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Rice straw biostimulants: Phytochemicals and effects on maize growth and tomato yield

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Abstract

Biostimulants offer sustainable alternatives to enhance plant growth, resilience and crop yield, especially in organic farming. This study aimed to develop a nutrient-enriched biostimulant from rice straw, a widely available agricultural by-product, often burned, leading to environmental pollution. Rice straw was collected, processed and extracted using cow urine and deionized water (1:50). The extracts underwent phytochemical analysis via GC-MS and LC-MS, identifying bioactive compounds such as fatty acids, sterols, phenols and flavonoids. Elemental analysis confirmed the presence of key nutrients and trace elements, including potassium and magnesium, essential for plant growth. *In-vitro* assays on maize (CO6 hybrid) evaluated the biostimulant's efficacy, showing a 12 % increase in germination and a 25 % improvement in seedling vigor at optimal cow urine extract concentrations (CE) (25 %). Root length and shoot biomass also exhibited significant improvements. Field experiments on tomato cultivar (Madhan hybrid) compared the effects of the rice straw-based biostimulant with panchagavya, a traditional organic preparation, alongside controls including cow urine and water. The findings revealed that the rice straw-derived biostimulant markedly improved fruit yield by 22 % compared to the control. This enhancement exceeded that of panchagavya, which achieved a 17 % increase in fruit yield. These results underscore the superior efficacy of the rice straw-based treatment, especially those extracted with cow urine, in enhancing crop productivity, reducing reliance on synthetic agrochemicals and promoting environmentally sustainable agricultural practices.

Keywords

rice straw; biostimulants; cow urine; organic farming; phytochemical profiling; maize; tomato

Introduction

In recent years, a plant biostimulant refers to a substance or microorganisms applied to plants or their root zone that enhances natural processes. This stimulation improves nutrient uptake, increases nutrient efficiency, boosts tolerance to environmental stress and enhances crop quality, regardless of the biostimulant's nutrient content. Biostimulants such as humic acids, protein hydrolysates and seaweed and agro waste derived stimulants have emerged as a vital tool for achieving sustainable yields in organic agriculture (1). These products enhance plant growth and development by improving nutrient absorption, increasing resilience to environmental stress and enhancing overall crop quality and yield. Unlike traditional fertilizers that directly supply nutrients, biostimulants stimulate the plant's natural biological processes, making them well-suited for organic farming (2). They are categorized into several types, such as humic substances, seaweed extracts, amino acids and protein hydrolysates, chitosan and other biopolymers, microbial inoculants and botanicals from plants (2), each with specific benefits for organic crops. Biostimulants derived from plant-based sources, particularly agricultural by-products or waste, are of considerable interest due to their environmentally friendly potential as alternatives to synthetic inputs (3, 4). Notably, rice straw emerges as a prominent resource, with Asia generating approximately 740 million tons annually (5). According to the Food and Agriculture Organization (FAO), 90 % of the world's rice is cultivated and consumed in Asia, in countries such as China, Thailand, Vietnam, the Philippines, Indonesia, India and Bangladesh. Unfortunately, much of this rice straw is burned in the fields, releasing particulate matter, aerosols and greenhouse gases, contributing to environmental pollution (6, 7). In India, rice residue burning is responsible for about 40 % of greenhouse gas emissions related to agricultural activities (8, 9). Shifting towards the use of these residues as biostimulants in organic farming could offer a sustainable solution for improving crop performance while minimizing environmental impact.

Rice straw is a valuable resource rich in biologically active compounds such as fatty acids, plant metabolites, phenols, flavonoids and amino acids (10), making it an excellent organic amendment in organic agriculture. It is commonly used as organic matter, mulch or compost to improve soil fertility, enhance nutrient cycling and promote soil health (11, 12). Additionally, rice straw can be processed into liquid biostimulants, containing a variety of nutrients and bioactive compounds that benefit crops. These biostimulants can be applied as a soil drench or sprayed as a foliar treatment to stimulate plant growth, improve nutrient uptake and enhance overall plant health (13). Biostimulants had positive and synergistic relationship with maize, enhancing its growth, nutrient efficiency, stress tolerance and overall productivity. Studies have shown that using rice straw compost in rice cultivation increased grain yield by 13-26 % compared to control (14). Similarly, the application of cow urine spray at concentrations of 50-100 % resulted in a 4.4-10 % increase in rice grain yield (15). Furthermore, the production of humic acid (HS)-rich plant growth promoters from rice straw has proven to be an eco-friendly approach to enhancing tomato yield and growth (3). By converting rice straw by soaking and fermentation into biostimulants, farmers can improve crop health while reducing reliance on synthetic fertilizers and chemicals, thus minimizing environmental impact. With this context, the present study aims to characterize rice straw for the development of nutrient-rich biostimulants using natural extractants and to evaluate their effectiveness on maize growth development under *in-vitro* state and on the performance of tomato under open field situation.

Materials and Methods

Rice straw collection, processing and extraction

The 10 kg of rice straw was collected from the wet land area of Tamil Nadu Agricultural University (TNAU), Coimbatore, India and dried in a hot air oven (NSW-143, Narang Scientific works, New Delhi) at 60 ± 2 °C and ground into powder using mechanical grinder and stockpiled in an airtight container until analysis at room temperature. The rice straw was soaked in cow urine and deionized water at specified ratio (1:50). Cow urine (*Holstein freisian*) was collected from dairy farm, Central farm unit, TNAU, Coimbatore and deionized water was utilized for extraction. At the end of the soaking period, all extracts were filtered through a clean muslin cloth, centrifuged and decanted to obtain 100 % stock solution. It was stored in an airtight container under refrigerated (-4 °C) condition for further use.

Chemicals and Reagents Used

The analytical chemicals of methanol, were purchased from Thermo Fisher Scientific and formic acid were obtained from E-Merck. Ultra-pure 0.2 µ filtered HPLC water (LaboStar TWF-UV 3, Ultra-pure Water System, with a maximum feed conductivity of 1400 µS/cm) was used for preparing standard stocks, secondary working standards and mobile phases. Other analytical chemicals, solvents and buffers were procured from SD Fine Chemicals, Himedia lab, India.

