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C Vennila
Associate Professor, Livestock Farm Complex, TANUVAS, Chennai, Tamil Nadu, India

T Muthuramalingam
Associate Professor, Livestock Farm Complex, TANUVAS, Chennai, Tamil Nadu, India

A Shanmuga Sundaram
Associate Professor, Livestock Farm Complex, TANUVAS, Chennai, Tamil Nadu, India

K Thilak Pon Jawahar
Associate Professor, Livestock Farm Complex, TANUVAS, Chennai, Tamil Nadu, India

S Meenakshi Sundaram
Associate Professor, Livestock Farm Complex, TANUVAS, Chennai, Tamil Nadu, India

Corresponding Author:
C Vennila
Associate Professor, Livestock Farm Complex, TANUVAS, Chennai, Tamil Nadu, India

Evaluation of liquid organic manures as biostimulants on the agronomic performance of spinach

C Vennila, T Muthuramalingam, A Shanmuga Sundaram, K Thilak Pon Jawahar and S Meenakshi Sundaram

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Abstract
Evaluation on the effect of organic biostimulants application on the performance of spinach was experimented. The organic biostimulants such as panchagavya and jeevamrutha prepared at the farm with products of native cattle breed of India, Gir was used for application. The treatments are T_1: Without nutrients, T_2: Recommended dose of nutrients, T_3: 100 % recommended dose of nutrients + Nano urea spray, T_4: 75% recommended dose of nutrients + Nano urea spray, T_5: 100% recommended dose of nutrients + Panchagavya spray, T_6: 75% recommended dose of nutrients + Panchagavya spray, T_7: 100% recommended dose of nutrients + Jeevamrutha spray, T_8: 75% recommended dose of nutrients + Jeevamrutha spray and replicated thrice in randomized block design. The data observed for the first and ratoon crop of spinach were interpreted for obtaining conclusions. The results indicated that maximum increase in growth parameters in first crop and ratoon crops of spinach such as plant height, leaf length, leaf breadth, number of leaves per plant, were obtained with the application of 100%RDF + Jeevamrutha spray (T_7). Shoot base diameter of spinach was not influenced by the application of nutrients. The root shoot ratio was found higher with the application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7), but on par with the application of 100% recommended dose of nutrients + Panchagavya spray (T_5) followed by the application of 100% recommended dose of nutrients + Nano urea spray (T_3) at first harvest and ratoon harvest. The physiological parameters such as leaf volume, leaf density, specific leaf area, leaf thickness, leaf area, relative water content and leaf weight were enhanced with the application of 100% recommended dose of nutrients + Jeevamrutha spray. The maximum shoot weight of 880.91 and 764.44 g, shoot dry weight (413.01 g and 370.52 g), root fresh weight (29.42 g and 25.08 g) and root dry weight (6.20g and 6.22 g) was obtained with the application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7) but on par with the application of 100% recommended dose of nutrients + Panchagavya spray (T_5) followed by the application of 100% recommended dose of nutrients + Nano urea spray (T_3) at first harvest and ratoon harvest od spinach respectively. The increase in yield per hectare is the maximum with T_7 resulting an increase by 122 % and 105% during the harvest of first and ratoon crop of spinach. The increase in percentage of nitrogen content was 20.61 during both the harvests, phosphorous was 26.31 and 19.64 percent and the potassium content was 17.08 and 19.0 % with the application of 100% recommended dose of nutrients along with Jeevamrutha spray (T_7) was compared to control during the first and ratoon harvests respectively. However, the quantity of nitrate accumulated increased with the application of recommended dose of nutrients along with the application of stimulants such as nano urea, panchagavya and jeevamrutha over the reduced quantity of nutrients and stimulants but found within the permissible limits.

Keywords: Spinach, liquid organic manure, panchagavya, jeevamrutha, biostimulants, yield and nutrients

1. Introduction
Ecological stability is required under intensified crop cultivation to feed the ever increasing population of the world. Ecological sustainability could be achieved through practicing organic farming that principally helps in maintaining soil health and rendering biodiversity (Bedoussac *et al*., 2015) [10]. Organic cultivation has considerably increased throughout the world and the corresponding increase is about 75% (Mäder *et al*., 2002) [29]. Now a day's farmers are aware of organic farming practices and are willing to adopt. Various organic fertilisers, manures are available for use as a nutrient source. Farmyard manure, poultry manure, goat manure, sheep manure, bio-fertilisers etc. are commonly used nutrient sources for enhancing crop productivity

and soil fertility by way of improving soil aggregate stability, aeration and water holding capacity. The production potential of organic manures is dependent on the quality of organic manure to its mineralisation (Heal *et al*., 1997). Besides these organic manures, liquid formulations are available to enhance crop productivity under organic farming. These organic manures can act as biostimulants and gaining momentum in crop cultivation now a day.

Biostimulants as substance or microorganism applied to plants in order to improve nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content (Yakhin *et al*., 2017) [49]. Formulations of biostimulants, either natural or synthesised containing organic and inorganic compounds are available (Kocira *et al*., 2018) [26]. Natural biostimulants such as amino acids, humic acids, chitin derivatives, effective microorganisms, sea weed extracts, fruit extracts etc are available. The naturally produced biostimulants are claimed to aid in sustainable and resilience crop production (Bonasia *et al*., 2022) [11]. Some of the biostimulants are naturally produced. As a natural or organic biostimulant, the products such as panchagavya, jeevamruth etc that are derived from the wastes and the products of livestock, could be used as biostimulants to enhance plant growth, stress tolerance, enhance nutrient use efficiency, and to produce economically important crops (Tsouvaltzis *et al*., 2014) [44]. These biostimulants when sprayed on plants improve the crop performance by way of increasing nutrient availability and increases the metabolic activity, increasing the uptake and thereby enhancing the yield of crops (Sabatino *et al*., 2021) [38]. Also noted that the biostimulants improve tolerance to stress by inducing the protective compounds and thereby enhances the crop productivity (Cristofano *et al*., 2021) [15]. Green leafy vegetables respond very well to application of biostimulants, however, the research on these are found scanty.

