

Evaluation of Biostimulants for low-cost input solution for onion crop (*Allium cepa* L.) cultivation in four districts of Maharashtra, by providing resistance or tolerance against abiotic and biotic stresses related to global climate change

ABSTRACT

The implementation of agronomic activities, based on the use of biostimulants, is an important element of agroecological practices. Therefore, a comprehensive field trial was carried out to evaluate the potential of biostimulants in growth promotion across four districts of Maharashtra (Jalna, Ahmednagar, Aurangabad and Nasik). The study aimed to evaluate the candidacy of selected biostimulants in improving onion (*Allium cepa* L.) extracted from proprietary technology involving adaptive molecular re-engineering for plant growth biostimulants category. Three biostimulants were preliminary analyzed for their nutritional content. Compositional analysis revealed the presence of several macro, micronutrients as well as humic, fulvic acid. The concentration of humic and fulvic acid in sediment extract were ranged from 34-38 and 10.5-10.8 g/100g respectively. The organic carbon, total nitrogen, phosphorus, and ash contents ranged between 54-57, 0.4-0.6, 0.3-0.4, and 3.3-7.2% respectively. Ash and total nitrogen content were the same for the extract 0.2% and 7.8% respectively. Onion crops were treated in three different modes, seed treatment, root drenching and foliar spray. Biostimulants were applied in combination with three different levels of chemical fertilizer 50%, 75%, and 100% RDF and compared with control (100% RDF without biostimulants). Several qualitative and quantitative parameters such as firmness, pungency, neck diameter, bulb diameter, and yield were analysed.

It was observed that all three biostimulants (Asaava, Somrith, and Yuvaani) significantly ($P < 0.001$) affected all the measured plant traits. In addition to this, overall, we found excess chemical fertilizer imposed adverse effect on plant growth parameters. Moreover, it was concluded that with seed treatment followed by root drenching and foliar spray and optimized dosage scheduling, yield can be

further improved. The best effect in overall growth and yield potential of the plants were noticed in Asaava treated plants.

Keywords: Biostimulants, Adaptive molecular re-engineering, Pesticide, Chemical Fertilizer, Environment

1. Introduction

Climate change has become a global brainstorming topic as well as a major challenge to face especially in agriculture industry. In the next century, the global temperature could increase up to 4.8°C and if it rises by 2°C or more, the production of the world's major crops such as wheat, rice, and maize will be hampered. Over the last century, the global mean surface temperature has increased by 0.6°C (Kumar et al., 2009). Though, the impact of climate change is not distributed evenly across the world (Chand, 2009). Globally, the impact of climate change on the agriculture sector has become a matter of thinking from a food security point of view because it can cause significant crop failures, shortage of yields, reduction in quality, and increasing pest and disease problems (Ayyogari et al., 2014).



Figure 1: MAHAFPC trial summary of 4 districts (Ahmednagar, Aurangabad, Nashik, Jalna) at Maharashtra

In Maharashtra, the country's biggest producer of onion, recent rains have reduced production of late kharif crop which according to MFPI report 'could cause reduced supply in the later weeks of January and February, 2022'. The benchmark prices of onion at Lasalgaon, Nasik, the hub of trade, on Wednesday was 1,850 per quintal against 1,350 per quintal prevailed a year ago. According to official sources, the retail prices of onion in key cities like Delhi, Chennai, Hyderabad and Kolkata have risen to Rs 40 a kg from Rs 33 a kg prevailed a week back. "Rains have adversely impacted the yield of kharif onion crop thus pushing up prices as the crop enters the market after the completion of harvest," Balasaheb Misar, an onion farmer from Manmad, Nashik district told FE.

Traders at Azadpur Mandi, the country's biggest wholesale market for fruits and vegetables, said that rainfall and hailstorm along with cold waves have adversely impacted the standing rabi or winter crop of tomato in Rajasthan, Punjab and Haryana. "If such erratic weather conditions could continue, the yield losses would be higher," a trader told FE. The benchmark mandi prices of tomato at Kollur, Maharashtra, was Rs 1,660 per quintal against Rs 860 per quintal a year ago. The retail prices across major cities are Rs 40-50 a kg.

Onion is currently being sown as rabi or winter crop and then the harvested crop will be kept in cold storage for ensuring supplies throughout the year, the MFPI report has noted that "erratic weather is posing the threat to the crop in Uttar Pradesh, Bihar and Madhya Pradesh as crop is at tuber forming stage and foggy weather and low temperatures will affect the formation of tuber". In onion seed production, it requires cool weather prior to and during early growth of seed stalk (Hazara and Som, 2009). The rising temperature during seed stalk growth period can affect it adversely and ultimately seed yield will be affected. Therefore, in current study we have chosen onion as an indicator crop.

Earlier under the similar situation, to cope up the increasing demand and attain self-sustainability, India agrarian system adopted several new initiatives. Agrochemicals played the prime role in increasing agricultural productivity, but its improper usage severely affected the soil health by reducing beneficial microbes and total organic carbon^{1,2}. One of the significant concerns with Indian agriculture is the decrease in productivity and production due to the increase in the degree of land degradation and area of degraded land³. Similarly, climate change and altered rain pattern imposing a severe threat to plant health and productivity. Among all the abiotic stresses, drought and soil salinization are the major obstacles for plant growth and health⁴⁻⁶. Tiwari et al 2018

Plant biostimulants, also known as "materials containing component(s) and/or microbes whose purpose when applied to plants or the rhizosphere is to stimulate natural processes to enhance/prefer plant growth and health through improving nutrient uptake, nutritional effectiveness, tolerance to abiotic biotic stress, and/or plant quality, irrespective of their nutrient constrained state," are one of the most innovative and effective solutions to address these important challenges (Yakhin et al. 2017). Algal extracts, complex organic materials, plant hormone-like chemicals, amino acids, and humic acids, among many other plant biostimulants, have been successfully proved for their beneficial qualities (De Vasconcelos and Chaves 2019).

However, nutrient-rich hidden sources of biostimulants like peat and sapropel have received far less attention. Specific biostimulants activities, such as improved root and shoot development, abiotic stress tolerance, water uptake, transplant shock reduction, and so on, have also been described (Adani et al. 1998; Petrozza et al. 2014). In hydroponic systems, biostimulants can also reduce fertilizer use and nutrient solution concentration (Vernieri et al. 2006).

Given the foregoing, we anticipate biostimulants playing a critical role in future agriculture. The biostimulants market was worth \$1,402.15 million in 2014, and it is predicted to grow at a CAGR of 12.5 percent to \$2,524.02 million by 2022. The expected drivers of this growth are (i) the growing importance of bioproducts in agriculture, (ii) the increased use of biostimulants in developing countries, and (iii) the increased global biostimulants presence and adoption by customers, as market players have developed a range of innovative products to meet specific crop needs (Povero et al. 2016).

While consumer awareness of the benefits of biostimulants on plants is growing, little attention has been paid to the critical scientific steps required for optimal source material selection, chemical-free extraction, and detailed biostimulant characterization in order to develop optimal solutions for specific agronomic needs. We can develop a list of natural sources or active substances that can be included in a future prototype based on further investigation of the desired attribute, a complete review of the scientific literature, and interaction with scientific experts in the research area of interest.

To identify, describe, and conserve certain active chemicals that can help plants achieve the desired physiological responses, a thorough understanding of the biological and chemical features of raw materials is required. As a result, it's critical to pick the correct location and extraction technology to get the best yield of certain biomolecules and compounds from the raw materials for the activities being investigated (Parys et al. 2009; Apostolidis et al. 2011).

In this context, sediment material such as sapropel, also known as biodeposit, which is made up of the remains of water plants, plankton, and benthic organisms, can be analyzed to see if it has the potential to be employed as biostimulants. Microorganisms, chemical and physical reactions (typically in an oxygen-free environment) change sapropel, which is then blended with mineral components derived from the water basin (Vincevica-Gaile and Stankevica 2018).

Sapropels have a complicated chemical composition with a wide range of values that are dependent on the location of the occurrence zone. Sapropel is often used in soil amendments to improve nitrogen, phosphorus, humus, and microelement content because of its organic-rich nature. It is a safe, efficient, and environmentally beneficial natural resource that is utilized as a biofertilizer and soil conditioner in agriculture (Stankevica et al. 2016).