Phyto-chemical profiling of rice straw by GC-MS and LC-MS

One-gram powdered rice straw was immersed in 50 mL of 95 % methanol (HPLC grade) at 24 to 25 °C for approximately 72 h using a rotary shaker (REMI-RS-36BL) set at 160 rpm (16). Following maceration, the mixture was centrifuged at 4000 rpm for 10 min. The resulting supernatants underwent evaporation under reduced pressure, were filtered through an Agilent 0.2 µm nylon membrane filter and then subjected to phytochemical analysis using both GC-MS and LC-MS techniques.

An Agilent GC-MS system (Model: GC 7890A / MS5975C) equipped with an EI triple-axis detector was utilized to quantify analytes. Separation was conducted on a standard non-polar DB-5MS capillary column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness, Agilent Co., USA) and helium as the carrier gas at a flow rate of 1.0 mL/min. Subsequently, 1 mL of the sample was loaded into a 2 mL screw top vial using an auto injector and 1 µL of the sample was injected into the system. The oven temperature was set at 60 °C and mass scanning ranged from 50 to 350 amu. Bioactive molecules were identified by comparing their mass spectra with those in the NIST (National Institute of Standards and Technology - USA) library.

For non-volatile compound analysis, the Shimadzu LC-MS/MS-8040 system (Shimadzu UFLC- LC-20 AD) equipped with a triple-axis detector was employed. Liquid chromatographic separations were carried out in reversed-phase C 18 column (4.6 mm × 250 mm, 5 µm particle size, Shimadzu) maintained at 35 °C for the run time of 20 min with the injection of 10 µL sample volume and the flow rate of 0.2 mL/min. The mass scanning range was set from m/z 100 to 1000 and scanned in positive ionization mode by using the mobile phase composition of 0.1 % formic acid in deionized water (A) and methanol (B) under gradient mode. Identification of non-volatile compounds involved comparing their mass spectra with those in the PmDB (Plant Metabolite Database) and NIST Mass Spectra Library. Additional information such as compound name and molecular weight was obtained from NIST, PubChem, Chemsphere.

Elemental analysis of raw rice straw and its extracts

The measured amount of rice straw and its extracts underwent digestion using triple acid (a mixture of concentrated nitric, sulfuric and perchloric acids in a 9:2:1 ratio) for total nitrogen determination and diacid (a blend of concentrated sulfuric and perchloric acids at a 5:2 ratio) for total sulfur determination (17). The digested samples were then diluted, filtered through Whatman No. 42 filter paper and adjusted to a final volume of 100 mL using appropriate diluents. Nitrogen content was determined using the Kjeldahl method, while phosphorus (P) and potassium (K) were quantified using a UV-Visible spectrophotometer (UV-1800, SHIMADZU, Japan) at 420 nm and a flame photometer respectively. Secondary nutrients, micronutrients and heavy metals were analyzed using an inductively coupled plasma optical emission spectrometer.

Phytotoxicity evaluation

A laboratory-based *in-vitro* assay was conducted with maize (CO6 hybrid released by TNAU) as the test species. Ten maize seeds were placed on germination paper in petriplates. Different concentrations (2.5 %, 5.0 %, 7.5 % and 10 %) of aqueous and cow urine extracts derived from rice straw were applied along with a control treatment (water alone). Seed germination progress was monitored and counts were recorded on the 7^{th} and 10^{th} day after treatment. Germination was considered successful when the radicle grown 2 mm or more. The plates were maintained at a temperature of 27 ± 1 ° C under a 16 h light/8 h dark cycle.

Using the collected data, various germination parameters including Germination % (GP), Germination Index (GI), Mean Germination Time (MGT), Seedling Vigour Index (SVI) and Germination Energy (GE %) were calculated following standard methods (18-20). After 10 days, maize seedlings were carefully removed from each plate and their growth parameters such as seedling length, biomass and root characteristics were meticulously measured. Root parameters including length (cm), surface area (cm^2), average diameter (mm), number of tips and volume (cm^3) were analyzed using root image analyzer with WinRHIZOPro software.

Field evaluation study

Based on the *in vitro* assay outcome, the best performing cow urine-based rice straw biostimulant concentrations (5 and 7.5 %) was evaluated with tomato under field conditions through foliar application alternating with and without panchagavya, an organic biostimulant routinely used by the farmers of south India alongside controls such as cow urine and water. A field experiment was conducted from February to May 2024 in Dharmapuri, Tamilnadu- India, adopting Randomized Block Design (RBD) and imposing treatments in triplicate. The experimental soil was classified as red sandy loam with a neutral pH (7.01), optimal electrical conductivity (0.37 dS m^{-1}) and medium levels of available nitrogen, phosphorus and potassium ie.,454, 79.76 and 208 kg ha^{-1} respectively. Additionally, the soil contained sufficient secondary nutrients (0.50 % calcium, 0.10 % magnesium and 0.41 % sulfur) and micronutrients (154 mg iron, 23 mg zinc, 19 mg copper and 86 mg manganese/kg of soil), with heavy metal concentrations within permissible limits. Field preparation, intercultural practices, crop management and protective measures strictly adhered to the standard operating protocols for organic tomato cultivation as recommended by Tamil Nadu Agricultural University, India. Atlast ploughing, farmyard manure (FYM) was applied at a rate of 25 tons/ha and biofertilizers including *Azospirillum* and phosphobacteria were incorporated at 2 kg/ha each, mixed thoroughly with 50 kg of FYM. Madhan hybrid tomato seedlings were procured from green gold nursery, Coimbatore, Tamilnadu-India and transplanted at a spacing of 45 cm by 60 cm, with irrigation provided through a drip irrigation system to ensure optimal water management. Experimental treatments were administered during the vegetative, flowering and fruiting stages of the crop. To support vertical growth, the tomato plants were trellised using wooden stakes and metal twine, starting 45 days after transplantation. Growth and yield parameters were recorded at the peak flowering (45^{th} days after transplanting) and harvest stages (60^{th} days after transplanting).

Statistical analysis

The Analysis of Variance for Completely Randomized Block Design and RBD was worked out to examine the collected data from the study. The treatments with significant variations were subjected to F-test (p = 0.05) using OPSTAT (21) for comparison.