Spinach, a leafy vegetable plays an important role in the diet of human (Chung *et al*., 2005) [14]. Spinach (*Spinacia oleraceae*) is commonly called as palak, widely cultivated in India, belongs to Amaranthaceae family. Produces rosette in the vegetative stage, harvested and used as green leafy vegetable. It's an excellent source of nutrients with sufficient quantities of protein, calcium, iron, vitamin A and vitamin C. However, it also contains higher level of nitrates, nitrites and oxalates (Santamaria *et al*., 2006) [40], mainly contributed by nutritional, environmental and physiological factors (Anjana *et al*., 2007) [5]. Spinach is a crop considered a slightly sensitive to abiotic stress such as salinity (Lucini *et al*., 2015) [28] that would hinder the yield of crops. It is proved that the bio active molecules in the biostimulants could improve the metabolism and physiology of plants, improves the root weight and shoot weight and thereby alleviate yield loss from the plants (Srivastava, 2022) [41].

Based on the review and the consideration for use of naturally derived biostimulants that help in improving the yield and quality of crops, the experiment was designed to evaluate the efficacy of organic biostimulants on the growth and yield performance of spinach.

2. Materials and Methods

The present investigation was conducted at the Instructional Livestock Farm Complex, Tamil Nadu Veterinary and Animal Sciences University to evaluate the efficacy of organic biostimulants on the growth and productivity of spinach. The organic biostimulants such as panchagavya and Jeevamrutha were prepared from the dung, urine and the derived products such as milk, ghee and curd of Gir cattle, a native breed of India, present at the farm.

Panchagavya was prepared by mixing the five products of cattle such as cow dung, cow urine, milk, curd and ghee and allowed for fermentation for about 7 days following the procedure from crop production guide. After 7 days, the fermented product of panchagavya was filtered and used for taking up spraying on crops.

Jeevamrutha was prepared by mixing cow dung, cow urine, jaggery, pulse flour and handful of garden soil, along with water and kept the container under shade covered with gunny bag. The mixture was stirred and incubated for seven days before use (Dev *et al*., 2022) [17]. Samples of panchagavya and jeevamrutha were drawn seven days after preparation for analysis of physical, chemical and microbial properties and the results presented in Table (1).

Table 1: Physio chemical and biological properties of organic biostimulants

Organic stimulants	Panchagavya	Jeevamrutha
N fixers (*10^4)	28	59
Bacteria (*10^5)	645	746
Fungi (*10^4)	16	22
Actinomycetes (*10^3)	3	8
P Solubilisers (*10^4)	36	49
pH	5.32	4.98
N		
P	0.145	0.186
K	0.320	0.398
Mg (ppm)	20	42
Cu (ppm)	38	46
Moisture (%)	91.27	97.07
Nitrogen (%)	1.96	2.86
Crude fibre (%)	3.86	3.57
Ether extract (%)	58.70	8.89

*Colony forming units

N-fixers like Bacteria - *Azatobacter sp*., *A. chroococcum*, *Bacillus sp*., *Beijerinckia sp*., Actinomycetes - *Streptomyces sp.*

Bacteria like- *Bacillus sp*., *Pseudomonas sp*.

The observations on different fungi were: *Fusarium sp*., *Trichoderma sp.* and P- solubilisers fungi like - *Penicillium sp.* and *Aspergillus sp*.

The soil samples from the experimental plot of the livestock farm complex, TANUVAS were collected for analysis of physio chemical properties before taking up sowing of spinach. The textural class of the soil is sandy loam, EC – 0.42 (dS m^{-1}), pH – 7.58, organic matter – 0.23%, available nitrogen – 183.6 kg ha^{-1}, available phosphorous – 14.68 kg ha^{-1} and available potassium – 218.65 kg ha^{-1}.

All green variety of spinach, obtained from Tamil Nadu Agricultural University, Coimbatore was used for sowing. Field experiment was conducted at the instructional livestock farm complex, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India. The efficacy of the biostimulants was experimented in field with the treatments T_1: Without nutrients, T_2: Recommended dose of nutrients, T_3: 100 % recommended dose of nutrients + Nano urea spray, T_4: 75% recommended dose of nutrients + Nano urea spray, T_5: 100% recommended dose of nutrients + Panchagavya spray, T_6: 75% recommended dose of nutrients + Panchagavya spray, T_7: 100% recommended dose of nutrients + Jeevamrutha spray, T_8: 75% recommended dose of nutrients + Jeevamrutha spray and repeated thrice in randomized block design.

Each plot size adopted was 2 m X 2 m and the seed rate followed was 25 kg ha^{-1}. The recommended dose of nutrients for

spinach is 60: 60 and 60 kg NPK ha^{-1} and supplied through urea, single super phosphate and murate of potash. The recommended dose of nitrogen (half dose of N), phosphorous (Full dose) and potassium (Full dose) were applied in the field before taking up sowing through, urea, single super phosphate and murate of potash respectively as per the treatment schedule. The remaining half the dose of nitrogen was applied on 15 days after sowing.

The biostimulants panchagavya and jeevamrutha were sprayed at 3% concentration and nano urea at 1% concentration as per the treatment schedule. The intercultural operations such as weeding, irrigation and pest management were carried as and when required as per the crop production guide of Tamil Nadu Agricultural University, Coimbatore, India. The growth, physiological parameters and yield, chemical parameters were recorded at the time of harvest. The plants were harvested at 45 days after sowing by cutting the stems of each plant leaving about 2 cm from the ground level. Harvested crop of spinach was allowed to grow as a ratoon crop and the agronomic practices were followed as mentioned for the first crop.

The growth parameters were measured in 5 plants per plot. Leaf length and petiole length were measured on fully developed third leaf of the spinach plant. The number of leaves, root length, stem length and stem diameter per plant were measured. Li-Cor 3100 area meter was used to measure the leaf area per plant. Stem and root fresh weight were measured and dry weight of the same were measured after drying in oven at 70 °C till the stable weight was obtained.

The physiological parameters such as membrane stability index and relative water content, yield and chemical analysis of plants were done at the time of crop harvest.

The membrane stability index was calculated as described (Sairam and Saxena, 2000), by measuring

$MSI = (1-C_1/C_2) *100$

C_1 = Electrical conductivity of the leaf samples before heating

C_2 = Electrical conductivity of the leaf samples after heating

The relative water content of the leaf samples was estimated using the method suggested (Lazcano-Ferrat and Lovatt, 1999).