Sapropel accumulation, formation, and intensive use in agriculture and energy have been reported in temperate regions of Asia and Europe, particularly in Latvia, Bulgaria, Ukraine, Russia, Lithuania, the Scandinavian Peninsula, Poland, France, Germany, and Belarus, and Canada and the United States from the continent of America in the Great Lakes region as well as most Middle Eastern countries such as Jordan and Saudi Arabia (Stankevica et al. 2016; Rummyantsev et al 2017).

Extraction technology is equally significant as the exploration of sapropel as a new raw material for biostimulant synthesis. Several researchers are conducting research to extract useful compounds from such materials; nevertheless, the bulk of them use chemical solutions for extraction or purification. Aqueous alkaline solution is used to extract HA from raw materials containing humic compounds in industrial operations (Saito and Seckler 2014; Zou et al. 2021).

The development of technology that does not use chemicals in the process but is yet efficient enough to create high-quality nutrient-rich extract is a promising direction. Adaptive molecular re-engineering® is the name we give to a highly differentiated biostimulants research, development, and production platform that we offer. This method uses hydrodynamic, flow pulsations to disintegrate big particles, enhance surface area and cavitation, and entail deep penetration of the water solvent into particle pores. These properties allow for the preparation of aqueous extract solutions including humic acids, fulvic acid, amino acids, and other compounds without the use of chemicals.

Our analysis seeks to shed light on the extent to which the adaptations of onion growers are effective in reducing vulnerability to climate change. Overall, we argue that the knowledge and adaptations of farmers, such as those in India, need to be taken into account when considering responses to climate change. Over centuries of pursuing livelihoods in the region, local people have experience of dealing

with unpredictable and highly variable weather conditions. Their knowledge and adaptations, may contribute to the formulation of appropriate strategies, to mitigate and adapt to climate change. In addition, these locally based initiatives offer the possibility of cost-effectiveness, as they will not require the government to invest substantial sums in creating entirely new strategies.

In this paper, we presented (i) a patented technology for spropel extraction that is robust, efficient, and chemical-free, (ii) compositional and ultrastructural analyses, and (iii) a plant growth bioassay to assess its potential for usage as biostimulants for plant growth promotion. The methodology is based on a combination of technology, know-how, and methods that have been accumulated over a decade of expertise in researching and developing effective biostimulants products.

2. Material and methods

2.1. Experimental site:

The field trial study was carried out during the Rabi season from October 2020 to May 2021 in a total of 36 hectares of area. The trial was conducted at 43 villages (Table 3) of Jalna district. Composite soil samples were collected before the trial for site characterization from 0-20 cm soil depth and analyzed for pH, Phosphorus, Nitrogen, Organic Carbon (OC), Potassium, and Texture (Table 1). Baseline data was also captured during the entire trial period. The mean annual rainfall in this region is 990 mm and the average annual minimum and maximum temperatures were 21° C and 42° C, respectively.

2.2. Experimental materials

Bombay Red variety of onion was used as an experimental material for experiment. It was released by Melkasa Agricultural Research Center during 1980. The variety has a characteristic of medium-red bulb color, erect leaf arrangement and flat globe bulb shape. It is an early maturing variety taking less than 120 days to reach maturity (EARO 2004; MoANRS 2011). Seeds were obtained from Shire-Maitsebri Agricultural Research Center. The inorganic and organic fertilizers used were N in the form of Urea ($\text{CO}([\text{NH}_2]_2)$ (46% N) and animal dung were used in the experiment. Animal dung was collected and composted in a pit for five months to produce well decomposed FYM. The FYM was collected from Shire-endasselassie Agricultural, Technical, Vocational and Educational Training College. Recommended dose of 100 kg ha⁻¹ phosphorus in the form of TSP (Triple super phosphate) (46% P₂O₅) was applied uniformly to all plots at the time of planting.

Organic biostimulant applied in this study was extracted from the highest-grade Russian peat through a 100% organic proprietary process called “Hydrodynamic Pulse Technology” which uses high-intensity ultrasound to change organic material without chemical additives and breaks peat substances to nano and micron-level size particles.

Seeds of Rice *Oryza sativa* L certified non-GMO were procured from seed stock maintained at seed distribution centre, Oman. The seeds were surface sterilized with 1% sodium hypochlorite for 1 min followed by three-time washing with tap water, blot-dried and used throughout the experiment²².

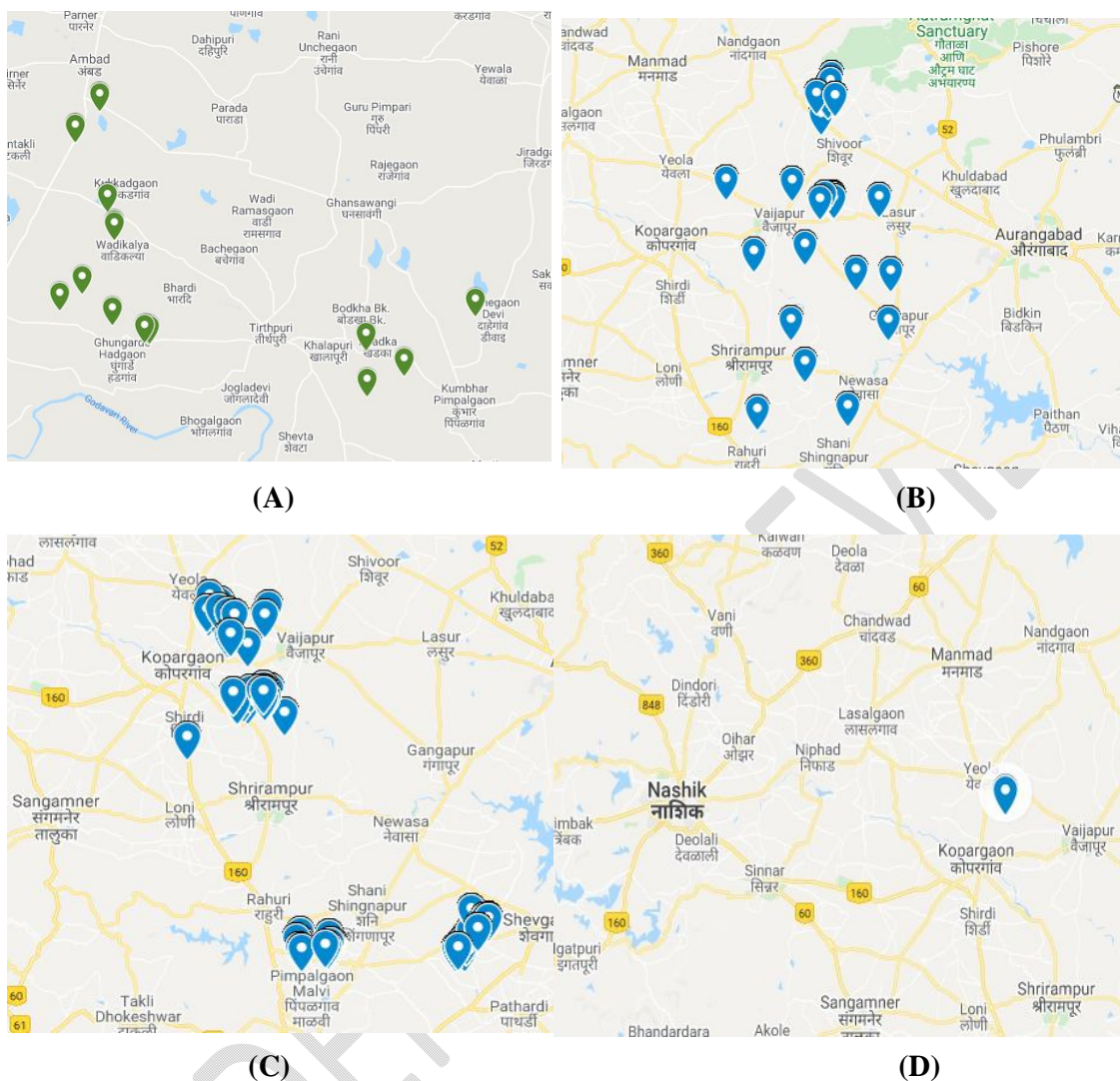


Figure 2: Different trial coordinates at Jalna (A), Aurangabad (B), Ahmednagar (C) and Nashik (D) district

2.3. Preliminary analysis

For all chemical analyses samples were used after being air dried at room temperature until constant weight reached. The extracted samples were then ground and sieved through 1mm sieve. The sediment samples were stored in sealed bottle for further analysis.