Results and Discussion

Elemental composition

The physico-chemical properties and elemental composition of rice straw, cow urine and the extracted biostimulants are presented in Table 1. Rice straw exhibited slightly acidic pH (6.78) and relatively high values of electrical conductivity (6.17 dS m^{-1}), organic carbon (54.48 %), and major nutrients including N, P, K, Ca, Mg and S at 1.06 %, 0.034 %, 1.25 %, 0.048 %, 0.11 % and 0.13 % respectively. Micro-nutrients such as Fe, Cu, Mn, Zn and B were present at 268.65 mg kg^{-1}, 49.05 mg kg^{-1}, 61.25 mg kg^{-1}, 28.3 mg kg^{-1} and 5 mg kg^{-1} respectively. Organic carbon content ranged from 16.37 % to 20.02 %, nitrogen from 0.66 % to 0.73 %, phosphorus from 0.11 % to 0.12 % and potassium from 0.40 % to 0.63 % (14). The results show the significant presence of essential nutrients and bioactive compounds in rice straw (22). Cow urine used for extraction exhibited basic pH (8.12) and lower EC (3.25 dS m^{-1}) compared to rice straw, with very low organic carbon content (1.59 %) and other essential nutrients. Heavy metal concentrations in both rice straw and cow urine were found below permissible limits. Among the major nutrients, N, K and S were present in higher quantities, with iron content exceeding that of other micro-nutrients.

The mineralogical composition of cow urine extract

(CE) and aqueous extract (AE) from rice straw was analyzed and compared to the composition of raw rice straw. Cow urine demonstrated significantly higher extraction efficiency for nutrient elements, ranging from 0.57 % to 59.20 % in CE and 1.82 % to 22.31 % in AE for macronutrients (N, P, K, Ca, Mg, S), micronutrients (Fe, Zn, Cu, Mn, B) and heavy metals (Pb, Cr, Ni, Cd) (Table 1 and Fig. 1). It has been documented that cow urine contains essential salts, metals, minerals, vitamins, enzymes and other bioactive compounds in trace amounts (22, 23). The study found that cow urine extracted significantly higher mineral elements from rice straw compared to AE. The extraction efficiencies for primary nutrients, secondary nutrients, micronutrients, and heavy metals were 30.82 %, 4.11 %, 6.22 % and 6.30 % for CE and 5.56 %, 10.82 %, 1.53 % and 7.59 % for AE respectively (Fig. 1). This enhanced solubility of minerals facilitated by the organic composition of cow urine (15) likely contributed to the higher nutrient content observed in the cow urine-based rice straw extract in this study.

Phytochemicals in rice straw by GC/MS and LC/MS analysis

In Table 2 and Fig. 2 shows the GC-MS mass spectra of rice straw along with the intensity of detected compounds. A total of 45 compounds were identified and categorized into groups such as fatty acids, alcohols and butyric acid derivatives (Fig. 3). Sterols and fatty acid derivatives constituted the majority

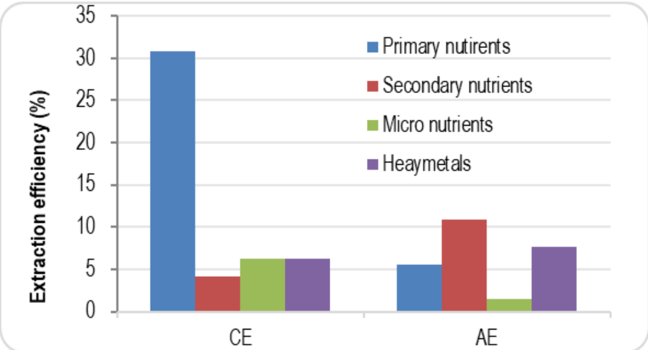

Fig. 1. Graph showing the class-wise nutrient extraction efficiency by cow urine and deionized water from rice straw (AE- aqueous extract; CE- Cow urine extract).

(>37 %) of these compounds, followed by sugar compounds (4.7 %) and amino groups (3.8 %). Notably, significant concentrations of growth-promoting and protective compounds like n-hexadecanoic acid, pentadecanoic acid, octadecanoic acid, sterols and organo-silicon were detected in rice straw. The most abundant compounds identified were n-Hexadecanoic acid (17.96 %), Gamma-Sitosterol (16.81 %), Stigmasterol (12.04 %) and Campesterol (7.64 %). The presence of free fatty acids and sterols as major bioactive compounds in rice straw was documented (24).

The LC-MS mass spectrum (m/z) of rice straw (Fig. 4). The total ion chromatogram (TIC) revealed over 50 compounds and about 28 compounds (Fig. 5) were identified and classified into various metabolomic groups (Fig. 6). Predominantly, these compounds belonged to phenols and cinnamic acid derivatives (45 %), including licarin A, epigallocatechin and catechin, known to enhance plant resilience, growth and yield (25-27). Additionally, benzoic acid and coumarins (23 %) were identified that can contribute to plant growth benefits. Flavonoids and isoflavonoids such as rutin, kaempferol, apigenin, daidzein and myricetin, which aid in plant defense against biotic and abiotic stresses, were also detected in rice straw (28). The presence of amino acids such as tyrosine, phenylalanine, valine and cystine, along with plant growth hormones like indole acetic acid, was also detected in rice straw.

Maize germination parameters

The biostimulants derived from rice straw, CE and AE, were evaluated for their stimulatory effects on maize germination at various concentrations (0, 2.5, 5.0, 7.5 and 10 %). Germination and growth parameters were assessed on the 3rd and 10th days after treatment application. The stimulant effects were analyzed for crucial parameters as provided in Fig. 7. Regardless of the concentration applied, aqueous extract (AE) showed lower germination % (GP: 50-75 %) compared to cow urine extract (CE: 75-100 %). At a concentration of 2.5 %, CE exhibited significantly (P≤0.05) higher germination (P≤0.05) of 100 %, while AE showed 75 %, both outperforming the control.

Table 1. Physico-chemical characteristics and elemental composition of rice straw, cow urine and extracted biostimulants and nutrient elements extraction efficiency of cow urine and deionized water from rice straw.