RWC (%) = (Fresh weight- Dry weight/Turgid weight – Dry weight) *100

Leaf volume, leaf density and average leaf thickness were measured as given (Ilya Raskin, 1993). Leaf volume was calculated based on weight of leaf in air and its weight in water. Leaf density was calculated based on weight of leaf and volume of leaf. Average leaf thickness is determined from the selected leaves using micro-meter.

Mineral contents were determined by wet acid digestion method followed by atomic absorption spectroscopy (AOAC, 1990) [6]. Nitrate content of leaf of spinach was estimated following the method described (Cataldo *et al*., 1975) [12].

The experiment was done in randomized complete block design with 8 treatments and 3 replicates (5 plants/replicate). The data were subjected to ANOVA using the R–Project statistical software, and differences between treatments were considered statistically significant at probability level of 5% using the Least Significant Difference (LSD) test.

3. Results and Discussion

Organic biostimulants such as panchagavya, jeevamrutha and inorganic stimulant nano urea were experimented along with the nutrients applied to the soil for their influence on growth, yield and physiological parameters of the first crop and ratoon crop of spinach. Application of stimulants prepared from the direct and by products of indigenous cattle breed shown significant results in terms of growth, physiological parameters, yield and nutrient content of spinach. Results of the liquid biostimulants tested for their efficiency on growth and productivity of spinach is presented here under.

3.1 Effect on growth Parameters

The data obtained on growth parameters of spinach for the first crop and the ratoon crop of spinach are presented in Table 2 and Table 3. The growth parameters of spinach such as plant height (cm), leaf length (cm), leaf breadth (cm), number of leaves per plant, shoot base diameter (cm), petiole length (cm) and root shoot ratio resulted in a significant difference with respect to soil application of nutrients and foliar spray of organic biostimulants and inorganic stimulant compared to control and soil application of nutrients alone.

The maximum increase in growth parameters of spinach was obtained with the application of 100%RDF + Jeevamrutha spray (T_7), on par with the application of 100% recommended dose of nutrients + Panchagavya spray (T_5) followed by the application of 100% recommended dose of nutrients + Nano urea spray (T_3) at first harvest. Shoot base diameter of spinach was not influenced by the application of nutrients. The root shoot ratio was found higher with the application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7), on par with the application of 100% recommended dose of nutrients + Panchagavya spray (T_5) followed by the application of 100% recommended dose of nutrients + Nano urea spray (T_3) at first harvest and the increase over control was 89.57%. Similar trend was observed in the results of growth parameters of spinach as influenced by nutrients in ratoon spinach as well. Significant influence of growth parameters was obtained with application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7) over other treatments during both the harvests of spinach.

Now a day`s, farming is turning towards the use of organic products for improved quantity and quality of farm produce. The organic substances, applied on plants to improve various functions of the plants such as nutrients use, water use, stress tolerance, quality traits irrespective of the nutrient load in the soil are called stimulants (Waleed Fouad Abobatta, 2020) [48]. Biostimulants are plant or animal products based. Humate substances, sea weed extracts and amino acid containing products are available as biostimulants (Kauffman *et al*., 2007) [25]. The stimulants for plant growth and yield are applied as foliar or in soil (Zhang *et al*., 2014) [51] to improve upon growth, yield and quality of crops (Ertani *et al*., 2016) [20]. Though the benefits of using plant biostimulants can be obtained through use of chemicals, it has adverse effects on soil and plants and in turn to the environment (Mustafa *et al*., 2019) [31], hence the use of organic products is advisable. The organic products appear to be economically viable, non-phytotoxic and easily biodegradable (Karthikeyan and Shanmugam, 2016) [24]. Organic biostimulants such as panchagavya and jeevamrutha can be applied on foliage as foliar spray and this acts as a nutrient source (Baroccio *et al*., 2012) [8]. Biostimulants is gaining momentum worldwide as it seems safe to improve quality and resistance to stress due to abiotic factors (De Lucia and Vecchietti, 2012) [16]. However, the organic biostimulants use in plants need to be studied elaborately for their enhanced crop growth, yield and quality. The present study reveals that use of organic biostimulants such as jeevamrutha and panchagavya along with the application of inorganic nutrients enhanced the growth, yield and mineral

content of spinach compared to foliar spray of nano urea. Nutritive evaluation of panchagavya and jeevamrutha showed the availability of mineral nutrients such as nitrogen, phosphorous, potassium, growth promoters such as Indole acetic acid, gibberlic acid and the availability of bacterial population, P solubilisers, N fixers, fungi and actinomycetes. Biostimulants contains higher nutrients and beneficial organisms essential for crop growth as studied by (Devakumar *et al*., 2014) [18]. The biostimulants favour the regulation of physiological process to absorb more quantity of nutrients from the soil and thereby increases the crop yield (Yakhin *et al*., 2017) [49].

The organic biostimulants jeevamrutha and panchagavya contains indole acetic acid and gibberlic acid that improves cell division, cell multiplication and cell elongation resulting in improved plant height, number of leaves per plant, leaf length, leaf breadth, petiole length, shoot base diameter and root shoot ratio. The increase in growth parameters with application of biostimulants along with inorganic nutrients as basal application has been reported in spinach by (Ramya *et al*., 2015) [34]. Similar results were reported (Tiamiyu *et al*., 2012) [42] in okra

Table 2: Effects of different stimulants along with the nutrients on the growth parameters during first crop of spinach

