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Evaluation of bio-efficacy and phytotoxicity of humic acid and fulvic acid bio-stimulants on performance of chilli (*Capsicum annum* L.)

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Abstract
A field experiment was conducted during Summer season 2024 at A field experiment was conducted during Summer 2024 at Agricultural and Horticultural Research Station, Honnavile, Shivamogga in Red sandy loam soil which comes under Southern Transition Zone of Karnataka. The experiment was laid out in RCBD with 9 treatments replicated thrice. The treatment composed of five different doses of Humic acid + Fulvic acid based Biostimilant *viz.*, 0, 3,5,7& 10 ml per litre of water and 2 stages of Biostimulant application *viz., either* at Establishment stage (7 DAT) or both Establishment and active vegetative stages (21 DAT). The variety used was Green thunder-2006. The results revealed that, a foliar application of Humic acid + Fulvic acid based Biostimulant at a concentration of 7 ml per litre in the spray solution applied during establishment stage (7 DAT) and active vegetative stage (21 DAT) can enhance total fruit yield (10.15 tonnes per ha) significantly followed by a foliar application of Biostimulant at a concentration of 5 ml per litre in the spray solution applied during establishment stage and active vegetative stage (9.84 t/ha). However they were significantly superior over Control-only water spray (8.27 t/ha) and single foliar application treatments (8.35 to 8.85 t/ha). Similarly, the above best treatment recorded higher net returns and B:C ratio.

Keywords: Chilli, bio-stimulant, foliar application, establishment stage, active vegetative stage

Introduction
Vegetables play a major role in Indian agriculture and responsible in solving problems of malnutrition among human population. Growing vegetable crops generate greater employment potential in rural areas bringing national security. India is the second largest producer of vegetables after China and contributes about 12 percent of the world vegetable production. Chilli (Capsicum annum L.) is one of the important vegetable crops of the Solanaceae family and is grown worldwide in large scale. It originates from South and Central America where it is still under cultivation. It is well documented, chilli is the first spice to have been used by human being and there is archaeological proof of consumption of chillies of about 6000 years ago. India is the world's largest producer, consumer, and exporter of chillies, renowned globally for its vibrant colour and varied pungency levels. Indian chillies are primarily exported to Asian countries, including China, Sri Lanka, Malaysia, Bangladesh, Singapore, Thailand, and the UAE. In 2023, the global chilli cultivation area was 18.03 lakh hectares, with a production of 58.22 lakh tonnes and an average productivity of 3,229 kg per hectare. India led the world in chilli production with 32.08 lakh tonnes from 9.90 lakh hectare and having a productivity of 3240 kg per hectare (Anonymous 2023-24) [2]. Chilli contains proteins, vitamins including vitamin A and C and is also a rich source of various minerals including calcium, phosphorous and irons. In addition, hot types of chillies are rich in digestive stimulant capsaicin. Chilli forms an essential ingredient of Indian curries. It is famous for this for its pleasant aromatic flour, pungency and high colouring substance. It is widely used in culinary, pharmaceutical and beverage industry. Application of commercial inorganic fertilizers has resulted in drastic reduction in soil microbial population and whole rhizosphere is getting polluted. Continuous

deterioration in soil physical properties, nutrient imbalance and rapid depletion of soil fertility are added disadvantages of chemical fertilization. The growth, development and yield of chilli can be increased by the balanced application of N, P and K nutrients. However, growth regulation of any crop is manipulated by the exogenous application plant growth regulations (PGR). But in the recent past instead of PGR, biostimulants are being used for crop regulation. The synergistic and complementary effect of biostimulants and essential nutrients is utilized for the synthesis of proteins which eventually leads to stimulated growth and yield. The biostimulants are substances that are derived from plants or microorganisms, when applied to plants or soil, enhance plant growth, stress tolerance and crop quality (Jardin, 2015) [12]. Yeast extract (Saccharomyces cerevisiae) is a type of protein hydrolysate biostimulant, rich in phytohormones (such as gibberellins, auxins, and cytokinins), amino acids (like lysine and tryptophan), minerals and vitamins (Surendar *et al*., 2020) [23]. Humic acid supports both shoot and root growth and enhances nutrient uptake in vegetable crops. It acts as a bio stimulant and promote plant growth (Fathima and Denesh, 2013) [8]. Seaweed extracts have been shown to have anti-stress and biostimulant effects potentially playing a role under stress conditions (Jardin, 2015) [12]. Gluconate is a salt of gluconic acid which helps in increasing the efficiency of micro nutrients (Gourkhede *et al*., 2020) [9] and increase the quality of produce. High yielding varieties and hybrid chilli has got good market preference due to its size and appealing attractive colour (Chakravarthy and Mohan, 2023) [10]. Hence, there is a need to increase the production by boosting up the yield and also quality by using biostimulants.