Parameters	Nutrient elements composition				Nutrients extraction efficiency (%)	
	Rice straw	Cow urine	AE*	CE*	AE	CE
pH (1 % solution)	6.78	8.10	5.89	8.67	-	-
Electrical conductivity (dS m^{-1})	6.17	3.25	2.97	51.8	-	-
Organic carbon (%)	54.48	1.57	1.01	1.05	-	-
Nitrogen (N) (%)	1.06	0.63	0.014	0.034	1.87	2.50
Phosphorous (P) (%)	0.034	0.005	0.003	0.012	8.82	30.77
Potash (K) (%)	1.25	0.50	0.075	1.03	6.00	59.20
Calcium (Ca) (%)	0.048	0.027	0.004	0.005	8.33	6.67
Magnesium (Mg) (%)	0.11	0.067	0.002	0.001	1.82	0.57
Sulfur (S) (%)	0.13	3.63	0.029	0.19	22.31	5.09
Iron (Fe) (mg kg^{-1})	268.65	58.45	4.2	47.5	1.56	14.52
Copper (Cu) (mg kg^{-1})	49.05	1.05	0.1	0.5	0.20	1.00
Manganese (Mn) (mg kg^{-1})	61.25	2.13	0.2	4.4	0.33	6.95
Zinc (Zn) (mg kg^{-1})	28.3	7.30	1.0	0.3	3.53	0.84
Boron (B) (mg kg^{-1})	5.0	14.29	0.1	1.5	2.00	7.79
Nickel (Ni) (mg kg^{-1})	7.2	6.20	1.1	1.5	15.28	11.19
Chromium (Cr) (mg kg^{-1})	16.45	10.23	0.6	0.7	3.65	2.63
Lead (Pb) (mg kg^{-1})	3.5	0.90	0.4	0.5	11.43	11.36
Cadmium (Cd) (mg kg^{-1})	ND	ND	ND	ND	-	-

*AE- aqueous extract; CE- Cow urine extract; ND- not detected

Table 2. Major phytochemicals profile identified in rice straw by GC-MS analysis.

Sl. No.	Rice straw	Retention time (min)	m/z range	Class
	Dimethylamine, N-(neopentyloxy)	4.520	61.10	Amino group
	2H-Pyran, 2-(8-dodecynyloxy)tetrahydro-	1.79	117.00	-
	Benzofuran, 2,3-dihydro-	6.775	120.10	Derivative of benzofuran
	1-Butyl(dimethyl)silyloxypropane	7.053	120.00	Organosilicon compound
	2-Methoxy-4-vinylphenol	7.697	150.10	Phenols
	2- Chloropropionic acid, octadecylester	8.286	55.10	Ester derivatives
	Vanillin	8.531	151.10	Benzaldehydes
	2-Deoxy-D-galactose	8.953	57.10	Deoxygalactose
	1,2-Dimethyl-3-nitro-4-nitroso-benzene	9.831	180.10	Benzene derivative
	d-Glycero-d-tallo-heptose	10.008	61.10	Sugar molecules with carbon
	D-Arabinitol	10.197	61.10	Sugar molecules with carbon
	Sorbitol	10.375	61.10	Sugar molecules
	2-Octenoic acid, ethyl ester	10.675	57.10	Fatty acid
	n-Pentadecanol	10.786	55.10	Fatty alcohol
	n-Decanoic acid	11.286	73.10	Fatty acid
	3-(4-Fluoroanilino)-1-(3-nitrophenyl)-1propanone	11.575	124.10	-
	2-Pentadecanone, 6,10,14-trimethyl	11.864	58.10	-
	Diepi-.alpha.-cedrene epoxide	12.364	157.10	Terpenoid
	Pentadecanoic acid, 14-methyl-, methyl ester	12.419	74.10	Fatty acid
	n-Hexadecanoic acid	12.663	73.10	Fatty acid
	9,12-Octadecadienoic acid, methylester, (E,E)-	13.519	67.10	Fatty acid
	cis-13-Octadecenoic acid, methyl ester	13.563	55.10	fatty acid
	Phytol	13.619	71.10	Fatty alcohol
	9,12-Octadecadienoic acid (Z,Z)	13.797	55.10	Fatty acid
	9,17-Octadecadienal, (Z)-	13.952	55.10	Fatty acid
	Octacosanol	14.519	55.10	Fatty alcohol
	5,6-Dihydro-4-ethyl-2-phenylamino-4H-1,3-thiazin-5-one	14.685	55.10	Amino group
	11,13-Dimethyl-12-tetradecen-1-ol acetate	14.908	55.10	-
	2-Nonyl methylphosphonofluoridate	15.041	99.10	-
	1-Docosene	15.796	55.10	Hydrocarbon
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	15.919	55.10	Fatty acid
	2-Piperidinone, N-[4-bromo-n-butyl]-	16.008	57.10	Organobromine
	Pyrido[2,3-d]pyrimidine, 4-phenyl-	16.674	207.10	-
	1,3,12-Nonadecatriene	16.841	55.10	Hydrocarbon
	9-Borabicyclo[3.3.1]nonane, 9-[3-(dimethylamino)propyl]-	16.974	55.10	Amino group
	Benzenamine, 4-(2-phenylethenyl)-N -(3,5dimethyl-1-pyrazolylmethyl)-	17.018	207.10	Amino group
	9-Octadecenamide, (Z)-	17.274	207.10	Fatty amide
	Silane, 1,4-phenylenebis [trimethyl	18.252	207.10	Silicon derivatives
	1,2-Bis(trimethylsilyl)benzene	19.529	207.10	Silicon derivatives
	Campesterol	20.440	55.10	phytosterols
	Stigmasterol	20.685	55.10	Phytosterols
	Gamma-Sitosterol	21.318	55.10	Phytosterols
	Stigmasterol	21.462	207.10	Steroid
	1,2-Bis(trimethylsilyl)benzene	21.785	207.10	Silicon derivatives
	Silane, 1,4-phenylenebis[trimethyl	22.896	207.10	Silicon derivatives