The pH, electrical conductivity (EC) and bulk density of the sediments were analyzed by FCO method methods. Carbon, Hydrogen, and present in the sample of lake sediment are analyzed by Perkin-Elmer 2400 CHN analyzer. Moisture content determined by the method of Meyer and Ishiwatari (1993). After taking moisture content, the remaining residue was used for ash content analysis by heating the samples overnight in a muffle furnace maintained at 600 °C (FCO 1985). The

carbohydrate and protein content of the sediment was measured by phenol sulphuric acid (Masuko et al 2005) and AOAC method (AOAC 2011). Total organic carbon and total carbon was estimated by FCO method (FCO 1985). Over 30 Macro and micronutrients (Cu, Fe, Zn, Mn, Cd, B, Ni, Pb etc) were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer) (Potortì et al. 2020) after microwave (Anton Paar Make model: Multiwave PRO) digestion with concentrated HNO₃. Macronutrients such as total nitrogen, phosphate, sodium, magnesium, total sulphur, calcium, etc. were estimated by FCO method (FCO, 1985). All determinations were performed in triplicate unless specified.

2.4. Field trial setup

The experiment was conducted with three biostimulants products treatment (Asaava, Somrith, and Yuvaani) along with three different doses of chemical fertilizer input (50% RDF, 75% RDF, and 100% RDF). Control i.e. 100% recommended dosage of fertilizer was maintained for each treatment.

Cultivation was done as per standard cultivation practices. A standard dosage of chemical fertilizer was incorporated by all farmers. In the whole life cycle of crop incorporated 100Kg N: 50Kg P: 50Kg K per ha as a 100% of RDF and accordingly 75% & 50% RDF was given. Seeds were sown in October Seeds were treated with working solution of biostimulant @ 40ml diluted in 1 litre water for 4hr. The compositional analysis of organic biostimulant has been done and given in table 1. A week before transplantation, chopped (2-3cm size) manure crops (Alfalfa, broad beans and sesbania aculeata) were added separately to the pot containing experimental soil and tilling was performed to incorporate the manure in the soil adequately. Thirty-five days old seedlings of basmati rice long-grained FL478 (Indica) variety were then root drenched with organic stimulant @500ml diluted in 100L water + 25L cow urine and transplanted on 1 February 2020. First and second foliar spray was done on 30th day @ 600ml (diluted in 120L water + 30L cow urine/acre) and 60th day @900ml (diluted in 180L water + 45L cow urine/acre). Irrigation was done till harvest, maintaining the water level in the pot at a depth of 2-3 cm above the surface of the soil.

Weeds were removed by hand and no formal pest control method was followed.

2.5. Growth parameter analysis

Soil samples from treated and non-treated plots were collected and analyzed for parameters such as texture, N, P, K, organic carbon (OC), and pH (Table 1). Similarly, onion plant samples were randomly collected from treated and non- treated plots and analyzed for quantitative and quantitative parameters such as plant height, no. of leaves, neck length, neck diameter, bulb diameter, bulb color,

pungency, firmness, yield. Bulb harvesting was done once plants were at proper physiological maturity (75% leaves withering). During harvesting, the bulbs were categorized into marketable and non-marketable yields. Marketable bulbs were greater than 20mm in diameter and free of cracking, diseases, insect, and mechanical damage. The plant height from the soil surface to the topmost growth is measured from randomly selected plants from the harvesting area of each treatment. The neck and bulb diameter were measured by using vernier scales. The number of leaves was calculated from randomly selected plants from sampling area of treated plots and compared with control. Qualitative parameters such as pungency was measured by cutting the onion into two halves followed by smell, whereas firmness and bulb color measured by pressing a onion through fingers and skin through visual observation.

Data on plant height, leaf number per plant, leaf length, leaf diameter, bulb diameter, neck diameter, average bulb freshweight, above ground dry biomass were collected from ten randomly selected plants. However, days to maturity, marketable, unmarketable and total bulb yields were recorded per plot base. Plants in the central four rows were used for data collection, leaving aside plants in the border rows and those at the end of each row.

Days to maturity The number of days from seedling transplanting to a day at which more than 70% of the plants in a plot showed yellowing of leaves or attained physiological maturity.

Plant height (cm) was measured using a scale ruler from the ground level to the tip of the terminal leaves of ten randomly selected plants at the time maturity. The average of ten plants was used for statistical analysis.

Leaf number per plant The total number of leaves per plant was counted from ten randomly selected plants at maturity. The average of ten plants was used for statistical analysis.

Leaf length (cm) This was measured at physiological maturity from the sheath to tip of the leaf of ten plants using a ruler and the average of ten plants was used for statistical analysis.

Leaf diameter (cm) diameter of leaves at three different parts were measured from ten randomly selected plants using digital caliper and the average of ten plants was used for statistical analysis.

Bulb diameter (cm) The mean bulb diameter of ten sample bulbs was measured at the wider portion of matured bulbs at harvest using digital caliper. The average of ten plants was used for statistical analysis.

Neck diameter (cm) The average neck width of ten randomly selected mature bulbs were measured using digital caliper and expressed in centimeter at harvest. The average of ten plants of bulbs were used for statistical analysis. Average bulb weight (g) The average fresh weight of ten randomly taken mature bulbs were measured using sensitive balance and finally then expressed in grams. The average of ten plants was used for analysis. Aboveground dry biomass (g) The above ground-biomass of ten plants were separated chopped and dried in an oven at 65 °C until constant dry weight was attained and immediately weighed and recorded as aboveground dry biomass. The average of ten plants was used for statistical analysis.

Marketable bulb yield (t ha⁻¹) This referred to as the weight of healthy and marketable bulbs that range from 20 to 160 g in weight of bulbs from the net plot area at the time of harvesting. Unmarketable bulb yield (t ha⁻¹) The total weight of unmarketable bulbs that are under sized (< 20 g), diseased, decayed and bulbs from plants with physiological disorder such as thick neck and split were measured from a net plot at final harvest and expressed in t ha⁻¹. Total bulb yield (t ha⁻¹) The total bulb yield was measured from the total harvest of the net plot as a sum weight of marketable and unmarketable bulb yields that were measured in kg per plot and finally converted into t ha⁻¹. Harvest index (%) This was expressed as the ratio of total bulb fresh weight to the total biomass fresh weight at harvest maturity and expressed in percentage. Harvest index was calculated as total fresh bulb weight divided to total fresh biomass weight multiplied hundred.

2.6. Economic analysis

The economic effect of biostimulants application was computed based on the value of yield increase resulting from the use of biostimulants and costs of their application.

Costs of cultivation in treated plot the (CoC) were calculated according to the following formula: $CoC = cs + cml + cm + cp + ci + cb$ (INR acre⁻¹),

where CoC is the cost of cultivation, (INR acre⁻¹), cs is the cost of seed, cml is cost of machinery and labour, cm is cost of miscellaneous, cp is cost of pesticides, ci is cost of inorganic fertilizer, cb is cost of biostimulant.

The average purchase price for all cultivation assets was based on information from wholesale markets. The cost of purchasing biostimulants was calculated as a retailer price.

$$\text{Net profit (}^{INR}\text{)} = \frac{\text{Gross profit (INR)}}{\text{acre}} - \text{Cost of cultivation (INR)}$$

$$\text{Gross profit (} \text{)} = \frac{\text{Yield achieved per acre (Qtl)} \times \text{Rate per Quintal (INR)}}{\text{acre}}$$

2.7. Statistical analysis

Weightage score was calculated as per Tiwari et al., 2018. Collected data were subjected to analysis of variance (One way ANOVA) using JMP Stat software version 16. Treatment means were separated using all pairs, Tukeys HSD test at 0.05% probability level. The data from 43 villages and 129 farmers were analyzed for plant height, root length, no. of leaves, neck length, neck diameter, firmness, skin colour, pungency, bulb diameter, yield and shelf life.

3. Results and Discussion

3.1. Compositional analysis:

The biostimulants sector needs better products for enhancing crop productivity. There are numerous reports in the literature of biostimulants increasing crop yields, improving growth (Yakhin et al. 2017), enhancing plant resistance to frost and protecting against pathogens (Rhods et al. 1986) but very few studies reported on the biostimulants like bioactivities in sediments. Organic carbon, and Humic substances were the major components of extract (Table 1 and 2). On the other hand, sediment extracts were also found enriched with macro and micronutrients (Table 3).