Materials and Methods

The present investgation was conducted at Agricultural and Horticultural Research Station, Honnavile, Shivamogga during January 2024 to June 2024. The topography of the experimental field was uniform with adequate irrigation and drainage facility. The experimental soil was red sandy loam in texture, medium organic carbon (0.67%), slightly acidic pH (5.66), medium in available-N (260.28 kg/ha), available Phosphorous (64.12 kg/ha) and available potassium (164.37 kg/ha). The experiment was laid out in Randomized Block Design (RBD) with 9 treatments replicated thrice. The field layout and randomization of treatments were made with plot size of 6.0 m × 4.5m (27 m^2). The seedlings of Chilli cv. Green thunder-2006 of one month old were raised in the nursery. They were transplanted in the well prepared main field at a spacing of 75 cm × 45 cm. Farm yard manure was applied @ 20t/ha as soil application at last ploughing. The fertilizers at 150:75:75 kg NPK per ha were applied as 50 per cent N, full dose of P and 50 per cent K as basal dose at the time of transplanting. The remaining 50 per cent N was applied in two equal splits at 30 DAT and at 60 DAT as top dressing. The fertilizers sources like urea, diammonium phosphate (DAP) and muriate of potash (MOP) fertilizers at 150:75:75 kg NPK per ha were applied to experiment. The crop was raised under protected irrigation scheduled at 15 days interval. Weeds are effectively managed by Pre-emergent application of Pendimethalin (30 EC) at 1 litre a.i. per ha was sprayed in moist soil Before transplanting of chilli seedlings followed by two hand weeding's at 30 and 60 DAT. The timely plant protection measures were taken up to avoid occurrence of pest and diseases, the chemicals *viz*., monocrotophos, chloripyriphos and carbendazim were applied as per recommended dose. The biostimulant was a Humic substances and fulvic acid formulation in equal proportion that is capable of aiding the crops to overcome physiological stress caused by climate changes and soil limitations. The treatments of present experiment comprised of T_1-0 ml/lit (only water spray) -Control, T_2 -3 ml/lit at Establishment stage (7 DAT), T_3 -5 ml/lit at Establishment stage (7 DAT), T_4 -7 ml/lit at Establishment stage (7 DAT), T_5-10 ml/lit at Establishment stage (7 DAT), T_6 -3 ml/lit at Establishment stage (7 DAT) and 3 ml /lit at active vegetative stage (21 DAT), T_7-5 ml/lit at Establishment stage (7 DAT) and 5 ml /lit at active vegetative stage (21 DAT), T_8-7 ml/lit at Establishment stage (7 DAT) and 7 ml /lit at active vegetative stage (21 DAT) and T_9 - 10 ml/lit at Establishment stage (7 DAT) and10 ml /lit at active vegetative stage (21 DAT). The biometrical parameters *viz*, Plant height (cm), Number of branches per plant, Days to 50% flowering, number of fruits per plant, fruit length (cm), fruit girth (cm), individual fruit weight (g), fruit yield per plant and total fruit yield tonnes per hectare at harvest were recorded. The Phytotoxicity observations were taken at 3, 6, 9 and 12 days after spraying. Observations for the specific parameters like chlorosis, necrosis, wilting, scorching, hyponasty and epinasty was noted. The growth and yield observations recorded were statically analysed using the simple randomized block design, for significance and critical difference values of the experiment were computed using duncan's multiple range test (DMRT) as suggested by Panse and Sukhatme (1985) [16].