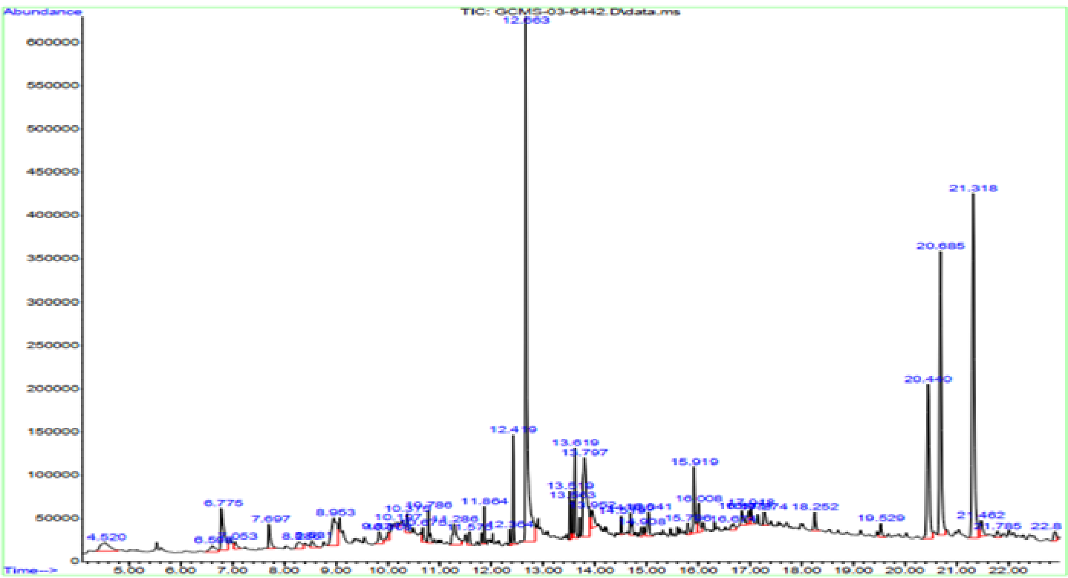

Fig. 2. GC-MS chromatogram of rice straw showing the phytochemical compounds.

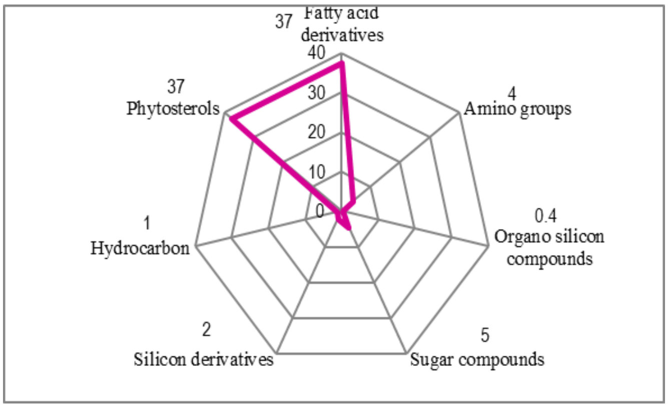

Fig 3. Radar plot illustrating the % of commonly identified bioactive compounds classes in rice straw by GC-MS.

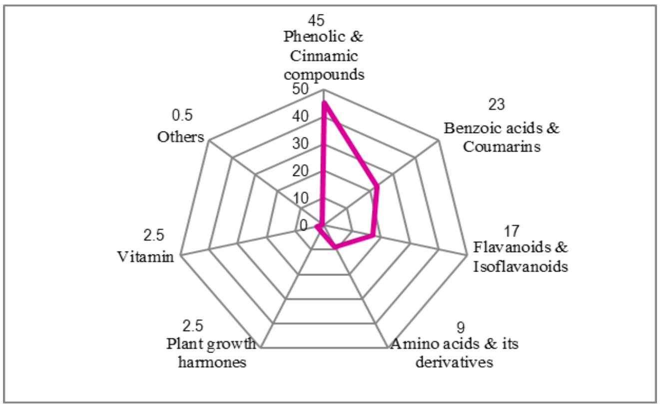

Fig. 6. Radar plot illustrating the % of commonly identified bioactive compounds classes in rice straw by LC-MS.

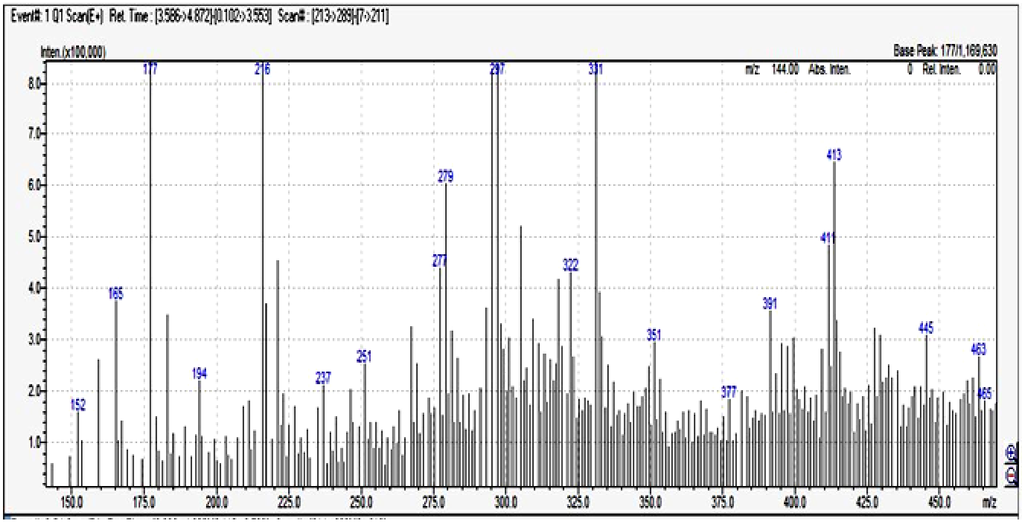

Fig. 4. The total ion count (TIC) chromatogram of rice straw analyzed by LC-MS-ESI detector.