Treatments	Plant height (cm)	Leaf length (cm)	Leaf breadth (cm)	Number of leaves	Shoot base diameter	Petiole length (cm)	Root shoot ratio
Control (T_1)	19.24±0.43 e	16.20±0.53 e	6.94±0.43 c	9.14±1.02 c	12.98±5.03 a	6.33±0.17 e	0.10±0.0031 c
recommended dose of nutrients (T_2)	21.63±0.48 d	18.01±0.59 de	6.68±0.50 c	10.98±1.23 c	16.02±6.21a	7.12±0.19 d	0.09±0.0029 c
100% recommended dose of nutrients + Nano urea spray (T_3)	25.78±0.57 ab	21.34±0.70 b	8.79±0.65 ab	16.44±1.83 ab	21.16±8.20 a	8.73±0.24 b	0.14±0.0045 b
75% recommended dose of nutrients + Nano urea spray (T_4)	23.57±0.53 c	18.97±0.62 cd	7.09±0.53 bc	11.20±1.25 c	17.03±6.60 a	7.34±0.20 d	0.12±0.0038 bc
100% recommended dose of nutrients + Panchagavya spray (T_5)	26.17±0.58 ab	22.24±0.73 b	8.91±0.66 ab	17.43±1.94 a	21.63±8.38 a	10.84±0.29 a	0.16±0.0050 ab
75% recommended dose of nutrients + Panchagavya spray (T_6)	23.84±0.53 c	20.10±0.66 bcd	7.92±0.59 bc	11.84±1.32 bc	17.98±6.97 a	7.80±0.21cd	0.13±0.0040 bc
100% recommended dose of nutrients + Jeevamrutha spray (T_7)	26.89±0.60 a	25.86±0.84 a	10.14±0.75 a	17.49±1.95 a	24.13±9.35 a	11.52±0.31a	0.19±0.0337 a
75% recommended dose of nutrients + Jeevamrutha spray (T_8)	25.06±0.56 bc	20.32±0.66 bc	8.33±0.62 abc	13.44±1.50 abc	19.33±7.49 a	8.34±0.23 bc	0.13±0.0040 bc
CV %	4.14	6.03	12.98	20.82	7.86	4.83	16.07
LSD ($p \leq 0.05$)	1.74	2.15	1.84	4.92	NS	0.72	0.04

The values following ± represent the standard deviation. The columns with different letters are significantly different at $p \leq 0.05$.

Table 3: Effects of different stimulants along with the nutrients on the growth parameters during ratoon crop of spinach

Treatments	Plant height (cm)	Leaf length (cm)	Leaf breadth (cm)	Number of leaves	Shoot base diameter	Petiole length (cm)	Root shoot ratio
Control (T_1)	21.24±2.44 c	15.74±0.43 f	5.911±0.060 g	7.59±0.14 f	12.05±0.15 f	5.42±0.09 h	0.10±0.0028 c
RDF (T_2)	22.77±2.61 c	17.54±0.45 e	5.691±0.058 f	9.46±0.17 e	14.46±0.23 e	6.21±0.11 g	0.09±0.0016 c
100%RDF + Nano urea spray (T_3)	27.21±3.12 bc	20.86±0.49 b	7.487±0.076 b	15.01±0.27 b	19.10±0.31 b	7.80±0.14 c	0.14±0.0048 bc
75%RDF + Nano urea spray (T_4)	26.62±3.05 abc	18.85±0.38 d	6.040±0.061 e	9.68±0.18 de	16.04±0.42 d	6.42±0.11 f	0.12±0.0048 bc
100%RDF + Panchagavya spray (T_5)	29.09±3.34 ab	21.40±0.75 b	7.603±0.077 a	16.00±0.29 a	19.52±0.31 ab	9.89±0.17 b	0.15±0.0025 ab
75%RDF + Panchagavya spray (T_6)	25.12±2.88 a	19.78±0.41 c	6.739±0.068 d	10.33±0.19 d	17.21±0.41 c	6.88±0.12 e	0.13±0.0027 bc
100%RDF + Jeevamrutha spray (T_7)	28.46±3.27 ab	25.39±0.54 a	7.652±0.038 a	16.06±0.29 a	20.40±0.60 a	10.56±0.19 a	0.18±0.0069 a
75%RDF + Jeevamrutha spray (T_8)	26.99±3.10 ab	19.64±0.62 c	7.088±0.072 c	11.95±0.22 c	17.12±0.36 cd	7.41±0.13 d	0.13±0.0028 bc
CV %	11.868	1.907	0.422	0.79	3.889	0.722	6.05
LSD ($p \leq 0.05$)	5.155	0.665	0.05	0.17	1.157	0.096	0.04

The values following ± represent the standard deviation. The columns with different letters are significantly different at $p \leq 0.05$.

3.2 Effect on Physiological parameters

Physiological parameters were found significantly improved by the application of nutrients as soil and foliar application of stimulants in comparison with control (Table 4 and Table 5). Leaf volume, leaf density, specific leaf area, leaf thickness, leaf area and leaf weight were significantly improved with the application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7) but on par with the application of 100% recommended dose of nutrients + Panchagavya spray (T_5) followed by the application of 100% recommended dose of nutrients + Nano urea spray (T_3) at first harvest. Similar trend on physiological parameters were observed during ratoon harvest of spinach. However, relative water content increased with application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7) and on par with the application of 100% recommended dose of nutrients + Nano urea spray (T_3) and 100% recommended dose of nutrients + Panchagavya spray (T_5) during ratoon crop of spinach.

The increase in physiological parameters such as leaf volume, leaf density, specific leaf area, leaf thickness, relative water content, leaf area and leaf weight also influenced the yield of spinach and obtained with the application of inorganic nutrients and stimulants. The increase in translocation rate improved the quantity or the weight of spinach and thereby the yield of the crops. The increase in the ratio of the dry matter accumulation to the leaf area as indicated by specific leaf weight denotes that there occurs the increased photosynthetic rate resulting in enhanced accumulation of dry matter (Abdelaziz, 2003) [2]. The application of stimulants in comparison to basal application of nutrients alone could have resulted increased respiration rate rather than photosynthetic rate and reduced nutrient uptake consequently resulting in reduced specific leaf weight (Aktsoglou *et al*., 2021, Abdelaziz, 2014) [3, 1].

Relative water content of spinach increased with the application of stimulants jeevamrutha and panchagavya in this experiment and decreased with nano urea application as foliar spray. The result revealed that relative water content increased with decreased water stress condition due to decreased water loss through transpiration and increased uptake of eater from plants (Ryan *et al*., 2001) [37]. Further, the stimulants also increase the leaf area and leaf turgor thereby increasing the photosynthetic efficiency in spinach leading to improved yield (Bashan *et al*.,

2014, Zahir *et al*., 2018) [9, 50].

The highest nutrient content such as nitrogen, phosphorous and potassium in spinach observed with application of nutrients in soil and foliar application of jeevamrutha and panchagavya might be due to the rapid penetration of stimulants in leaves and faster translocation resulting in higher uptake of nutrients. This is in line with the findings (Rouphael *et al*., 2017 and Pereira *et al*., 2019) [35, 32]. It is also suggested that fertiliser addition to plants via soil enhances the uptake of nutrients by plants (Mugnai *et al*., 2008 and Jadhav *et al*., 2017) [30, 23].