Table 1 Preliminary analysis of Peat and sapropel

S. No	Parameters, units	Sediment 1	Sediment 2
1	pH (1:2) @25°C	5.4±0.03	6.12±0.03
2	Electrical conductivity (1:2) @ 25°C (kg/l)	0.205±0.06	0.31±0.06
3	Total Carbon (%)	34.3±1.01	31.8±1.01
4	Total Organic Carbon (%)	57.62±1.01	54.8±1.01
5	Total Ash content (%)	3.34±0.8	7.2±0.8
6	Moisture (%)	40.6±0.8	42.28±0.8

*Data presented in table are mean (n=3) ± standard error

It was clearly apparent from analysis that both sediment samples have pH range suitable (5.4-6.0) for maximum nutrient and beneficial rhizospheric microbial availability. Organic acid content was also found signatory in both sediment sample, which make them more suitable for agriculture purpose.

Total organic carbon was the dominant element in both extracts recorded with 57.62 and 54.8 for biostimulants extract (BS) 1 & 2 respectively. The values of micronutrients such as Silicon (103.9 and 84.28 mg/kg), Aluminum (500.2 and 370.5 mg/kg), Calcium (0.12 and 0.18g/100g), sulfur (0.31 and 0.25 g/100g), potassium (0.017 and 0.015g/100g) and iron (4045 and 4647 g/100g) were found in both sediment extract tested. Organic acids such as humic and fulvic acid from sediment extract are expressed as a percentage.

According to the results presented in Table 2, humic acids concentration was high, 34.73% in case of BS1 and 37.31% for BS2 as compare to fulvic acid (10.51% and 10.69% for BS1 and 2 respectively).

Further ash content (3.34% for BS1 and 7.2% for BS2) was also found in line with findings of Sharma H.S.S. et al., 2016.

The protein contents were same for both the extract 4.81 and 4.03 g/100g for BS1 and BS2 respectively whereas carbohydrate content was 55.18% higher in BS1 in comparison to BS2. Densities (g/cm^3) of the biostimulants extract 1 and 2 was 1.8 and 1.01 g/cm^3 respectively indicating that biostimulants 1 was denser than the BS2. Variations in the macro and micronutrients are listed in Table 3, showing a relationship between high ash contents in BS1 (Table 2) and high concentrations of micronutrients.

Table 2 Results on biochemical analysis of surficial extract

S. No.	Parameters	Quantity	
		BS1	BS2
1	Humic acid, g/100g	34.73±0.03	37.31±0.03
2	Fulvic acid, g/100g	10.51±0.009	10.69±0.009
3	Protein g/100g	4.81±0.009	4.03±0.009
4	Carbohydrate g/100g	16±0.009	10.31±0.009
5	Bulk density (gm/cm^3)	1.8±0.009	1.01±0.009

*Data presented in table are mean (n=3) ± standard error

*BS1: Biostimulant extract 1

*BS2: Biostimulant extract 2

Table 3 Summary of results on macro and micronutrient content analysed in BS1 and BS2

S. No.	Parameters	BS1	BS2
1	Total Phosphorus (as P ₂ O ₅), g/100g	0.32±1.02	0.41±1.03
4	Total Potassium (as K ₂ O), g/100g	0.017±0.8	0.015±0.7
5	Total Nitrogen as N, g/100g	0.67±0.02	0.47±0.04
6	Ammoniacal Nitrogen, g/100g	0.018±0.003	0.011±0.004
7	Chloride as Cl, g/100g	1439±0.001	1290±0.002
8	Total Sulphur as S, mg/kg	0.31±0.003	0.25±0.006
9	Sodium as Na, g/100g	0.016±0.05	0.018±0.03
10	Calcium as Ca, g/100g	0.12±0.001	0.18±0.001
11	Magnesium as Mg, g/100g	0.02±1.03	0.052±1.03
12	Iron as Fe, g/100g	4045±0.001	4647±0.001
13	Boron as B, mg/100g	0.1±0.003	0.2±0.002
14	Lead as Pb, mg/kg	11.3±0.006	14.7±0.004
15	Zinc as Zn, mg/kg	6.8±0.03	7.6±0.04
16	Copper as Cu, mg/kg	1.4±0.15	0.5±0.18
17	Cobalt as Co, mg/kg	BDL (DL-0.01)	BDL (DL-0.01)

18	Nickel as Ni, mg/kg	BDL (DL-0.01)	BDL (DL-0.01)
19	Manganese as Mn, mg/kg	11.6±0.11	43.5±0.13
20	Chromium as Cr, mg/kg	BDL (DL-0.01)	BDL (DL-0.01)
21	Silicon as SiO ₂ mg/kg	103.9±0.06	84.28±0.08
22	Fluoride as F, mg/kg	112.15±0.04	165±0.03
23	Vanadium as V, mg/kg	BDL (DL-0.01)	BDL (DL-0.01)
24	Lithium as Li, mg/kg	BDL (DL-0.01)	BDL (DL-0.01)

*Data presented in table are mean (n=3) ± standard error

The structure and composition of biostimulants plays important role in controlling the behavior and mobility of minerals in the environment and contributes substantially to improving the global soil fertility status. These features together with major demand for safe food and sustainable agriculture have contributed to enlarge the environmental significance of peat and sapropel based biostimulants.

The presence of macronutrients and micronutrients, humic fulvic acids, and amino acids in both sediment extracts revealed physicochemical profiles that could make them useful as biostimulants in agricultural practices due to their positive effects on plant biomass production, nutrition, and activation of enzymes involved in nitrogen metabolism and glycolysis. Several studies have found that biostimulants like humic acid can cause morphological changes in plants, particularly a considerable increase in root biomass and stimulation of root hair growth, resulting in improved plant nutrient uptake and accumulation (Zandonadi et al. 2007, 2010; Nardi et al. 2016).

Baseline data analysis

The result of the physical and chemical analysis of experimental soil before planting had been depicted in Table 4. The textural class was found to be clay loam with a particle size distribution of 40% sand, 38% silt, and 22% clay (Hazelton and Murphy 2016). The pH value range was 5 – 9.0 which is neutral according to the rating of (Bruce and Rayment 1982). The optimum pH for onion production ranges between 6 and 8 (Nikus and Mulugeta 2010). Variable range of soil organic carbon content range was found between 0.02-7.5%, which is rated low to high according to Maria and Yost (2006). Maria and Yost (2006) stated that organic carbon content of < 1.5, 1.5–2.5, and > 2.5% grouped as a low medium, and high respectively. Since the trial was conducted at different locations, different factors such as soil, onion variety, cultivation practices, irrigation scheduling, plant spacing, transplantation date, no. of treatments, humidity, temperature, wind speed, precipitation etc. have been considered for the data analysis process. It was found that FarmicsTM Biostimulants products positively affected the onion qualitative and quantitative parameters in all the fields compared to control plots (Figure 3). Statistical analysis showed that the positive performance of Biostimulants products was found on plant growth parameters (Figure 4). It was also observed that Biostimulants along with high RDF (100%) showed decreased plant attributes as a comparison to low RDF (50% & 75%) which is also in line with the finding of several studies (Zia-Ur-Rehman et al., 2016; Wang, X et al., 2019; Guo, J. et al., 2019; Muhammad Hasnain et al.,

2020).

