auxin-like and gibberellin-like activity as well as indoleacetic acid (IAA) (Pizzeghello *et al.,* 2001). The beneficial effects of the humic acids towards its growth promotion activities such as seed germination rate, root initiation and seedling growth was documented in various agricultural crops such as rice (Olk *et al.,* 2007); wheat (Killi, 2004; Antošová *et al.,* 2007) ; maize (Nardi *et al.,* 2002); sunflower (de Sanfilippo *et al.,* 1990); vegetables such as lettuce and tomato (Lulakis and Petsas, 1995; Turkmen *et al.,* 2004) and leguminous plants (Ferrara *et al.,* 2004). Foliar application studies of humic acids also has evidenced significant increase in the chlorophyll content and yield of pepper (Karakurt *et al.,* 2009).

Humic acids derived from organic source such as leonardite was found to increase the macro and micro nutrient content such as nitrogen, phosphorus, potassium, calcium, magnesium, iron and zinc of leaves and scapes and root growth of *Gerbera jamesonii* L (Nikbakht *et al.,* 2008). Prakash *et al.,* 2014 has documented that humic acids derived from leonardite has pronounced effect on germination rate, root and shoot development of seedlings of *Raphanus sativus* L. Quite interestingly, humic acids derived from decomposition of organic matter such as compost reported to promote several biological functions. Humic acids derived from vermicompost prepared using cattle, food and paper waste upon addition to plant growth media (Metr-mix 360) at different concentrations were found to increase the root growth of marigold and pepper and fruit numbers in strawberries (Arancon *et al.,* 2006). The positive effect of humic acids isolated from vermicompost towards stimulating lateral root development of *A. thaliana* and *Zea mays* was reported (Canellas *et al.,* 2010). Similarly, another study on vermicompost extracted humic acids were found to promote shoot length and lateral root development of *Brachiaria brizantha* cv. MG5 (Amorim *et al.,* 2015).

The present study aims to study the treatment of different concentrations of humic acids derived from co-compost developed using biological trashes derived from marine fish processing waste and sugarcane bagasse on the growth promotion activities of seeds belonging to Fabaceae family.

Materials and Methods

Isolation of humic acids

The co-compost samples were prepared using marine fish processing waste and sugarcane bagasse based on our previous study (Aranganathan *et al.,* 2019). Ten gram of the co-compost sample was added to 100 mL of 0.1 M Sodium hydroxide solution in an screw capped bottles in 1:10 proportion under N_2 atmosphere (Yamamoto and Fukushima, 2013). The bottle was kept shaken well in an orbital shaker for 24 h. After shaking, the suspension was transferred to polyethylene centrifuge bottles and centrifuged at 10,000 rpm for 10 min. The dark brown supernatant solution was collected and transferred to beaker. Few drops of 6 M hydrochloric acid (HCl) was added and adjusted to pH 1 and left for 24 h to allow precipitation of humic acids. The sample was centrifuged at 3500 rpm to separate humic acid portion from fulvic acids. The brown colloidal suspension was then rinsed thrice with distilled water to remove impurities and freeze dried to obtain dry powder.

Collection of seeds and preparation of humic acids

Dried seeds of plants belonging to Fabaceae such as *Vigna radiata* (Green gram); *Vigna mungo* (Black gram); *Vigna unguiculata* (Cow pea) and *Cicer arietinum* (Chick pea) were purchased from local market of Chennai. The healthy seeds were carefully separated and stored for the experiment. Various concentrations of the humic acids solution such as 0.02 %, 0.04 %, 0.06 %, 0.08 % and 0.1 % was prepared and stored in screwed capped bottles at room temperature.

Seed germination assay

The germination test was carried out by between paper (BP) method (International Seed Testing Association, 1993). Prior to the experiment, the seeds were sterilized with 5% sodium hypochlorite solution and rinsed well with sterile distilled water. Ten seeds of each plant were placed on the surface of the double sheets of the paper towel which were moistened with different concentrations of the humic acids and control was maintained. The seeds were covered with another sheet of paper towel and covered with polythene bags. The seeds were placed in a dark compartment at room temperature. The paper towels were periodically checked to ensure uniform moisture content. The seeds were considered germinated with the growth of emerging radical having 2 mm long (Murillo-Amador and Troyo-Diéguez., 2000).