Table 4: Effects of different biostimulants along with the nutrients on the physiological parameters during first crop of spinach

Treatments	Leaf volume (mm^3)	Leaf density (mg mm^{-3})	Specific leaf area (cm^2 g^{-1})	Leaf thickness (mm)	Relative water content (%)	Leaf area (cm^2)	Leaf weight (g)
Control (T_1)	5.28±0.17 f	0.366±0.0086 d	5.96±0.17 f	0.47±0.016 e	68.63±3.07 c	252.18±10.30 c	2.38±0.31 e
RDF (T_2)	6.69±0.21 e	0.374±0.0087 cd	7.04±0.20 fe	0.50±0.017 de	73.71±3.30 bc	252.45±10.31 c	2.63±0.08 de
100%RDF + Nano urea spray (T_3)	11.23±0.36 b	0.441±0.0103 b	9.58±0.27 b	0.63±0.022 b	91.94±1.75 a	307.52±12.56 ab	4.77±0.11 b
75%RDF + Nano urea spray (T_4)	6.36±0.20 e	0.381±0.0089 cd	8.42±0.24 d	0.54±0.018 cd	82.64±3.70 ab	260.62±10.64 c	2.95±0.09 cde
100%RDF + Panchagavya spray (T_5)	11.90±0.36 ab	0.441±0.0103 b	10.36±0.30 a	0.71±0.024 a	82.07±1.56 ab	312.40±12.76 ab	5.33±0.30 b
75%RDF + Panchagavya spray (T_6)	8.37±0.27 d	0.394±0.0092 cd	8.49±0.24 cd	0.58±0.020 bc	78.18±3.50 bc	278.38±11.37 bc	3.11±0.09 cd
100%RDF + Jeevamrutha spray (T_7)	12.73±0.09 a	0.491±0.0115 a	10.41±0.30 a	0.77±0.026 a	88.66±3.97 a	314.27±12.83 a	6.41±0.19 a
75%RDF + Jeevamrutha spray (T_8)	9.51±0.30 c	0.403±0.0094 c	9.20±0.26 bc	0.61±0.021 bc	83.27±3.72 ab	281.18±11.48 ab	3.49±0.10 c
CV %	5.31	4.214	4.81	6.34	7.27	7.17	8.66
LSD ($p \leq 0.05$)	1.16	0.042	1.02	0.09	14.34	49.18	0.82

The values following ± represent the standard deviation. The columns with different letters are significantly different at $p \leq 0.05$.

Table 5: Effects of different stimulants along with the nutrients on the physiological parameters during ratoon crop of spinach

Treatments	Leaf volume (mm^3)	Leaf density (mg mm^{-3})	Specific leaf area (cm^2 g^{-1})	Leaf thickness (mm)	Relative water content (%)	Leaf area (cm^2)	Leaf weight (g)
Control (T_1)	4.65±0.04 g	0.318±0.006 g	4.12±0.10 f	0.51±0.09 g	64.95±0.99 h	228.47±5.92 g	1.22±0.05 d
RDF (T_2)	6.02±0.05 f	0.326±0.007 f	5.21±0.12 e	0.55±0.09 fg	70.11±1.07 g	228.74±5.92 f	1.67±0.07 d
100%RDF + Nano urea spray (T_3)	10.18±0.09 c	0.393±0.008 b	7.75±0.18 b	0.70±0.12 c	79.17±1.20 d	284.18±7.36 b	4.13±0.17 b
75%RDF + Nano urea spray (T_4)	5.96±0.05 f	0.333±0.007 e	6.59±0.15 d	0.60±0.10 ef	88.60±1.35 a	236.97±6.14 e	2.55±0.11 cd
100%RDF + Panchagavya spray (T_5)	10.96±0.10 b	0.393±0.008 b	8.53±0.20 a	0.79±0.13 b	74.64±1.13 f	289.09±7.49 a	4.21±0.41 b
75%RDF + Panchagavya spray (T_6)	6.65±0.06 e	0.346±0.007 d	6.65±0.15 d	0.64±0.11 de	78.59±1.19 e	254.84±6.60 d	4.25±1.26 b
100%RDF + Jeevamrutha spray (T_7)	11.95±0.11 a	0.442±0.009 a	8.58±0.20 a	0.86±0.14 a	79.80±1.21 c	290.98±7.54 a	5.95±0.04 a
75%RDF + Jeevamrutha spray (T_8)	8.97±0.08 d	0.355±0.007 c	7.37±0.17 c	0.67±0.11 cd	85.28±1.30 b	257.67±6.67 c	3.65±0.15 bc
CV %	0.51	0.407	0.921	5.214	0.26	0.46	13.42
LSD ($p \leq 0.05$)	0.072	0.003	0.111	0.061	0.35	2.08	0.82

The values following ± represent the standard deviation, where $n = 3$. The columns with different letters are significantly different at $p \leq 0.05$.

3.3 Effect on Yield parameters and yield

The yield parameters presented in table 6 and table 7 show that all the treatments with nutrients applied in soil and along with stimulants resulted in a significant increase compared to control in the first and ratoon crop. The maximum shoot weight of 880.91 g, shoot dry weight (413.01 g), root fresh weight (29.42 g) and root dry weight (6.2g) was obtained with the application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7) but on par with the application of 100% recommended dose of nutrients + Panchagavya spray (T_5) followed by the application of 100% recommended dose of nutrients + Nano urea spray (T_3) at first harvest. Similarly, during the second harvest or ratoon crop, the shoot weight (764.44 g), shoot dry weight (370.52 g), root fresh weight (25.08 g) and rot dry weight (6.22 g) was obtained with the application of 100% recommended dose of nutrients + Jeevamrutha spray (T_7), which is found significant and maximum compared to the remaining treatments given for spinach.