There is no single reason responsible for growth promotion by biostimulants. Numerous research and field study proved the role of biostimulants in plant growth promotion and how they play important role in environmental sustainability and soil quality restoration (Govindasmy and Chandrasekran, 1992; Boguta P. and Sokołowska Z., 2013; Taimur Ahmad et al., 2018; Wang D et al., 2019). Nutrients supplied by biostimulants release slowly compared with chemical fertilizer, resulting in less loss of Agri input and better preservation of the soil nutrients. The biostimulants also catalyze the production of stress hormones and provide tolerance against abiotic stress (Nardi S et al., 2015; Vasconcelos ACFD and Chaves LHG, 2019). This could also be the reason all treated plots performed better in a varied range of temperature stress (21-42°C). Biostimulants trigger effect-oriented action via the involvement of different but integrated mechanisms, which function as sequential events of complex networks at transcriptional and post-transcriptional levels (Garcia- Mina et al., 2004; Mora et al., 2014). In addition to this, it was observed that the enhancement in onion plant traits, could be due to the enhanced soil nutrient availability, plant nutrient uptake, and assimilation, and

increase mineral content through Biostimulants application. 30+ macro, micronutrients, 18+ analysed amino acids, humic, fulvic acid, and other trace minerals presence in our Biostimulants products positively affected root growth and nutrient uptake, improved plant nutrition by affecting soil properties (improvement of the soil structure or improvement of micronutrient solubility in the soil) and by directly affecting the plant's Agronomy physiology (changes in root morphology). *Further from the trial, it was also concluded that excess chemical treatment (control) leads to decreased productivity and quality.* Similar results were also in concurrence with several findings (Liu, E et al., 2010; Li, Z et al., 2017; Hasnain M. et al., 2020). Significant increase in disease resistance and enhanced vigour was also reported in all plots.

Table 4. Soil Physiochemical properties of soil collected from different locations

S. No	Parameters	Values
1	Texture	Clay loam soil
2	pH	4.5-8.5
3	Organic Carbon (%)	0.02-7.5
4	Nitrogen (Kg/ha)	100-700
5	Phosphorus (Kg/ha)	7-60
6	Potassium (Kg/ha)	60-300

Table 5. Baseline data details during trial period (Dec 2020 – Jun 2021)

S. No	Parameters	Values
1	Temperature (°C)	21- 42
2	Humidity (%)	35-69
3	Wind speed (Km/h)	5-35
4	Precipitation (mm)	2-6

Plant growth parameter

UNDER PEER REVIEW

Table 6: Effect of Farmics™ biostimulants on Onion's (*Allium cepa* L.) plant growth parameters

Treatment	Root length	No. of leaves	Plant height after 30 days (cm)	Seedling stand count	Seedling vigour index	Germination	Pungency	Firmness	Bolting percentage (%)	Number of leaves/plant
	Asaava									
Control	7.94±0.12	6.88±0.48	36.4±0.26	94.089	6.02±0.29		3.82±0.21	5.4±0.09		8.333
50% RDF	8.86±0.15	11.2±0.25	49.3±0.31	94.479	8.77±0.34		7.5±0.18	8±0.11		6.667
75% RDF	9.72±0.19	9.85±0.45	42.9±0.42	94.360	7.43±0.41		5.53±0.09	6.63±0.12		8.667
100% RDF	8.87±0.20	6.93±0.34	38±0.22	94.301	6.84±0.3		5.16±0.12	6.92±0.08		7.833
	Somrith									
Control	8.23±0.09	6.58±0.52	34.7±0.34		5.94±0.45		4.1±0.09	5.4±0.09		
50% RDF	10.5±0.12	10.8±0.34	52.2±0.37		8.56±0.33		8.2±0.11	7.8±0.15		
75% RDF	9±0.13	9.43±0.39	40.2±0.42		7.17±0.38		5.8±0.13	7±0.18		
100% RDF	9.58±0.15	6.66±0.37	36.3±0.45		6.54±0.42		5.56±0.17	7.24±0.16		
	Yuvaani									

Control	7.99±0.06	7.43±0.48	36.7±0.53		6.2±0.62		4.39±0.13	5.72±0.09		
50% RDF	9.19±0.08	7.92±0.38	39.4±0.42		6.3±0.56		5.5±0.16	7.5±0.12		
75% RDF	9.74±0.09	9.97±0.44	41.3±0.28		7.05±0.73		4.64±0.12	6.64±0.13		
100% RDF	8.69±0.11	7.45±0.49	41±0.32		6.9±0.45		5.29±0.09	6.71±0.14		

UNDER PEER REVIEW

Plant height

Plant height is an important growth parameter. The mean plant height at physiological maturity was significantly affected by Biostimulant treatment over control with maximum value (52.2cm) in Somrith+50% RDF followed by Asaava+50% RDF (49.3cm) > Asaava+75% RDF (42.9cm). The results also showed that the product Yuvaani requires more RDF to exert beneficial effects similar to the other two products (Asaava & Somrith). In these trials the crucial seed soaking application phase was not done due to the late start of trials. The onion seed variety/genotype can also lead to difference in growth performance. Interestingly, same observation was found in stagewise analysis. No significant growth was observed in plant height over control at the vegetative stage, whereas immediately after root drenching and first foliar spray, significant difference was found. The increase in height could be due to biostimulant application due to presence of 18+ analysed amino acids which are the building block of protein. These proteins involve in several metabolic processes required for plant growth. Similar results have been reported by Amans et al. (1996), Kumar et al. (1998), Khan et al. (2002), El-Shaikh (2005), Shaheen et al. (2007) and Abdissa et al. (2011).

Similar conclusions were obtained from other studies with various plant species (Brunetti, G et al., 2019). Biostimulants used as biostimulants and the method of their application (seed treatment, rootdrenching, and foliar) significantly affected the growth, physiology, and yield of onion as well as the quality characteristics. Our study was also in line with the findings of Kocira et al. (2020) where he observed a significant increase in yield and nutraceutical properties of the bean by biostimulant treatment. Biostimulants are also known for their auxinic activity which helps in root development and nutrient absorption required for plant growth. According to the study of Zandonadi et al. (2007) where he comparatively evaluated the effects of indole-3-acetic acid and humic acids (HA) isolated from different soil substances on maize root development and activities of the plasmalemma and tonoplast H⁺ pumps. They observed that HA, as well as low Indole acetic acid concentrations, stimulated root growth by inducing the proliferation of lateral roots along with a differential activation not only of the plasma membrane but also of vacuolar H⁺-ATPases and H⁺-pyro-phosphatase.

No. of leaves

The data concerning no. of leaves are presented in Figure 4. Overall, all three products performed best in combination with RDF. Asaava and Somrith at the combination of 50% RDF showed the best result (11.2 & 10.8) as compared to control (6.88 & 6.58). Similar to plant height results, here as well Yuvaani performed less and maximum results were found with 100% RDF. The possible reason could be due to high particle size, absence of functional groups required for metal mobility and inability to rejuvenate soil microbiome. Another reason could be due to difference in transplantation dates. This result is also aligned with the findings of Khadrah SHA et al (2017) where he found the effect of early transplantation on plant height, leaf area, bulb size, and yield. Transplanting dates of onion seedling reflecting the effect of edaphic factor and all environmental conditions on large scale on growth, bulb yield, bulb quality which differ from one region to another. Thus, optimum transplanting dates have a vital role in maximizing plant growth (Bharti and Ram, 2014, Mishra et al., 2014, Ali et al., 2016).

In addition to this, during the trial period, no chlorosis symptoms were noticeable in Biostimulants treatments however in some control plots chlorosis symptoms were observed in the control-treated plots of some farmers. The biostimulants products provide multiple benefits to crops, apart from nutrition, increased mobility of essential metals, smaller loss of the nutrients it activates several signals for abiotic stress tolerance (Garcia et al., 2020; Ossten et al., 2017).

Overall, plants accumulated more biomass in Biostimulants treatments than plants with chemical fertilizer in this study and even other scientific trials (Ravindran, B et al., 2019; Zandvakili, O.R et al., 2019). The trial suggests that the biostimulants improved the productivity and total biomass of plants. The humic and fulvic acid content in biostimulant has been reported to improve soil fertility by increasing the soil's water-holding capacity, porosity, aeration, and reducing nutrient leaching, which in turn results in better plant physiology (Trevisan S et al., 2010). Here our Biostimulant range is not only acting as a direct impact facilitator for better nutrient absorption by plants, but also indirectly affecting the morphology and physical parameters of the plants by influencing the beneficial soil organism community structure and nutrient turnover (Canellas, L.P. et al., 2015; K. Stankevica et al., 2019).