The percentage seed germination (SG %) was calculated using the formula,

Seed germination (%) = No.of germinated seeds / No.of total seeds X 100 % (Luo *et al.,* 2018).

Measurement of growth parameters

Seedling root length

The seedlings treated with different concentration of the humic acids were measured for root length by between paper (BP) germination test. Three replicates of the seeds treated with humic acids in various concentrations was placed at the centre of the paper towels in a straight line with the end of the radicale placed towards the bottom of the towels. Then the towels were covered in a rectangle shape and incubated at 25 ºC in the dark germinator. The seedling root length of well grown seeds after 6-7 days and the means of each seedling root length was calculated in cm per seedling.

Seedling shoot length

The seedlings that were treated with the various concentrations of the humic acids grown in the paper towels by between paper germination test was measured for seedling root length. The means of the seedling shoot length was calculated in cm per seedling.

Seed vigour index (SVI)

The seedling vigour index of the humic acid treated seedlings was calculated based on Abdul-Baki and Anderson, 1973

SVI= Germination percentage x Total seedling length (cm).

Results and Discussion

Effect of humic acid on seed germination (SG)

The leguminous seeds treated with various concentrations of humic acids isolated from sugarcane bagasse and marine fish waste co-compost showed different effects on the percentage of seed germination (%) (Table 1). Among the different seeds tested, SG 100 % was observed in *Vigna radiata* seeds treated with 0.02% concentration of humic acids and SG 90 % was identified in 0.06 % and 0.1 % of humic acids. In *Vigna mungo* seeds, maximum SG 90 % was found in highest concentration (0.1 %) of humic acids. In *Vigna unguiculata* seeds, SG 70 % was identified in 0.06 % of humic acids. Among all the seeds tested, SG % was significantly lower in *Cicer arietinum*. SG 50% was recorded in *Cicer arietinum* seeds treated with 0.02 % and 0.06 % concentration of humic acids respectively. The SG% of control treatment is represented in Table 1. From the observed results, it was evident that humic acids has influenced good germination effects of *Vigna radiata*; *Vigna mungo* and *Vigna unguiculata* seeds but moderate effect on *Cicer arietinum* seeds.

Effect of humic acid on root length (cm)

The growth promotion effect of the humic acids on different types of seedlings was measured in terms of root length (cm). The root length of the control is represented in Fig.1. Among the seeds tested with different concentration of the humic acids, maximum root length was observed in *V.radiata* (11 ±1.61 cm) and *V.mungo* (10.3 ± 0.35 cm) seeds treated with low concentration (0.02%) of humic acids. In contrast, low root length (5.8 ± 2.16 cm) was observed in *V. unguiculata* and *Cicer arietinum* seeds treated with 0.02 % humic acids. More specifically, high root length was recorded in *V. unguiculata* (8 ± 1.1 cm) and *Cicer arietinum* (7.3 ± 0.41 cm) seeds treated with 0.06 % humic acids. Although, the root length of all the seedlings decreased at highest concentration of humic acid (0.1 %), *Vigna mungo* recorded maximum root length (6.1± 0.9 cm) respectively (Fig.2). Based on the obtained results, it was identified that the humic acids treated in the concentrations ranging from 0.02-0.06% was found to promote the root growth of all the seedlings. The variation in the root growth depends upon the type of seedlings and their interaction with the treatment of humic acids. The obtained result was in accordance with the root growth promotion effects of humic acids on *Triticm aestivum* L. seedlings (Malik and Azam, 1985). The growth stimulation activity of the humic acids on radical length development could be due to the increasing absorption capacity of the roots (Vaughan and MacDonald, 1976). Humic acids extracted from vermicompost and urban organic waste composts were documented to affect root architecture and promote proton pump activation of Tomato and maize vesicles which might be attributed to its auxin like effects (Dobbss *et al.*, 2010; Jindo *et al.*, 2012).