Table 6: Effects of different stimulants along with the nutrients on the yield parameters during first crop of spinach

Treatments	Shoot weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Yield (t)per hectare
Control (T_1)	167.65±17.65 g	64.24±1.64 f	10.82±0.38 d	4.46±0.155 g	16.34±0.69 c
RDF (T_2)	302.56±13.91 f	144.10±3.67 e	20.33±0.71 c	5.69±0.198 f	16.54±0.70 c
100%RDF + Nano urea spray (T_3)	708.95±16.89 bc	298.72±7.61 b	23.84±0.83 b	6.28±0.218 c	24.55±1.04 b
75%RDF + Nano urea spray (T_4)	412.88±23.16 e	224.66±5.73 d	20.43±0.71 c	5.97±0.208 e	18.03±0.90 c
100%RDF + Panchagavya spray (T_5)	816.51±37.55 ab	415.64±10.59 a	24.26±0.84 b	6.48±0.226 b	33.85±1.69 a
75%RDF + Panchagavya spray (T_6)	412.88±23.16 e	224.66±5.73 d	20.43±0.71 c	5.97±0.208 e	19.59±0.83 c
100%RDF + Jeevamrutha spray (T_7)	880.91±22.39 a	413.01±3.02 a	29.42±1.02 a	6.72±0.234 a	36.39±1.54 a
75%RDF + Jeevamrutha spray (T_8)	770.43±35.43 c	348.10±8.87 c	22.08±0.77 bc	6.24±0.217 c	23.90±1.01 b
CV %	7.35	1.98	6.48	0.696	8.62
LSD ($p \leq 0.05$)	73.38	9.26	2.48	0.073	4.95

The values following ± represent the standard deviation, where $n = 3$. The columns with different letters are significantly different at $p \leq 0.05$.

Table 7: Effects of different stimulants along with the nutrients on the yield parameters during ratoon crop of spinach

Treatments	Shoot weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Yield (t) per hectare
Control (T_1)	145.48±2.29 g	57.63±2.12 f	9.22±0.31 e	4.13±0.11 f	15.81±1.61 e
RDF (T_2)	262.55±4.14 f	129.28±4.76 e	17.33±0.58 d	5.27±0.14 e	16.00±1.62 e
100%RDF + Nano urea spray (T_3)	615.21±9.69 d	267.99±9.87 c	20.32±0.68 b	5.81±0.16 c	23.76±2.41 c
75%RDF + Nano urea spray (T_4)	358.29±5.64 e	201.55±7.43 d	17.41±0.58 d	5.53±0.15 d	17.45±1.77 de
100%RDF + Panchagavya spray (T_5)	708.55±11.16 b	372.88±13.74 a	20.67±0.69 b	6.01±0.16 b	32.76±3.33 b
75%RDF + Panchagavya spray (T_6)	358.29±5.64 e	201.55±7.43 d	17.41±0.58 d	5.53±0.15 d	18.96±1.93 d
100%RDF + Jeevamrutha spray (T_7)	764.44±12.04 a	370.52±13.65 a	25.08±0.84 a	6.22±0.17 a	35.22±3.58 a
75%RDF + Jeevamrutha spray (T_8)	668.56±10.53 c	312.29±11.51 b	18.82±0.63 c	5.78±0.16 c	23.13±2.35 c
CV %	1.30	3.01	1.42	0.55	5.76
LSD ($p \leq 0.05$)	11.07	12.61	0.45	0.05	2.31

The values following ± represent the standard deviation. The columns with different letters are significantly different at $p \leq 0.05$.

Spinach yield was positively influenced by the application of nutrients at different doses of nutrients along with stimulants compared to unfertilised treatment. Significant improvement in crop yield of spinach was obtained with 100% RDF along with organic stimulants during first and second harvest respectively (Figure 1 and Table 6 and 7).

At the time of first harvest, application of 100% recommended dose of nutrients along with jeevamrutha spray resulted in significant yield. The increase in yield per hectare is the maximum with T_7 resulting an increase by 122 %. However, the spinach yield is on par with the application of 100% recommended dose of nutrients with Panchagavya spray (T_5) with an increase in yield of about 107%. These treatments are followed by the application of 100% recommended dose of nutrients along with nano urea spray (T3) and 75% % recommended dose of nutrients with Jeevamrutha spray (T_8). During the harvest of ratoon crop, similar trend results were obtained. Application of 100% recommended dose of nutrients along with Jeevamrutha spray (T_7) resulted in increased spinach yield of 122% but followed by the application of 100% recommended dose of nutrients with Panchagavya spray (T_5) with an increase of 107% and application of 100% recommended dose of nutrients along with nano urea spray (T_3) (50%). However, the application of recommended dose of nutrients alone without the spray of stimulants resulted in minimum increase in yield and the results also shows that application of stimulants had an influence on increased yield either organic or inorganic.

The present study shows the increase in shoot fresh and dry weight, root fresh and dry weight was obtained with the application of inorganic nutrients along with application of jeevamrutha and panchagavya but on par with nano urea spray. This shows the significant crop improvement with the application of stimulants. The organic stimulants increased plant growth parameters due to the enhanced phytohormones and resulting in yield parameters of spinach (Tiamiyu *et al*., 2012, Priyanka Mishra and Koshy, 2016) [42, 33].

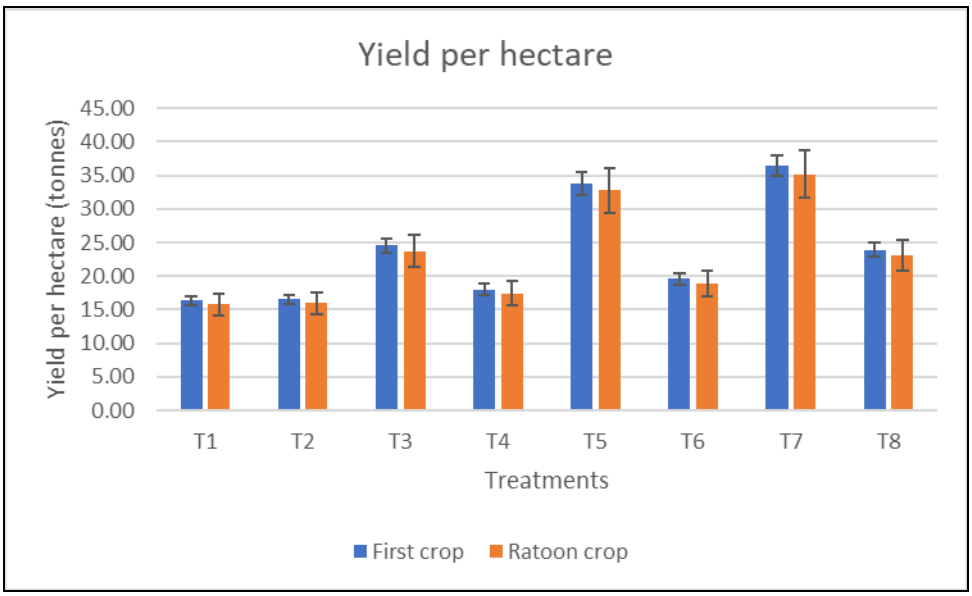

Fig 1: Effects of different stimulants along with the nutrients on the yield of spinach