Similar to plant height trait, excess chemical fertilizer had an adverse effect on the no. of leaves (Figure 4) as well. It has been widely reported that plants would maintain strong growth under appropriate levels of nitrogen fertilizer (Zia-Ur-Rehman, M et al., 2016; Chekli, L et al., 2017; Wang, Y. et al., 2019). One of the reasons for the unsatisfying plant growth with a higher ratio of chemical fertilizer might be the inhibition of root and shoot growth in response to a higher level of available nutrients that were directly provided by chemical fertilizer (Wang, X et al., 2016; Guo, J et al., 2019). Furthermore, the smaller number of micronutrients (compared with the biostimulants), that could not be provided by the chemical fertilizer was also inhibiting the growth of the plants (Zia-Ur-Rehman, M. et al., 2016). Therefore, a well-balanced macro and micronutrient solution are essential to ensure favorable plant growth and health (Chekli, L. et al., 2017). Similar to plant height (Figure 5) stagewise analysis of no. of leaves (Figure 4) showed more influence at bulb initiation and formation and lower in early-stage as seed treatment was not done in any plot which brought our attention to the importance of biostimulant

treatment at seed level. In the case of Asaava, maximum no. was found with 50% treatment followed by 75% > 100%. Comfortable plant growth concerning plant height, number of leaves per plant, enhanced vigor could also be attributed to an increased rate of photosynthesis and assimilation in plant tissues. Biostimulants improve the photosynthetic efficiency of the plant in several ways. Chlorophyll is the dominant pigment in a mature plant cell. Chlorophyll is synthesized within the chloroplast from a plentiful precursor, the amino acid glutamate which is provided by the FarmicsTM Biostimulants nutritional package. Furthermore, Biostimulants also take part in the structural formation of chlorophyll. This compound is consisting of a magnesium ion encased in a large ring structure known as a chlorin. (Robert D. Willows, 2006).

Neck length (NL) and Neck diameter (ND)

Similarly, both NL and ND were significantly affected by FarmicsTM Biotimulant treatment. Maximum results for NL were found with Asaava+50% RDF (3.9cm) followed by Somrith+75% RDF (3.8cm) which was high over control, 3.04cm in both case. However, for neck diameter,

Somrith along with 50% RDF performed best (3.44cm) followed by Asaava +50% RDF (3.38). Yuvaani like other parameters continuously performed better at high chemical fertilizer level (100% RDF). The lowest NL of 2.55cm was obtained with control and statistically at par with that obtained at combinations of Biostimulants with 50%, 75%, and 100%. In conformity, Yohannes et al. (2017) reported that neck thickness is one of the important parameters which is highly significantly influenced by the combined application of organic and inorganic fertilizers. The enhanced neck thickness could be due to high photosynthesis and respiration efficiency which leads to providing more energy for neck formation (Negasi et al., 2017).

Table 7. Effect of Farmics™ biostimulants on Onion (*Allium cepa* L.) neck length and diameter

Treatments	Neck length	Neck diameter	Bulb diameter (Vertical)	Bulb diameter (Horizontal)	Internal sprouting	Weight loss
Asaava						
Control	0.937±0.03	2.04±0.04	3.32±0.16	3.59±0.17		
50% RDF	1.210±0.028	3.36±0.045	4.61±0.09	4.99±0.18		
75% RDF	1.046±0.06	3.02±0.034	3.86±0.11	4.3±0.20		
100% RDF	0.988±0.0	2.88±0.03	3.48±0.17	4.26±0.21		
Somrith						
Control	3.04±0.05	2.55±0.054	3.79±0.08	3.73±0.07		
50% RDF	3.59±0.04	3.44±0.058	4.14±0.014	4.56±0.06		
75% RDF	3.83±0.047	2.94±0.057	3.96±0.07	4.92±0.12		
100% RDF	3.53±0.037	2.78±0.04	3.9±0.09	4.47±0.13		
Yuvaani						
Control	2.67±0.06	2.51±0.07	3.71±0.12	3.68±0.09		
50% RDF	2.75±0.07	2.7±0.067	3.88±0.13	4.26±0.12		
75% RDF	2.55±0.05	2.91±0.07	3.78±0.09	4.24±0.14		
100% RDF	3.13±0.04	2.86±0.042	4.03±0.11	4.89±0.13		

Root length

Roots have important roles for plants to withstand adverse environmental conditions.

Biostimulant treatment has significant effects on root length. The highest root length was found on Farmics™ Biotimulant treated crops. Root length increased and varied between 7.94 - 10.5% (Figure 5) in treated plots. The biochemical and molecular mechanisms underlying these events are only partially known. Our product contains humic and fulvic acid and they have been known to contain auxin and an “auxin-like” activity (Macdonald

H. 1997). They are known to be involved in root development and having potential antioxidant properties were identified. This was in accordance with research conducted by Suwardi and Wijaya where humic acid treatment can increase the length and dry weight of rice and corn roots. Anwar also stated that humic acid increased the dry weight of wheat. An experiment was conducted with a biostimulant and different water regimes (full, partial, and non-irrigated irrigation) to evaluate the action of this biostimulant on main rootlength. They promoted greater growth, both in plant height and in length of the main root. Biostimulants promotes a deepening of the roots and allows the capture of water in deeper layers of the soil, favoring the maintenance of its growth for a longer time. In addition, the hypothesis of a HS auxin-like activity was also supported by reports showing a positive effect of such substances on specific targets of auxin action.

Bulb Diameter (horizontal and vertical girth)

Bulb diameter (Horizontal and Vertical) at maturity stage as affected by biostimulants along with 3 different RDF levels (50%, 75%, and 100%) and their interaction are presented in Figure 5. Bulb diameter was markedly affected by biostimulant treatment and their combination. Application of Asaava product at a combination of 50%RDF gave the better bulb diameter (Horizontal- 4.99cm, Vertical-4.61cm) and minimum observed with control (Horizontal- 3.59cm and Vertical-3.32cm). Yuvaani also performed significantly high in comparison over control but with high chemical fertilizer (100%RDF) requirement. The beneficial effect of the other two biostimulants might be due to the optimum dose of RDF (50%), leading to an increase of nutrients elements in the soil, which ultimately affects the bulb diameter. These results are in agreement with those obtained by Mahmoud., et al. and Sahar., et al.

Regarding the effect of bio-stimulants on this criterion, Figure 5 shows that bulb diameter tended to be higher with root drenching and foliar spraying with biostimulants, than those treated with only chemical fertilizers. The difference between biostimulant treatments was significant at all growth stages. This favorable effect of biostimulants might have been due to their effective role in improving early onion growth, more dry matter accumulation, and stimulated the building of metabolic products that translocated to, which resulted in increasing bulb diameter. Such findings were reported also by El-Gabry., et al.

Further enhanced growth of root, also played a significant role in nutrient absorption, water transport to plants which ultimately helps in bulb formation.

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UNDER PEER REVIEW

Firmness, skin tightness and pungency

The number of growing points, bulb size and shape, number of scales, pungency, and bulb firmness are important bulb quality characters in the fresh market and onion processing industry. Onion (*Allium cepa* L.) is a vegetable crop grown for its pungent bulbs and flavorful leaves. Increasing bulbs Sulphur content that resulting from root drenching followed by spraying with the Farmics™ biostimulants can affect bulb quality, especially its pungency, which is defined as the combination of onion flavor and odor that are functions of the concentrations of sulphonic and thiosulphonic volatile acids containing Sulphur (De Souza et al., 2015). The obtained results probably due to various phytohormones signaling catalyzed due to biostimulant in addition to that rich in mineral, macronutrient and micronutrient contents that led to improve the formation of vigorous plant root system which increased water and nutrients absorption from the soil (Ocak and Demir 2012; Sanli et al., 2015; Dawood et al., 2019; Pyakurel et al., 2019).

The results of this study revealed that biostimulant application increased pungency level in crops treated with Somrith (8.2) and Asaava (7.5) followed by Yuvaani. Our Farmics™ product also contains optimum level of sulphur content and it is known that this is essential for increasing the onion yield and pungency level in soils having sufficient sulphur. However, excess sulphur application did not influence the yield and pungency level significantly. Several studies have been conducted to determine the influence of sulphur level on onion pungency (McCallum et al. 2005) who indicated increased pungency level with higher sulphur nutrition in the soil.

Pungent flavour of onion is derived from number of volatile sulphur compounds. These compounds are produced when the onion cell is mechanically disrupted, bringing the enzyme allinase into contact with flavor precursors such as S- alk(en)yl-L-cysteine sulfoxides (ACSOs). Besides the volatile sulphur compounds, the enzymatic break down of ACSOs also produces ammonia, pyruvic acids and lachrymator factor. The amount of pyruvic acid generated has been shown to be correlated with onion pungency. This enzymatically produced pyruvic acid is commonly accepted measure of pungency. Lancaster et al. (2001) also showed low sulphur supply reduced the sulphur in cell walls and also decreased the bulb hardness and the storage life of onion bulbs. Qureshi and Lawande (2006) also reported that the storage losses were decreased with application of sulphur.