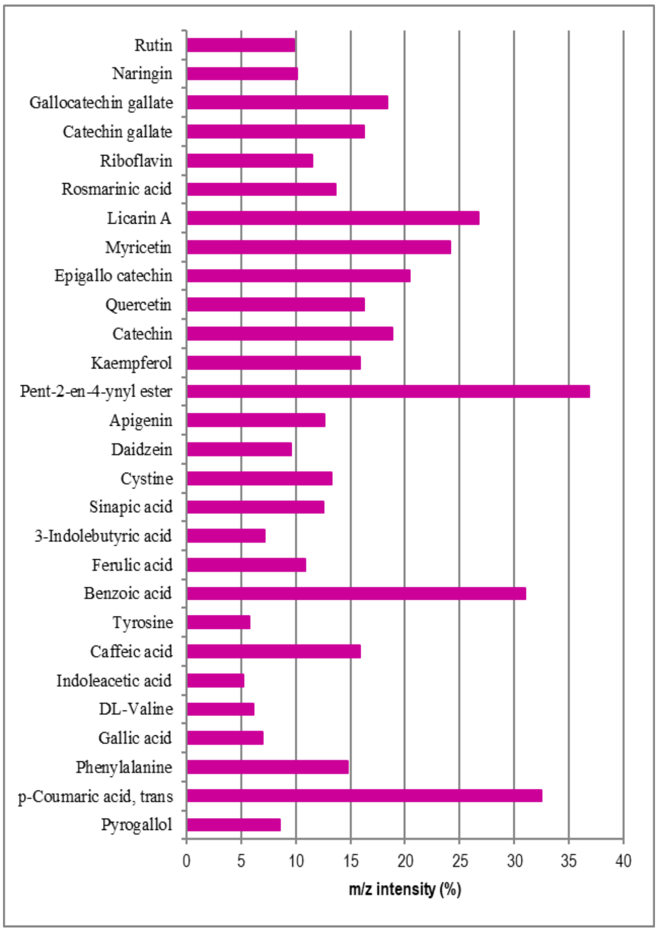

Fig. 5. Intensity of metabolites identified in rice straw by LC-MS.

Higher concentrations, however, led to reduced germination % (Fig. 7a). Similar trends were observed for the germination index (GI), where CE showed higher values (75-100 %) compared to AE (50-75 %). The highest and lowest indices were recorded at the 2.5 % concentration and in the control respectively (Fig. 7b). The CE biostimulant proved more effective in enhancing maize germination compared to AE, which exhibited negative effects at higher concentrations (7.5 % and 10 %), possibly due to phytotoxic effects of bioactive compounds present in AE at higher concentrations (29). Germination energy, reflecting the speed and uniformity of seed emergence, was higher with CE (30-80 %) compared to AE (25-55 %) based on data from the 3rd day. The 2.5 % concentration consistently showed higher values than the control, regardless of extract type (Fig. 7c). Mean germination time (MGT) did not show significant ($P \leq 0.05$) differences between CE and AE at all concentrations tested but varied depending on concentration levels, ranging from 3 to 4 days for both extracts (Fig. 7d). A lower MGT was observed at a 7.5 % concentration, while higher MGT was noted in the control group, regardless of the extract type used. Additionally, the seedling vigor index (SVI) increased with higher concentrations, peaking at 7.5 % concentration before declining (Fig. 7e). The CE biostimulant exhibited a higher SVI than AE. Similar findings, demonstrating improved germination rates, seedling length, root length and biomass in rice after applying aqueous extracts from rice straw substrates (30). Similarly, enhanced germination and seedling growth in papaya using cow urine as a growth-promoting substance (31).

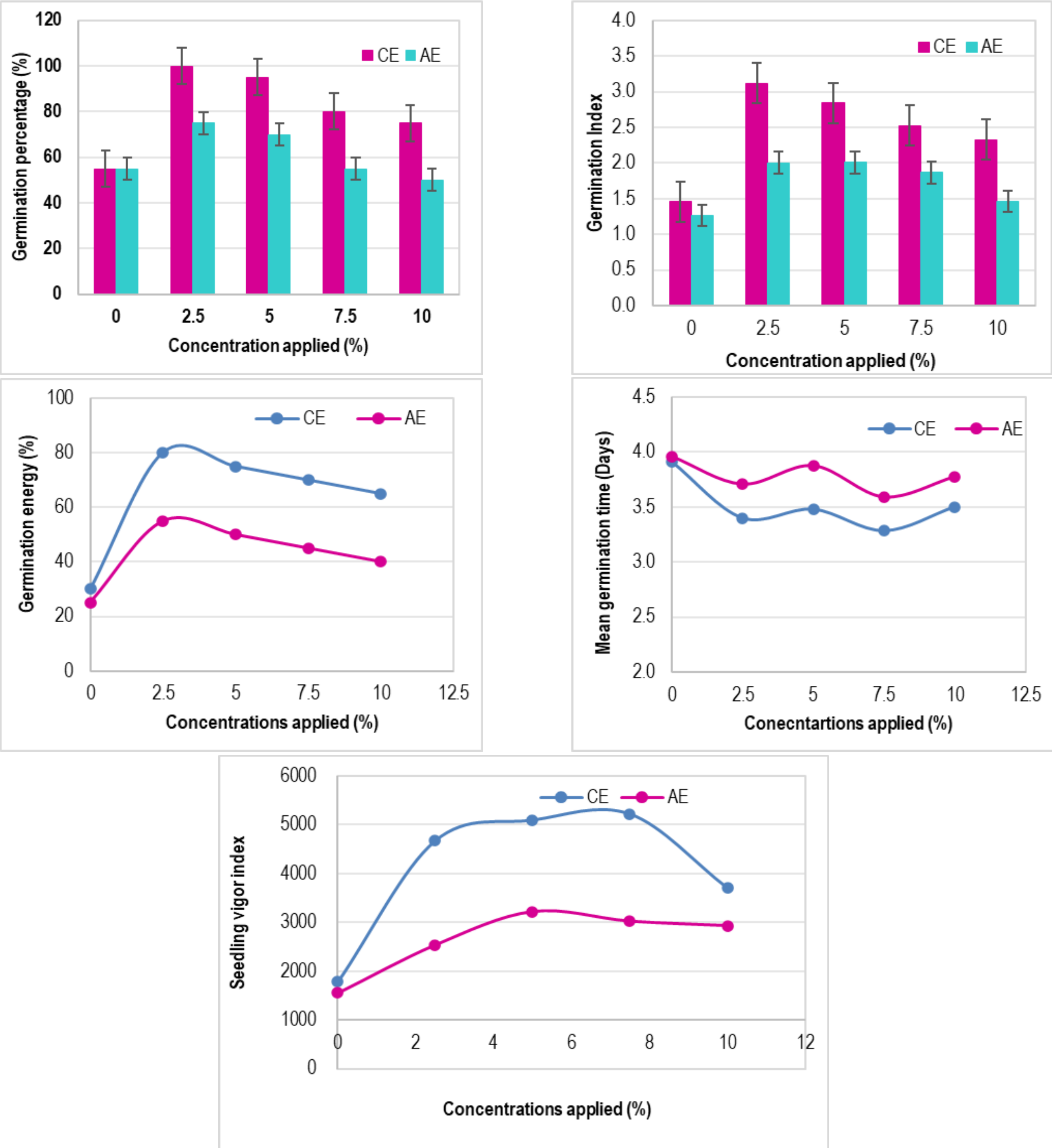

Fig. 7. Influence of various concentrations of aqueous and cow urine extracts on (a) germination percentage (%), (b) germination index, (c) germination energy (%), (d) mean germination time (days) and (e) seedling vigour index.