3.4 Effect on Nutrient content of spinach

Nutrient content of spinach as influenced by different treatments were presented in Figure 2, 3 and 4). Application of recommended dose of nutrients along with stimulants enhanced the nutrient content of spinach such as nitrogen, phosphorous and potassium. Nitrogen content was higher with the application of 100% recommended dose of nutrients along with Jeevamrutha spray (T_7) followed by the application of 100% recommended dose of nutrients with Panchagavya spray (T_5) and the least nitrogen content was found without nutrient application during both the harvests. The increase in percentage of nitrogen content was 20.61 during both the harvests compared to control. Similarly, the increase in percentage of phosphorous was 26.31 and 19.64 with the application of 100% recommended dose of nutrients along with Jeevamrutha spray (T_7) over control during first and ratoon harvest respectively. The increase in potassium content of spinach with the application of 100% recommended dose of nutrients along with Jeevamrutha spray

(T_7) was 17.08 and 19.0 compared to control during the first and ratoon harvests respectively.

Treatments had significant influence on the nitrate accumulation in green leaves of spinach plants (Figure 5). Application of nutrients influenced the availability of nitrate in spinach compared to unfertilised. The quantity of nitrate accumulated increased with the application of recommended dose of nutrients along with the application of stimulants such as nano urea, panchagavya and jeevamrutha over the reduced quantity of nutrients and stimulants. The application of 100% recommended dose of nutrients along with Jeevamrutha spray (T_7) resulted with significant increase in NO3-N compared to other treatments, but found within the permissible limits of European commission regulation.

The response of spinach in terms of growth, yield and nutrient content to application of biostimulants in addition to fertiliser application might be due to the availability of indole acetic acid and gibberlic acid in jeevamrutha and panchagavya helps in shoot and root development of plants (Vance and Chapin, 2001) [46] and instant availability (Asrar *et al*., 2012 and Van Oosten *et al*., 2017) [7, 45].

Though nitrogen is important for the crop growth, in shorter duration crops like green leafy vegetables, spinach, lettuce etc, nitrate accumulation poses a problem and varied with the soil organic matter content, quantity of fertiliser application, weather and climatic conditions (Edward Kunicki *et al*., 2010) [19]. However, various studies indicated that application of biostimulants helps in reduction of nitrate content (Vernieri *et al*., 2006, Amanda *et al*., 2009) [47, 4]. Studies in baby lettuce with application of biostimulants were found to reduce nitrate content by nitrate metabolism and increased the yield and quality of crop (Christophe El-Nakhel *et al*., 2022) [13], and also helps in reduced root nitrate uptake from soil (Ruiz *et al*., 2000) [36] in pepper.

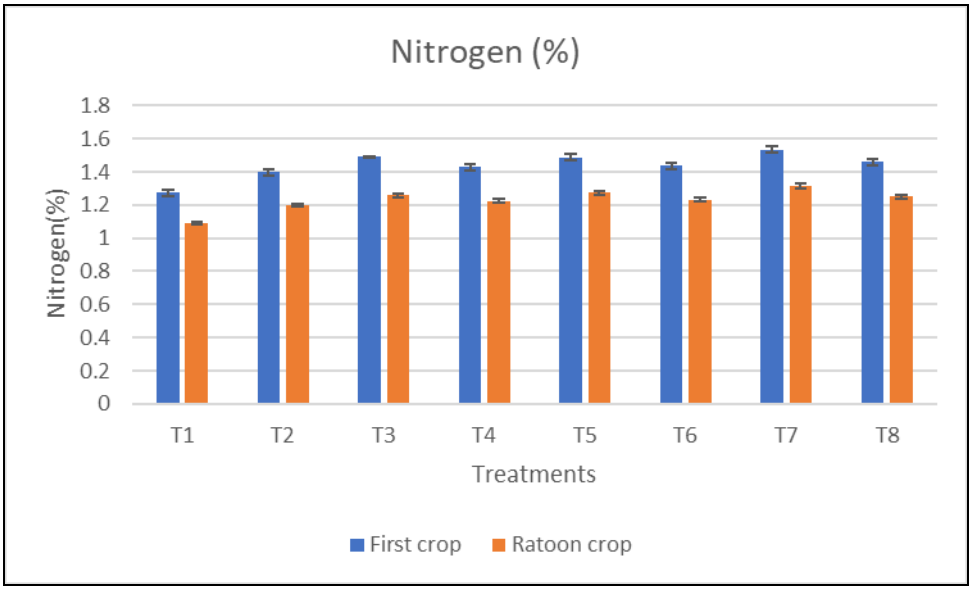

Fig 2: Effects of different stimulants along with the nutrients on nitrogen (%) during first crop and ratoon of spinach.