Results and Discussion

Plant height (cm)

The Result revealed that, with the application Bio-stimulant @ 7 ml/lit at Establishment stage and at active vegetative stage recorded significantly higher plant height (89.38 cm) followed by T_7: 5 ml/lit at Establishment stage and at active vegetative stage (88.49 cm), however the difference was non-significant. Even lowest plant height (69.27 cm) was recorded with T_2: 3 ml/lit at Establishment stage and T_1: Control (51.25 cm).

The increment in plant height of biostimulant applied treatments may be due to enhancement in photosynthetic and other metabolic activities which promote various plant metabolites responsible for cell division and quicker multiplication of cells in the shoot apex due to the auxin type of activity of humic acid on plant growth and the quick availability of nutrients especially nitrogen, the chief nutrient of protein for the formation of protoplasm leads to cell enlargement. Similiar findings were also reported by Fathima and Denesh (2013) [8], Gudapati Ashoka Chakravarthy *et al*., (2023) [3], Shailaja Nimmala *et al.* in chilli crop.

Number of branches per plant

Significant differences were found among the treatments w.r.t. number of branches per plant with foliar application of Humic acid and Fulvic acid bio-stimulant in Chilli. Among the treatments, T_8 and T_7 recorded the highest number of branches per plant (6.85& 6.26) and was followed by T_6 and T_9; but statistically they were at par. All the Humic acid and Fulvic acid bio-stimulant foliar spray at establishment and vegetative stage have shown a significantly higher number of branches per plant (5.01 to 6.85) than single foliar spray at establishment stage (4.12 to 4.92) and Control (3.82). The possible reason that the foliar application of the biostimulant product, which is composed of humic acid and fulvic acid, might influence vegetative growth by encouraging cell division and elongation and increased number of branches. These acid rises the chlorophyll and leaf N, P and K concentration increased number of branches was also reported by Hylaja Gangam *et al.*, Similar findings were also reported by Fathima and Denesh (2013) [8], Gudapati Ashoka Chakravarthy *et al*., (2023) [3].

Days to 50% flowering

As regards days to 50% flowering, T_8:7 ml/lit at Establishment stage and at active vegetative stage recorded the minimum number of days (72.66), followed by T_7 (73.33 days), T_6 (73.66 days) and T_9 (74.33 days) however they all were at par. Application of Humic acid and Fulvic acid bio-stimulant foliar spray at establishment and vegetative stage resulted in early flowering ranging between 7 to 9 days when compared with Control (81.66 days). The Humic acid and Fulvic acid present in the biostimulant is used to ameliorate or reduce the negative effect of stress. Significant superiority over control might be due to increased photosynthetic activity, hormonal activity and uptake of nutrients resulting in early flowering as reported by Patel *et al.*, (2018) in bhindi. These findings are in conformity with those of Gourkhede (2020) [9], Diksha Pundir *et al.*, Anbarasi and Venkatraman (2022) [5].

Number of fruits per plant

Among the treatments, T_8:7 ml/lit at Establishment stage and at active vegetative stage recorded the highest number of fruits per plant (148.36) which was at par with T_7: 5 ml/lit at Establishment stage and at active vegetative stage (145.27) Even lowest fruits per plant (134.22) was recorded with T_2: 3 ml/lit at Establishment stage and T_1: Control -control (102.38). This might be due to the accumulation of more photosynthates, amino acids and nitrates in the treated plants that resulted in making the plants resistant to pests and diseases, and subsequently producing a greater number of fruits per plant. These findings are comparable with that of Chakravarthy *et al.* (2023) [3] in chilli. Similar results were also reported by Chaudhari *et al.* (2023) [4], Hylaja Gangam *et al.* (2024) [11] in capsicum.

Table 3: Effect of Humic acid and Fulvic acid bio-stimulant on growth, yield and phytotoxic effect in Chilli crop