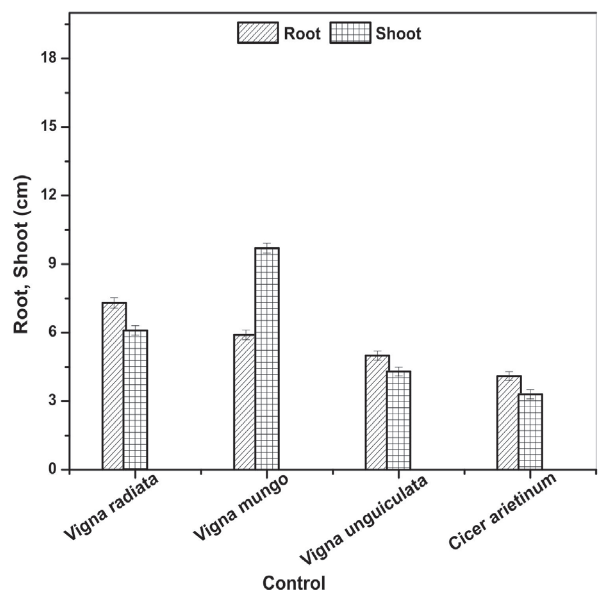

Fig. 1. Growth parameters of the seedlings in control treatment

In *Vigna radiata*, the root length of the seedlings was found to decrease with increase in the concentration of the humic acids. The reduction in the root growth length of all the seedlings was found to decrease in high concentration of humic acids: 0.08 % and 0.1% respectively. More specifically, decreased

Table 1. Seed germination percentage (SG %) observed in seeds treated with various concentrations of humic acids isolated from sugarcane bagasse and marine fish waste co-compost

	Concentration of humic acids (%)					
	Control	0.02%	0.04%	0.06%	0.08%	0.1 %
Vigna radiata	100	100	80	90	60	90
Vigna mungo	90	80	70	70	60	90
Vigna unguiculata	100	60	50	70	40	60
Cicer arietinum	80	50	60	50	40	60

root growth was well identified in *V.radiata*;*V. unguiculata* and *Cicer arietinum* seeds treated with 0.1 % concentration of humic acids. It has been documented that high concentrations of humic acids cause reduction in the plant growth of Tomato seedlings (Atiyeh *et al.,* 2002). Low concentrations of humic acids combined with calcium nitrate treatments had significant positive influence on root length of *Capsicum annum* cv Derme grown under saline conditions (Gulser *et al.,* 2010).

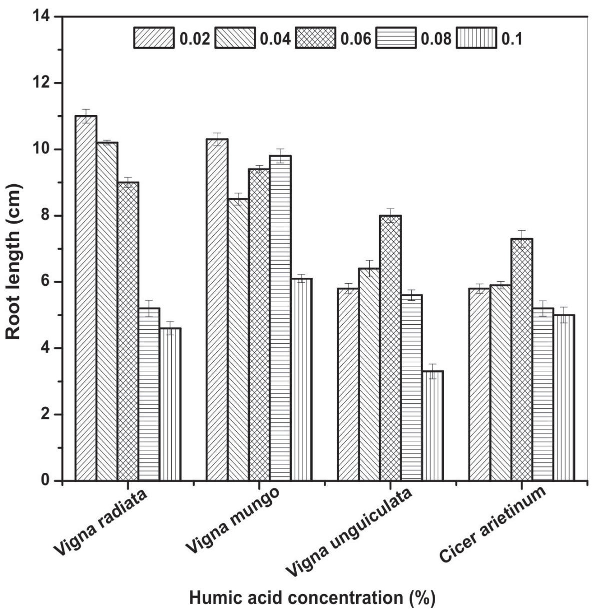

Fig. 2. Effect of various concentration of humic acid treatment on root length of the seedlings

Effect of humic acid on shoot length (cm)