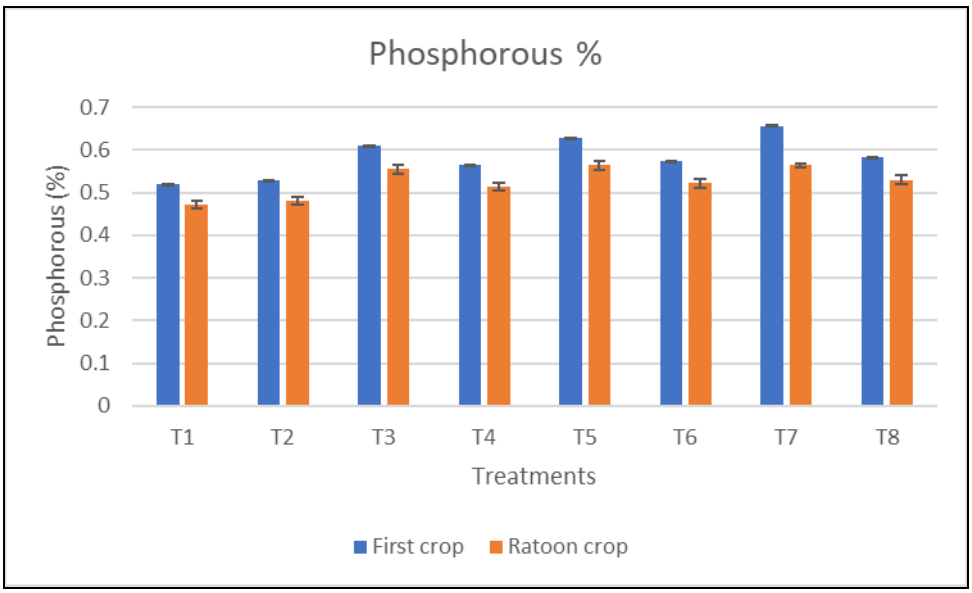

Fig 3: Effects of different stimulants along with the nutrients on phosphorous (%) during first crop and ratoon of spinach.

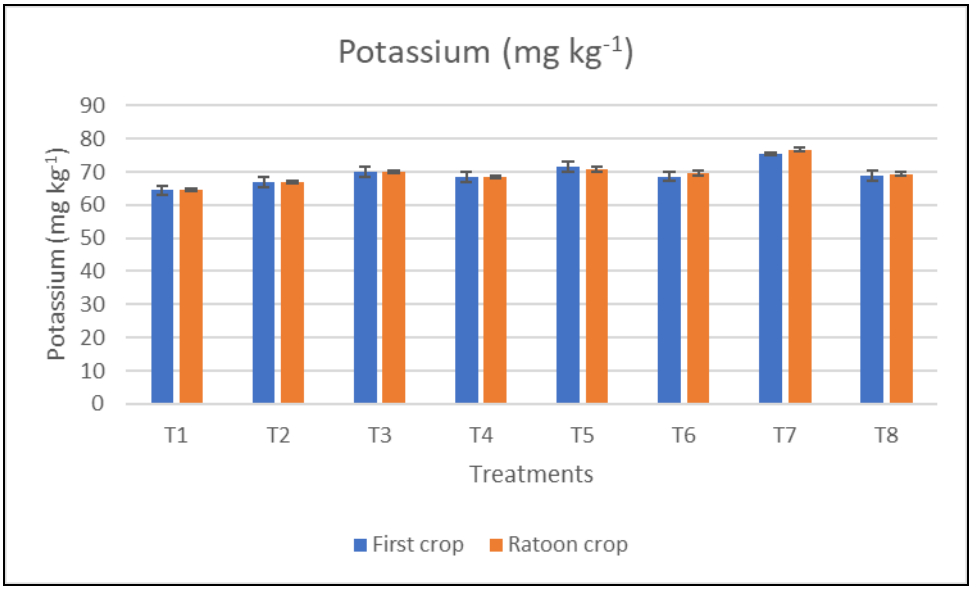

Fig 4: Effects of different stimulants along with the nutrients on potassium (mg kg^{-1}) during first crop and ratoon of spinach.

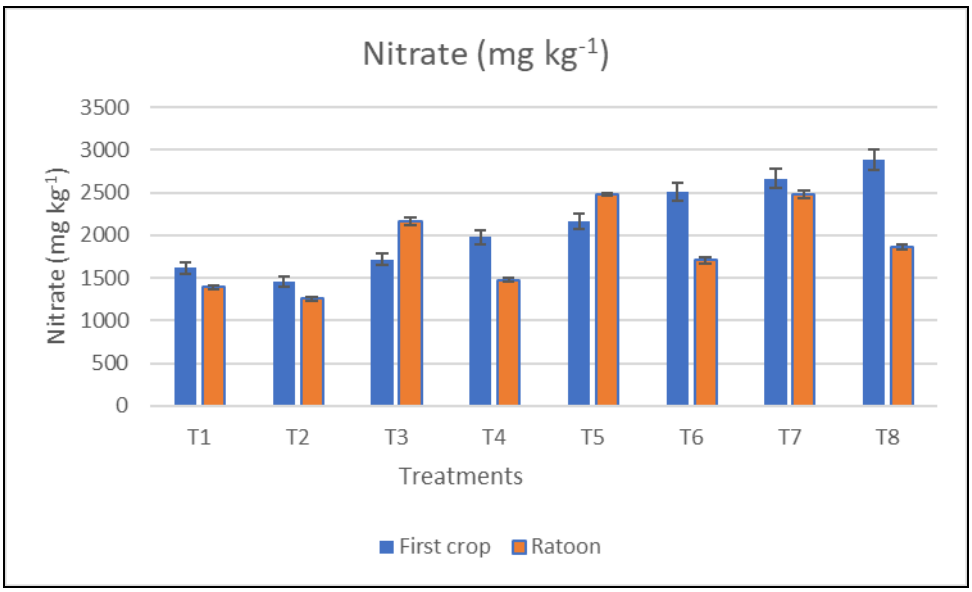

Fig 5: Effects of different stimulants along with the nutrients on Nitrate (mg kg^{-1}) during first crop and ratoon of spinach.

4. Conclusion and Suggestion

It is concluded from the results of the experimentation of first crop and ratoon crop of spinach, application of liquid organic manures enhanced the growth, yield, physiology and qualitative parameters of spinach, acting like biostimulants. Application of liquid organic manure, jeevamrutha as biostimulant along with the application of 100% recommended dose of nutrients resulted in significant increase in growth and yield of spinach, but on par with the application of panchagavya along with 100% recommended dose of nutrients over the application of nano urea along with recommended dose of nutrients. Application of jeevamrutha and panchagavya have resulted in improved physiological and qualitative parameters of spinach. Thus the liquid organic manures such as panchagavya and jeevamrutha can be utilised as biostimulant for improved and sustainable production of green leafy vegetables. Application of organic nutrients to foliage increases the absorbance of nutrients by the plants and enhances the nutrient availability in plants. The liquid organic manures possess the ability to enhance the nutrients thereby reflecting in the growth and yield of crops. Hence, suggesting that the liquid organic manures could be used as biostimulants.

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