Treatments	Plant height (cm)	Number of branches/plant	Days to 50% flowering	Fruits/ plant
T_1: 0 ml/lit (only water spray) -Control	51.25	3.82	81.66	102.38
T_2: 3 ml/lit at Establishment stage (7 DAT)	69.27	4.12	78.33	134.22
T_3: 5 ml/lit at Establishment stage (7 DAT)	74.18	4.31	76.33	139.67
T_4: 7 ml/lit at Establishment stage (7 DAT)	76.27	4.57	75.00	135.68
T_5: 10 ml/lit at Establishment stage (7 DAT)	78.25	4.98	74.66	140.33
T_6: 3 ml/lit at Establishment stage (7 DAT) and 3 ml /lit at active vegetative stage (21 DAT)	84.15	5.68	73.66	143.28
T_7: 5 ml/lit at Establishment stage (7 DAT) and 5 ml /lit at active vegetative stage (21 DAT)	88.49	6.26	73.33	145.27
T_8: 7 ml/lit at Establishment stage (7 DAT) and 7 ml /lit at active vegetative stage (21 DAT)	89.38	6.85	72.66	148.36
T_9: 10 ml/lit at Establishment stage (7 DAT) and10 ml /lit at active vegetative stage (21 DAT)	81.78	5.01	74.33	142.15
S.Em±	3.80	0.25	3.64	6.69
CD (P=0.05)	11.40	0.76	10.93	20.06

Fruit length (cm)

Application of Humic acid and Fulvic acid bio-stimulant resulted in increasing the length of Chilli fruit. The result showed that there was a significant difference found in fruit length with foliar application of Humic acid and Fulvic acid bio-stimulant in Chilli compared to control (T_1) plants and also with single foliar spray of Humic acid and Fulvic acid (T_2 to T_5). Among the treatments, T_8:7 ml/lit at Establishment stage and at active vegetative stage recorded the highest fruit length (8.35 cm) and was at par with T_7 (8.13 cm), Even the single foliar application of Humic acid and Fulvic acid bio-stimulant resulted in increasing the fruit length over T_1. This might be due to the effect of humic acid, which promotes root growth and development, leads to better nutrient absorption and also promotes cell elongation and cell division, which leads to larger and longer fruits. These findings are in line with those of Sakthivel and Manivannan (2021) [19], Saritha Sahu *et al.* (2019) [20] in chilli and El -Tohamy *et al.* (2008) [6] in Egg plant.

Fruit girth (cm)

The foliar application of Humic acid and Fulvic acid bio-stimulant was found to have a significant effect on fruit girth (cm). Among the treatments, T_8:7 ml/lit at Establishment stage and at active vegetative stage recorded the highest fruit girth (3.18 cm) and was at par with T_7 (3.10 cm) and T_6 (3.05 cm), Even the single foliar application of Humic acid and Fulvic acid bio-stimulant resulted in increasing the fruit girth (2.54 to 2.74 cm) over T_1 (2.41 cm). This might be due to the effect of humic acid in the biostimulant applied that caused rapid multiplication of cells in the reproductive organs and more accumulation of photosynthates in fruits than compared to absolute control. These findings are similar to those of Fadia *et al.*, (2019) [7] in cowpea, Gourkhede *et al.*, (2020) [9] and Madhu Singh *et al.*, (2017) [14] in chilli.

Individual fruit weight (g)

Application of Humic acid and Fulvic acid bio-stimulant resulted in increasing the individual weight of Chilli fruit. The result showed that there was a significant difference found in individual fruit weight with foliar application of Humic acid and Fulvic acid bio-stimulant in Chilli compared to control (T_1) plants and also with single foliar spray of Humic acid and Fulvic acid (T_2 to T_5). Among the treatments, T_8:7 ml/lit at Establishment stage and at active vegetative stage recorded the highest individual fruit weight (5.34 g) and was at par with T_7, (5.23 g) and T_6 (5.18 g), Even the single foliar application of Humic acid and Fulvic acid bio-stimulant resulted in increasing the individual fruit weight (4.36 to 4.85 g) over control (4.21 g). This might be due to humic acid leading to increased fruit weight through positive physiological effects such as the impact of metabolism of plant cells, photosynthesis and increasing the concentration of leaf chlorophyll (Sure *et al*.,2012) in cucumber. These findings are in close conformity with Pankaj Kumar *et al.*, (2015) [15] in Okra, Ruban *et al.*, (2019) [18] in Brinjal, Shankwar *et al.*, (2017) [22] in chilli and Yatagiri *et al.*, (2017) [25] in capsicum.