In addition to evaluating germination parameters, seedling length, biomass and root traits were measured to assess the potential impact of rice straw extracts on maize establishment and growth (Fig. 8). Increasing concentrations of biostimulants resulted in greater seedling length and biomass, with the maximum length observed at a 7.5 % concentration and the maximum biomass at 5.0 %. At a 7.5 % concentration, biostimulants increased seedling length by 132 % (CE) and 120 % (AE), while a 5 % concentration increased biomass by 71 % (CE) and 26 % (AE) compared to the control. This enhancement is attributed to the higher levels of essential mineral elements in CE, particularly amino and phospho compounds, along with other growth-promoting phytochemicals. Similar findings of (32), demonstrating that a fermented mixture of cow urine and medicinal plants can enhance plant growth, promoting healthy seedling growth in cluster bean and fenugreek. Our GC-MS and LC-MS analyses also confirmed the presence of phenols, flavonoids, isoflavonoids, benzoic-cinnamic-coumaric compounds, amino acids and plant growth hormones such as indole acetic acid (33, 34).

Maize root parameters, including root length (RL in cm), root surface area (RSA in cm^2), average diameter (AvD in mm), root hairs (RH tips) and root volume (RV in cm^3), exhibited significant (P≤0.05) variations based on the type and concentration of extractant applied (Table 3). Both root length and surface area were significantly (P≤0.05) higher with the 7.5 % solution, regardless of the biostimulant type. In contrast, the 5 % AE resulted in greater root diameter, more root tips and increased root volume. The control group showed lower values for all root traits. These significant differences among the biostimulant types and concentrations

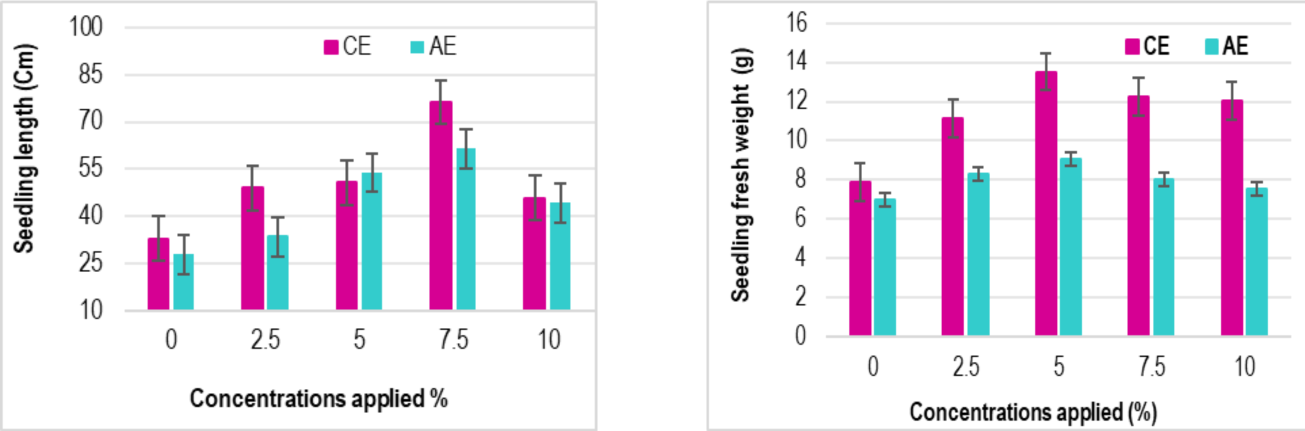

Fig. 8. Representation showing the influence of aqueous and cow urine rice straw biostimulants at varying concentrations on maize growth (a) Seedling length, (b) seedling fresh weight.

Table 3. Effect of aqueous and cow urine rice straw biostimulants on maize root traits.

Treatments	RL (cm)	RS (cm²)	AvD (mm)	RTs	RV (cm³)
			Cow Urine-Rice straw biostimulant		
0	171.01	32.89	0.61	952	0.58
2.5	312.88	62.94	0.71	1247	0.80
5.0	311.08	55.79	0.77	1249	1.23
7.5	330.83	77.96	0.62	1512	1.46
10.0	255.43	70.42	0.62	1435	1.26
Mean	**276.25**	**59.99**	**0.66**	**1279**	**1.07**
			Aqueous-Rice straw biostimulant		
0	170.78	32.13	0.61	914	0.50
2.5	212.33	51.13	0.57	1159	0.98
5.0	273.78	60.90	0.72	1240	1.08
7.5	323.14	62.64	0.78	1148	0.97
10.0	261.90	51.05	0.75	894	0.79
Mean	**248.39**	**51.57**	**0.69**	**1071**	**0.86**

	SE(d)	**CD (p=0.05)**	**SE(d)**	**CD (p=0.05)**	**SE(d)**	**CD (p=0.05)**	**SE(d)**	**CD (p=0.05)**	**SE(d)**	**CD (p=0.05)**
Stimulant type (S)	2.35	4.94	0.33	0.70	0.006	0.012	14.46	30.37	0.01	0.022
Concentration (L)	3.72	7.81	0.53	1.11	0.009	0.019	22.86	48.01	0.017	0.035
S x L	5.26	11.04	0.75	1.57	0.012	0.026	32.32	67.90	0.024	0.050

may be attributed to the enhanced extraction of various macro- and microelements, along with the stimulatory effects of phytochemicals such as sterols, fatty acids and other compounds present in their composition (22). Similarly, significant increases in Chinese cabbage yield, vitamin C content and soluble protein content after extracting wheat and maize straw biochar in hot water was also reported (35).