The shoot length of the seedlings treated with different concentrations of the humic acids is represented in Fig. 3. Among all the seedlings tested, humic acids showed enhanced growth of the shoot in *V. radiate* and *V.mungo* seeds respectively. In control, high shoot length was observed in *V.mungo* (9.7± 0.9 cm) whereas other seedlings showed relatively less shoot length (Fig. 1). The seedlings of *V.radiata* showed significant variations in the shoot length with increasing concentrations of the humic acids. Highest shoot length of *V. radiata* (14.1 ± 2.02 cm) was observed in the initial concentration of humic acids (0.02 %). Similarly, *V.mungo* also showed variations in the shoot length with respect to the increasing concentrations of humic acids. Maximum shoot length (15.4±3.27 cm) was observed in *V.mungo* seeds treated with 0.06 % concentration of humic acids. Among all the seedlings, less growth response was identified in *V. unguiculata* and *C.arietinum* seedlings respectively. The shoot length of *V. unguiculata* seedlings gradually decreased with increasing concentration of humic acids. Also, *Cicer arietinum* showed the same pattern with slight increase in the shoot length at 0.1 % concentration of humic acids. Among these two seedlings, highest shoot length (5.7 ± 0.64 cm) was observed in *V. unguiculata* seeds than *Cicer arietinum* (3.2± 2.01 cm) treated with 0.02 % concentration of humic acids.

Interestingly, the shoot length of the seedlings

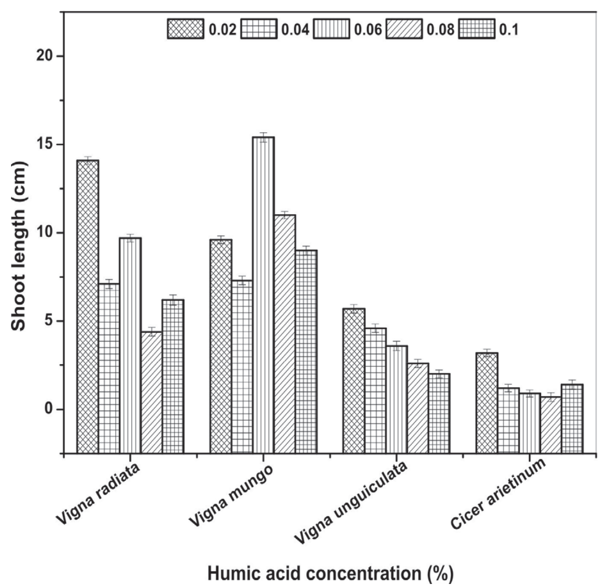

Fig. 3. Effect of various concentration of humic acid treatment on the shoot length of the seedlings

Table 2. Seed vigour index (SVI) of seedlings treated with various concentrations of humic acids.

	Concentration of humic acids (%)					
	Control	0.02 %	0.04 %	0.06 %	0.08 %	0.1 %
Vigna radiata	1340	2510	1384	1683	576	972
Vigna mungo	1404	1592	1106	1736	1248	1359
Vigna unguiculata	930	690	550	812	330	318
Cicer arietinum	592	450	426	410	236	384

significantly decreased at highest concentration of humic acid (0.1%) similar to the root length. Among all the seedlings that were treated with high concentration of humic acid (0.1%), maximum shoot length (9 ± 0.79 cm) was recorded in *Vigna mungo*. From the observed results, it was identified that the humic acid treatments has influenced better shoot growth of *V.radiata* and *V.mungo* seeds.

Comparison of root and shoot growth development

In the present study, it was obvious that humic acids isolated from marine fish waste and sugarcane bagasse co-compost significantly enhanced the root growth of the seedlings. The treatment of the seedlings with various concentrations of the humic acids showed significant variations in the development of root and shoot growth. Specifically, it was observed that 0.06 % concentration of humic acids demonstrated moderate growth promotion effect on root development. The high concentration of humic acids: 0.08 % and 0.1 % has demonstrated reduction in the growth activity of the roots in all the seedlings. It has been reported that humic acids shows growth inhibition effects when applied at high concentrations (Gulser *et al.,* 2010; Souguir and Hannachi, 2017). Although, high concentrations of humic acids resulted decrease in the root length, *V.mungo* seedlings showed high root length (11 ± 1.15 cm) in 0.08 % concentration of humic acids respectively.