In addition to pungency, Farmics™ also found effective in improving firmness, skin color as compare to control. This parameter is qualitative, and analysis varied from human to human.

Bulb firmness is desirable for minimizing quality losses caused by bruising, puncturing, and cutting during various harvesting and post harvesting operations. Even bulb firmness wise, Farmics™ biostimulant Asaava (8) and Somrith (7.8) along with 50%RDF performed better than control. Although this subjective rating of bulb firmness provides an estimate, variation exists between individual testers.

Table 8. Effect of Farmics™ biostimulants on pungency, firmness and bulb colour of Onion (*Allium cepa* L.) crop

Treatments	Firmness	Skin	Pungency
Asaava			
Control	5.4±0.09	4.16±0.11	3.82±0.21
50%RDF	8±0.11	6.25±0.12	7.5±0.18
75%RDF	6.63±0.12	6.52±0.09	5.53±0.09
100%RDF	6.92±0.08	3.48±0.14	5.16±0.12
Somrith			
Control	5.4±0.09	4.4±0.15	4.1±0.09
50%RDF	7.8±0.15	7±0.12	8.2±0.11
75%RDF	7±0.18	6.8±0.17	5.8±0.13
100%RDF	7.24±0.16	6.2±0.18	5.56±0.17
Yuvaani			

Control	5.72±0.09	4.56±0.11	4.39±0.13
50%RDF	7.5±0.12	7.5±0.12	5.5±0.16
75%RDF	6.64±0.13	6.64±0.09	4.64±0.12
100%RDF	6.71±0.14	5.76±0.13	5.29±0.09

Bulb yield

Data presented in Figure 5 show the effect of biostimulants and their different combination levels with chemical fertilizers on average bulb weight. In the present study, the fresh weight yield of bulbs, was higher in biostimulants treated plants than the mean yields in control plants. The obtained results clearly showed that from the three studied treatments of biostimulants (Asaava, Somrith and Yuvaani) and mineral fertilization, Asaava+50%RDF yielded highest onion bulb yield followed by Somrith but with 75%RDF. Although Yuvaani also performed better but with 100%RDF which is not economically beneficial to farmers.

Data in Figure 5 elucidate that, the interaction between chemical fertilizer levels and bio stimulant treatments had a significant effect on yield (Qtl/acre) as well as average bulb weight (g/100bulb). Maximum yield (135 Qt/acre) resulted from onion plots that were treated with Asaava> Somrith>Yuvaani. Similar trend was found for bulb size, 6.13, 5.68 and 5.65 respectively.

Root drenching followed by 1st and 2nd Foliar spray with biostimulant yielded positive effect in this trait. The detected positive effects of product on marketable bulbs yield might be related to its beneficial effects on vegetative growth characters, which probably supplied more photosynthates and hence, might help in increasing yield potential. These results are in line with those obtained by Bettoni et al.

This effect might also be due to applying bio-stimulants together with fertilizer, which increased microorganisms in the soil, and thus converting the ability of mobilizing the unavailable forms of nutrients elements to available forms. On the other hand, the microorganisms produced growth-promoting substances, which increase the plant growth. This increase in plant growth may be increasing the photosynthetic rates leading to an increase of the assimilation rates. Hence the average bulb weight increased, and consequently increased the marketable yield, as also reported by Hafez and Geries.

Table 9. Onion (*Allium cepa* L.) bulb colour and size in control vs biostimulants treated plot (Product used -Asaava)

Treatments	Bulb colour (white, light red, medium red, red and red dark)	Bulb size (small, medium and big)
Asaava		
Control	7.13±0.08	5.02±0.18
50%RDF	7.16±0.02	6.13±0.17
75%RDF	8.19±0.04	5.47±0.16
100%RDF	8.31±0.09	5.09±0.12
Somrith		
Control	7.17±0.12	5.2±0.16
50%RDF	7.84±0.14	5.68±0.09
75%RDF	7.98±0.08	5.07±0.12
100%RDF	8.44±0.13	5.3±0.14
Yuvanni		
Control	7.23±0.08	5.05±0.12
50%RDF	7.74±0.06	5.21±0.13
75%RDF	8.3±0.17	5.09±0.14
100%RDF	7.57±0.012	5.65±0.21

Table 10. Onion (*Allium cepa* L.) bulb yield and average bulb weight in Control vs Farmics™ Biostimulant treated plot

Treatments	Average bulb weight (g/100 bulb)	Yield in Qtl/acrE
Asaava		
Control	7598±0.08	106±0.18
50% RDF	8958±0.02	135±0.17
75% RDF	8795±0.04	119±0.16
100% RDF	8595±0.09	114±0.12
Somrith		
Control	8036±0.12	106±0.16
50% RDF	8378±0.14	121±0.09
75% RDF	9380±0.08	134±0.12
100% RDF	8628±0.13	109±0.14
Yuvanni		
Control	7688±0.08	109±0.12
50% RDF	8921±0.06	95.5±0.13
75% RDF	9156±0.17	114±0.14
100% RDF	7553±0.012	133±0.21

ECONOMIC ANALYSIS

The average purchase price for all cultivation assets was based on information from wholesale markets. The cost of purchasing biostimulants was calculated as a retailer price.

From the economic analysis we observed 11% to 19% difference in net profit through biostimulants application. The net profit depends on several factors such as farmer's cultivation practices, agroclimatic conditions and adherence level to Farmics™ user guide. Apart from net profit, overall district wise fertilizer cost was reduced upto 20.06 % on test plot where Farmics™ was used compared to control plot. In addition to yield increase, a reduction was found in insecticide and fungicide usage upto 42%. Our products also provide tolerance against abiotic and biotic stress, and hence reduction in insecticide and fungicide usage also was clearly apparent in treated plots.

Table 11. Gross and net profit analysis of plots treated with Farmics™ application vs control

Treatments	Net profit (Rs)	Gross profit (Rs)
Ahmednagar		
Farmics™ Treated	47738±0.08	109167±0.18
Control	39603±0.02	102895±0.17
Aurangabad		
Farmics™ Treated	62635±0.12	117609±0.16
Control	51962±0.14	108976±0.09
Jalna		
Farmics™ Treated	106795±0.08	142122±0.12
Control	68687±0.06	105945±0.13
Nasik		
Farmics™ Treated	27250±0.08	100894±0.08
Control	19872±0.08	94403±0.08

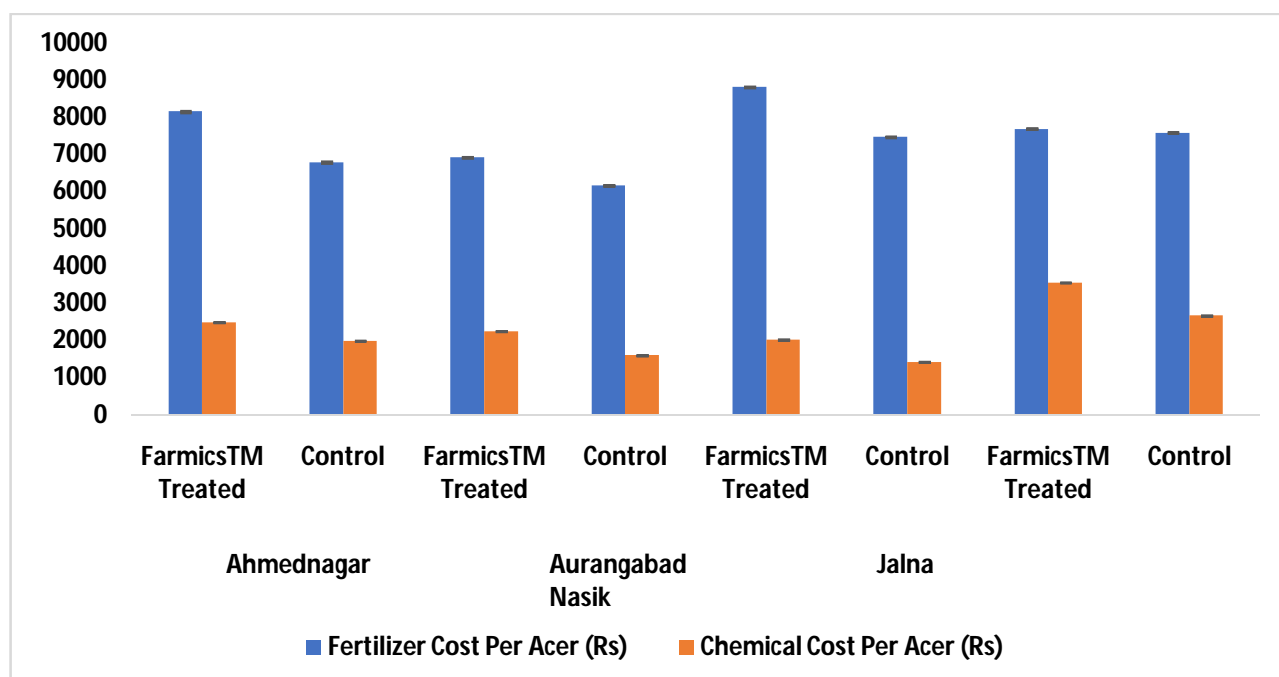


Fig.4. Fertilizer and Pesticide cost in Farmics treated and Control plot in 4 district of Maharashtra