Fruit yield per plant (g/plant)

The variations in fruit yield per plant were observed among the treatments. The foliar application of Humic acid and Fulvic acid bio-stimulant was found to have a significant effect on fruit yield per plant (g/plant). Among the treatments, T_8:7 ml/lit at Establishment stage and at active vegetative stage recorded the highest fruit yield per plant (287.25 g/plant) and was at par with T_7 (276.56 g/plant), Even the single foliar application of Humic acid and Fulvic acid bio-stimulant resulted in increasing the fruit yield per plant (233.21 to 259.34 g/plant) over Control (179.68 g/plant). These findings are in conformity with those of Gourkhede (2020) [9], Diksha Pundir *et al.,* Anbarasi and Venkatraman (2022) [1].

Total fruit yield (t/ha)

Plot wise yield extrapolated into yield in tonnes per ha presented in Table 2 revealed that Foliar application of Humic acid and Fulvic acid bio-stimulant directly resulted in increasing the total fruit yield of Chilli. The highest total fruit yield (10.15 tonnes per ha) was recorded in Treatment T_8:7 ml/lit at Establishment stage and at active vegetative stage followed by T_7 (9.84 t/ha), T_6 (9.72 t/ ha). Difference in total fruit yield among the treatments (T_8 to T_6) was non-significant; however they were significantly superior over T_1: Control (8.27). In general, the increase in total fruit yield of Chilli ranged between 14 to 22 percent with two spray of Humic acid and Fulvic acid bio-stimulant at Establishment stage (7 DAT) and at active vegetative stage (21 DAT) when compared with T_1 (Control). There was improvement of anabolic activities as well as redistribution of metabolites. Thus, the nitrogen which was earlier utilized by vegetative parts was translocated towards reproductive organs to form amino acids, which upon condensation formed proteins and ultimately used for early onset of flowering, increasing the number of flowers per plant and improves yield attributing characters ultimately leads to higher weight of the fruits. Similar findings were reported by Thouti Pavani *et al.* (2022) [24], Surendar *et al.,*(2020) [23] in chilli, Madhu Singh (2017) [14] in Capsicum and Karak *et al.,* (2023) [13] in Potato.