Similarly, the shoot length has also significantly reduced in the seedlings treated with high concentrations of humic acids. In terms of shoot growth development, the seedlings of *Vinga mungo* showed increased shoot length: 11 ± 1.15 cm and 9 ± 0.79 cm at high dose of humic acids: 0.08 and 0.1% respectively. Rodrigues *et al.,* 2017 has also reported increase in the shoot length of corn seedlings treated with high doses of humic acid. The increased shoot length of *V. mungo* seedlings may be attributed to the growth stimulation effects of humic acids on plant respiration such as increasing ATP production by stimulating oxidative phosphorylation, with a consequent increase in the absorption and transport of nutrients and also in the biosynthesis of compounds, which further results in increasing the shoot development (da Silva *et al.,* 1999). The decrease in the growth activities of *Vigna unguiculata* and *Cicer arietinum* seedlings could depend on the source and dose of humic acid treatment as well as the influence of humic acid specifically varies with the plant species (Muscolo *et al.,* 2007; Rodrigues *et al.,* 2017). From the observations, it was identified that *V.mungo* seedlings recorded high root and shoot growth in high concentration of the humic acids. In summary, the biological effects of humic acids on seed growth depends upon the concentration tested, plant species as well as the source of extraction (Amorim *et al.,* 2015).

Seed vigour index (SVI)

The seeds showing higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973). The application of humic acids has positive effect on the physiological processes of the plant to ensure high seedling vigour (Rodrigues *et al.,* 2017). A previous study by Ebrahimi, M. and Miri, E. 2016 has reported that humic acid treatment promotes the seed vigour index of *Cichorium intybus* seedlings. In the present study, significant differences in the SVI of each seedling treated with different concentration of humic acids. The SVI calculated for the seedlings treated with humic acids obtained from fish processing waste and sugarcane bagasse co-compost and control is represented in Table 2.

Among all the seedlings, highest SVI (2510) was identified in *V.radiata* seeds that were treated with 0.02% concentration of humic acids but the vigour index drastically reduced in high concentration of humic acids. In contrast, *V.mungo* showed slight variations in the SVI in all the treatments with maximum SVI (1736) observed in 0.06 % treatment of humic acids. *V. unguiculata* seedlings showed maximum SVI (812) at 0.06 % concentration of humic acids. However, *Cicer arietinum* recorded relatively low SVI compared to the other seedlings with high SVI (450) at 0.02 % of humic acids. The SVI index values were observed to be very low with increased concentration of humic acids in *V. unguiculata* and *Cicer arietinum* seeds respectively. The obtained result was in agreement with seed vigour index of Chilli seeds which was reduced with the increasing concentration of humic acids (Ananthi *et al.,* 2017). More specifically, the SVI was calculated to be maximum (1359) in seedlings of *V. mungo* treated with high concentration (0.1%) of humic acids. Comparison of the calculated results revealed that SVI index values were relatively in moderate levels for all the seedlings treated with 0.06 % concentration of humic acids.

Conclusion

The preliminary results of the study identified the treatment of seeds belonging to Fabaceae family with various concentrations of humic acids significantly promoted the growth parameters such as root and shoot length respectively. Noticeably, humic acids influenced root growth than shoot growth. Also, it was observed that low concentration of humic acids favoured the growth of root and shoots. In contrast, the growth of the seedlings has reduced with high doses of humic acids. Among all the seeds, *Vigna mungo* recorded maximum root and shoot growth at high dose- 0.08 % and 0.1 %. Moderate growth effects were observed in the seedlings treated with 0.06 % concentration of humic acids which could be considered as ideal concentration for seed treatment. The seedlings of *Vigna radiata* and *Vigna mungo* showed enhanced growth interms of root and shoot development compared to *V. unguiculata* and *Cicer arietinum* which showed less growth effects upon humic acid treatment. The seed vigour index has also reduced with increasing concentration of the humic acids. Hence it was concluded that humic acids derived from biological transformation of fish processing waste and sugarcane bagasse co-compost could be used as cost effective source of obtaining humic acids for assisting plant growth. Further, field studies have to be carried out to identify its application as “growth enhancing agent” to increase plant growth and crop yield.

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