Effect of CE rice straw biostimulant on tomato growth and yield

Among the treatments evaluated under field conditions, CE biostimulants applied at 5 % and 7.5 % demonstrated significant (P≤0.05) improvements in tomato growth and yield compared to the untreated control. The control treatment resulted in a plant height of 69.47 cm at flowering and 83.14 cm at harvest, with an average of 13 bearing clusters and 7 flowers per cluster. In contrast, the 5 % CE treatment significantly (P≤0.05) enhanced these parameters. The most pronounced effects were observed with the 7.5 % CE treatment, where plant height increased to 115.96 cm at flowering and 121.32 cm at harvest. Additionally, the number of bearing clusters increased to 27 and the number of flowers per cluster showed a substantial improvement (Table 4). These findings indicate that the 7.5 % CE treatment not only outperformed the control in all measured aspects but also demonstrated the most significant enhancement in tomato growth.

The improved performance of CE can be attributed to the synergistic role of cow urine in promoting the extraction of bioactive compounds from rice straw. Studies have also shown cow urine's ability to stimulate plant growth and its antimicrobial properties. Moreover, combining rice straw with cow urine has consistently led to increased yields, further validating its effectiveness in agricultural applications (36, 37). All treatments showed significant improvements in tomato growth compared to water spray alone. For example, plants treated with 3 % Panchagavya saw a 13.5 % increase in flower numbers, while the 5 % CE treatment resulted in a 23.1 % increase and the 7.5 % CE treatment led to a 32.7 % increase. Combinations of Panchagavya and CE also performed well, with flower numbers increasing by 21.2 % for T5 and 26.9 % for T6. These treatments also shortened the time to flowering and harvest, with the 7.5 % CE treatment reducing the time to flowering by 13.5 %. Yield improvements were significant, with T4 showing a 39 % increase over the control and combined treatments of PG and CE further boosting yields (Fig 10). Overall, higher concentrations of CE and combined treatments greatly enhanced flowering, accelerated growth and increased yields (Fig. 9).

Table 4. Effect of CE and Panchagavya (PG) on tomato plant growth.

Treatments	Plant height (cm)		No. of bearing clusters	No. of flowers/cluster
	Flowering	Harvest		
T_1- Absolute control	69.47	83.14	13.00	7.00
T_2- Panchagavya at 3 %	83.10	99.84	15.00	10.00
T_3- CE at 5 %	106.55	112.63	19.00	14.00
T_4- CE at 7.5 %	115.96	121.32	27.00	21.00
T_5- T_2 + T_5	106.21	119.48	21.00	18.00
T_6- T_2 + T_6	109.34	120.08	25.00	20.00
SE (d)	**2.09**	**2.56**	**0.51**	**0.29**
C.D. (p=0.05)	**4.72**	**5.81**	**1.16**	**0.65**

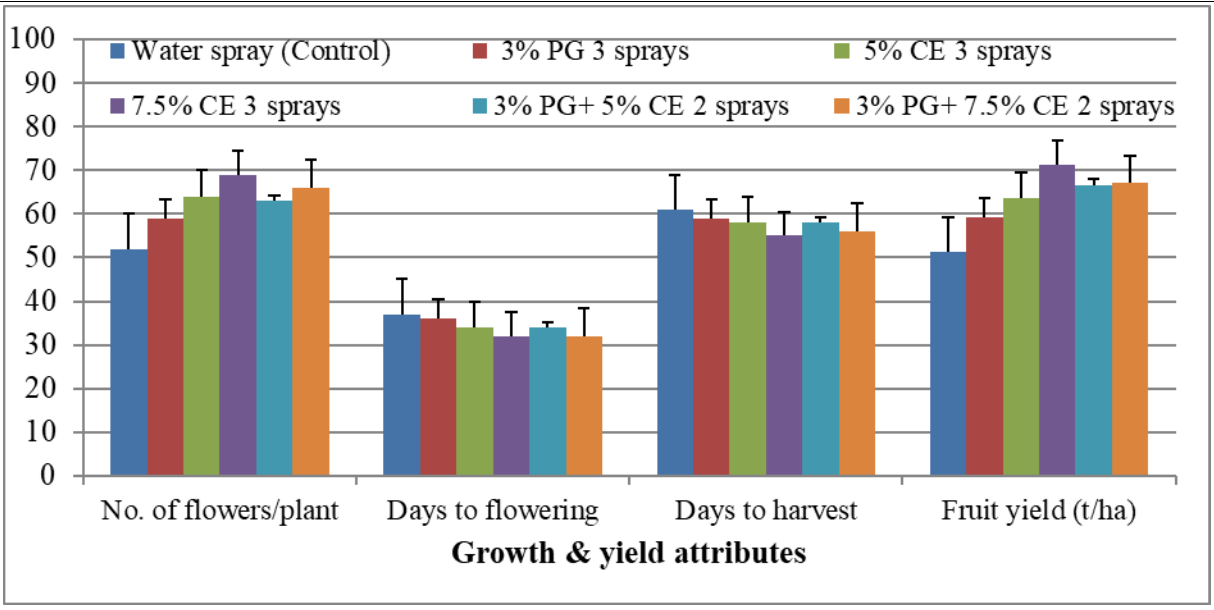

Fig. 9. Influence of CE and PG combinations on number of flowers / plant, days to flowering, days to harvest and yield of tomato.

Fig. 10. Harvested tomato fruits/plant under field experiments.

Conclusion

This study developed environmentally friendly biostimulants from rice straw using cow urine and deionized water, with their nutrient composition and phytochemical content thoroughly analyzed. The rice straw treated with cow urine demonstrated higher nutrient levels compared to those extracted with water. The study identified key phytochemicals, including amino acids like tyrosine and phenylalanine, plant hormones such as indole acetic acid and other compounds like phenols and flavonoids. Both cow urine- and water-based rice straw biostimulants were tested on maize at concentrations from 2.5 % to 10 %. Results showed that maize germination and growth improved up to 7.5 %, with the cow urine biostimulant at this concentration performing best in germination, biomass and seedling length compared to the water-based version. Field trials further validated the superior tomato growth and yield performance of cow urine-extracted biostimulants at 5 % and 7.5 % concentrations. These findings underscore the potential of rice straw-based biostimulants, particularly those derived with cow urine, as eco-friendly solutions for organic agriculture, offering a promising avenue for comprehensive utilization of rice straw in the future.

Authors' contributions

- VV carried out the experiment, analyzed the data and wrote the draft manuscript.
- PJ, TC, EP, MS and RK conceived, designed and coordinated the experiments and corrected the manuscript.
- All the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflict of interest to declare.

Ethical issues: None.

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