Conclusion

In these MAHAFPC trials undertaken towards low-cost onion cultivation, to determine an optimum dosage of fertilizer along with biostimulant application, a systematic integration of data from various sources like soil, water and plant growth parameters was involved. However, every criterion will contribute towards the suitability in variable degrees. The relative importance of these parameters can be well evaluated to determine the suitability of weightage techniques (Section 9.7).

In conclusion, the combination of fertilization of onion plants with 50% RDF in the case of Asaava, 75%RDF in Somrith and 100%RDF in Yuvaani, was found to be the best-combined rates in this study for giving the highest bulb yield, with high net returns under field environmental conditions.

Our ideology behind these trials was to increase the production, productivity and utilization of onion for economic livelihood and nutritional security. It is concluded that secondary micro nutrient imbalance is not only decreasing the vegetable yield, but also affected the quality of produce.

It was observed that all three biostimulants (Asaava, Somrith, and Yuvaani) positively affected all the measured plant traits such as plant height, no. of plants, neck length, neck diameter, bulb diameter, bulb colour, firmness, pungency, and bulb yield. Various critical environmental, physical, and chemical factors such as temperature, humidity, variety of onion seed, transplantation date, row spacing, soil type, soil - water quality and texture have been considered during decision making in the trial. The results clearly showed that the biostimulant products

with gowmutra, could be applied as a complete package to provide nutrients, vitamins, amino acids, abiotic and biotic tolerance to promote plant growth, increase bulb production, and improve soil fertility in clay loamy soil.

An optimal combination of Biostimulants and fertilizer, correct treatment application schedule, dosage, and modes of treatment play a critical role in commercial onion production. It was also concluded that since seeds were not treated with biostimulants, which is a crucial part of the application schedule, a substantial additional difference could be seen in treated crops in future trials. In addition to this, overall, we found excess chemical fertilizer imposed an adverse effect on plant growth parameters.

Further, the economic analysis indicated that with the application of Farmics™ 11% (100% RDF) to 19% (50% & 75% RDF) difference in net profit through biostimulant application was obtained. The economic effect of biostimulants application was computed based on the value of yield increase, resulting from the use of biostimulants and cost of their application.

The net profit depends on several factors such as farmer's cultivation practices, agroclimatic conditions and adherence level to Farmics™ user guide. Apart from net profit, overall district wise fertilizer cost was reduced upto 20.06 % on test plots where Farmics™ was used compared to control plots. In addition to yield increase, a reduction was found in insecticide and fungicide usage upto 42%. Our products also provide tolerance against abiotic and biotic stress, and hence reduction in insecticide and fungicide usage also was clearly apparent in treated plots.

All trial results were disseminated and shared between partners and farmer associations, through numerous dissemination and transfer actions. A crisp weightage average report covering Project web page and an app with an integrated Knowledge base will be established, containing all project results and external links. The best identified Farmics and cultivation strategies will be used for efficient Onion production.

A total of nine FPCs were involved under the MAHAFPC trials and were evaluated for weightage average scorecard. To undertake low-cost onion cultivation; to determine an optimum dosage of fertilizer along with biostimulant application, a systematic integration of data from various sources like soil, water, plant growth parameters was involved. Every criterion contributed their role to determine the potential of Farmics™ biostimulant beneficial effect, contributing to a significantly higher overall profit. In the current trials, the weighted averages have been calculated to total 80% since 10% weightage has been allotted to shelf life and 10% to crop nutrition. Both these values of shelf life and crop nutrition will be ascertained in Sep 2021 by precise evaluation with specialist instruments. The USP of Farmics™ based on past experimental data, shows that Farmics™ treated crops will show a very significant positive difference in both the shelf life and crop nutrition. Hence the weighted parameter average will significantly further increase to add further impetus to the very significant and enhanced performance shown by the Farmics™ range of biostimulants.

The weighted average data shows that Farmics™ treated plots have 88.71% better performance concerning qualitative and quantitative characters for a particular crop cycle (Annexure I). Farmics™ treated plots have performed superior irrespective of the FPC or farmer across the tested locations. The same was endorsed by all the involved 9 FPCs and 125 Farmers (Annexure II).

Overall, it was concluded that with seed treatment followed by root drenching and foliar spray and revised dosage scheduling, yield can be further improved for the oncoming Kharif season. The hesitancy by the farmer initially during the trials to proceed with 50% % 75% RDF can be obviated by enhanced farmer confidence in the next cropping season leading to the best yields. Not missing out on the crucial seed soaking application phase will also give significantly better trial results.

References

1. Kumar V, Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*. 2009 Feb;84(2):151-7.
2. Chand D, Wood R, Anderson TL, Satheesh SK, Charlson RJ. Satellite-derived direct radiative effect of aerosols dependent on cloud cover. *Nature Geoscience*. 2009 Mar;2(3):181-4.
3. Ayyogari K, Sidhya P, Pandit MK. Impact of climate change on vegetable cultivation-a review. *International Journal of Agriculture, Environment and Biotechnology*. 2014;7(1):145-55.
4. Tiwari GN, Meraj M, Khan ME. Exergy analysis of N-photovoltaic thermal-compound parabolic concentrator (N-PVT-CPC) collector for constant collection temperature for vapor absorption refrigeration (VAR) system. *Solar Energy*. 2018 Oct 1;173:1032-42.
5. Yakhin OI, Lubyantsev AA, Yakhin IA, Brown PH. Biostimulants in plant science: a global perspective. *Frontiers in plant science*. 2017 Jan 26;7:2049.
6. De Vasconcelos AC, Chaves LH. Biostimulants and their role in improving plant growth under abiotic stresses. *Biostimulants in plant science*. 2019 Nov 7:1-4.
7. Adani F, Genevini P, Zaccheo P, Zocchi G. The effect of commercial humic acid on tomato plant growth and mineral nutrition. *Journal of plant nutrition*. 1998 Mar 1;21(3):561-75.
8. Petrozza A, Santaniello A, Summerer S, Di Tommaso G, Di Tommaso D, Paparelli E, Piaggese A, Perata P, Cellini F. Physiological responses to Megafol® treatments in tomato plants under drought stress: A phenomic and molecular approach. *Scientia Horticulturae*. 2014 Jul 22;174:185-92.
9. Vernieri PA, Borghesi E, Tognoni F, Serra G, Ferrante A, Piaggese A. Use of biostimulants for reducing nutrient solution concentration in floating system. In *III International Symposium on Models for Plant Growth, Environmental Control and Farm Management in Protected Cultivation* 718 2006 Oct 29 (pp. 477-484).
10. Vincevica-Gaile Z, Stankevica K. Impact of micro-and macroelement content on potential use of freshwater sediments (gyttja) derived from lakes of eastern Latvia. *Environmental geochemistry and health*. 2018 Oct;40:1725-38.

11. Stankevica K, Vincevica-Gaile Z, Klavins M. Freshwater sapropel (gyttja): its description, properties and opportunities of use in contemporary agriculture. *Agronomy Research*. 2016;14(3):929-47.
12. Saito B, Seckler MM. Alkaline extraction of humic substances from peat applied to organic-mineral fertilizer production. *Brazilian Journal of Chemical Engineering*. 2014;31:675-82.

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