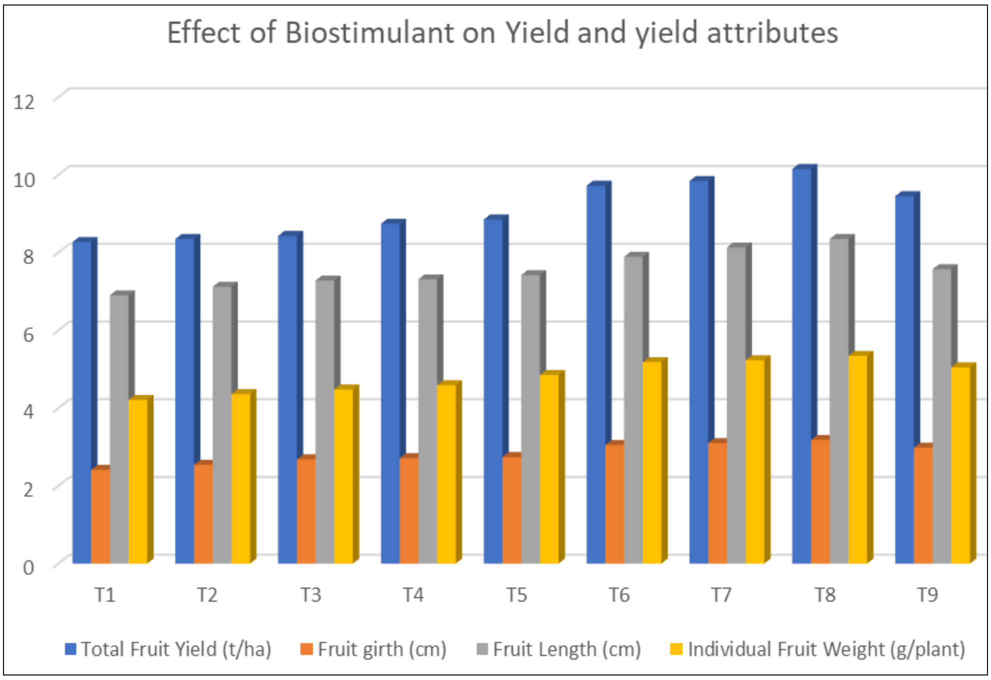

Fig 1: Effect of Biostimulant on Yield and Yield attributes of Chilli

Table 4: Effect of Humic acid and Fulvic acid bio-stimulant on yield in Chilli

Treatments	Fruit girth (cm)	Fruit length (cm)	Individual Fruit weight(g)	Fruits yield per plant (g/plt)	Total Fruit yield (t/ha)
T_1: 0 ml/lit (only water spray) -Control	2.41	6.90	4.21	179.68	8.27
T_2: 3 ml/lit at Establishment stage (7 DAT)	2.54	7.12	4.36	233.21	8.35
T_3: 5 ml/lit at Establishment stage (7 DAT)	2.68	7.28	4.48	245.39	8.43
T_4: 7 ml/lit at Establishment stage (7 DAT)	2.71	7.31	4.59	251.45	8.74
T_5: 10 ml/lit at Establishment stage (7 DAT)	2.74	7.42	4.85	259.34	8.85
T_6: 3 ml/lit at Establishment stage (7 DAT) and 3 ml /lit at active vegetative stage (21 DAT)	3.05	7.89	5.18	274.15	9.72
T_7: 5 ml/lit at Establishment stage (7 DAT) and 5 ml /lit at active vegetative stage (21 DAT)	3.10	8.13	5.23	276.56	9.84
T_8: 7 ml/lit at Establishment stage (7 DAT) and 7 ml /lit at active vegetative stage (21 DAT)	3.18	8.35	5.34	287.25	10.15
T_9: 10 ml/lit at Establishment stage (7 DAT) and10 ml /lit at active vegetative stage (21 DAT)	2.98	7.57	5.05	269.38	9.45
SEm±	0.14	0.37	0.24	12.46	0.44
CD (P=0.05)	0.42	1.10	0.71	37.37	1.33

The phytotoxicity results indicated that, there was less than 10 per cent phyto-toxicity observation recorded when chilli crop is applied with the higher dose of humic acid and fulvic acid 10 ml per litre at Establishment stage (7 DAT) and at active vegetative stage (21 DAT).

Table 5: Effect of evaluation of bio-efficacy of the Humic acid and Fulvic acid Biostimulant on phyto toxicity to Chilli crop

Treatments	Phytotoxicity (Days after spraying)			
	3 DAS	6 DAS	9 DAS	12 DAS
T_1: 0 ml/lit (only water spray) -Control	0	0	0	0
T_2: 3 ml/lit at Establishment stage (7 DAT)	0	0	0	0
T_3: 5 ml/lit at Establishment stage (7 DAT)	0	0	0	0
T_4: 7 ml/lit at Establishment stage (7 DAT)	0	0	0	0
T_5: 10 ml/lit at Establishment stage (7 DAT)	1	1	0	0
T_6: 3 ml/lit at Establishment stage (7 DAT) and 3 ml /lit at active vegetative stage (21 DAT)	0	0	0	0
T_7: 5 ml/lit at Establishment stage (7 DAT) and 5 ml /lit at active vegetative stage (21 DAT)	0	0	0	0
T_8: 7 ml/lit at Establishment stage (7 DAT) and 7 ml /lit at active vegetative stage (21 DAT)	0	0	0	0
T_9: 10 ml/lit at Establishment stage (7 DAT) and10 ml /lit at active vegetative stage (21 DAT)	1	1	0	0

Conclusion

The data obtained from the field trail of Humic acid and Fulvic acid -Biostimulant on Chilli crop during *Summer* season of 2024 to study the efficacy and Phytotoxicity showed that the T_8: 7 ml/lit concentration in the spray solution, when sprayed on the foliage during establishment stage (7 DAT) and active vegetative stage (21DAT) recorded highest yield per hectare due to enhanced growth and improved yield contributing factors on the crop.

The treatment T_7:5 ml/lit concentration recorded second highest yield on the crop. The T_7 and T_8 treatments were statistically significant against control and single application treatments. As far as phytotoxicity, no phytotoxicity was observed till 7 ml per litre concentration and at a concentration of 10 ml per litre, less than 10 per of the total population treated recorded mild phytotoxicity symptoms.

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