Preparation, optimization, and testing of biostimulant formulations as stress management tools and foliar applications on brinjal and onion for growth and yield

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Abstract
Not only would the application of biostimulant formulations boost vegetable yields under typical and different biotic stress circumstances, but it will also be user and ecologically friendly. Due to their hydrophobic character and huge molecular volumes, the production of highly stable (~2 years shelf-life) emulsifiable concentrate (EC) formulations is the bottleneck of plant growth regulator uses. Gibberellic acid (0.25% EC) and Brassinolide (0.15% EC) were developed using a variety of solvents [solvent naphtha (C9), toluene, dimethyl sulfoxide] and surfactants (calcium alkylbenzene sulfonate, nonylphenol ethoxylate-13). Emulsification (spontaneous), detergency, and quick wetting performance were all outstanding in laboratory adjusted phytohormone formulations (E). Highly stable oil in water emulsions with exceptional compatibility (without phase separation) and outstanding emulsion stability (24 h) have been created using secondary alcohol ethoxylates and sulfonate anionic (5:5), which are affected by the hydrophilic-lipophilic balance (HLB) value (12.52) and type of nonionic and anionic surfactants. The hormone content fluctuation was also estimated to be appropriate using liquid chromatography–tandem mass spectrometry (LC–MS/MS) (5.0%). Single and double dosed applications of 450 and 900 mL ha−1 in brinjal (muktakeshi) and 180 and 360 mL ha−1 in onion (sukhsagar) have significantly improved growth and yields above control (untreated) plants. Gibberellic acid boosted brinjal yields by 37.5%, while Brassinolide raised onion yields by 33.9%. Plant growth regulator formulations that have been thoroughly developed and evaluated might be a big step toward environmentally friendly agricultural production.

Keywords Phytohormones · Abiotic stress · Emulsifiable concentrate · Accelerated storage stability · Leaf area index · Absolute growth rates

Introduction
Agriculture is today faced with the problem of boosting output and productivity in order to fulfil the ever-growing global population’s need for food while simultaneously improving resource efficiency and reducing environmental risk to ecosystems and human health. Synthetic pesticides/chemicals and fertilizers play a critical role in agriculture, serving as a powerful tool for farmers to boost output and ensure consistent production throughout the seasons, in both poor and ideal conditions. Various novel methods have been launched in recent years to boost the sustainability of agricultural output by reducing the use of synthetic pesticides, chemicals, and fertilizers. Adapting biostimulants, which are capable of inducing a variety of physiological, biochemical, morpho-anatomical, and molecular plant responses that
enhance plant growth, flowering, fruit set, nutrient use efficiency and productivity, and are responsible for increasing abiotic stress resistance, would be an optimistic and environmentally sound innovative weapon (Colla and Rouphael 2015). Field crops including cereals, pulses, and oil seed crops, as well as greenhouse crops like berry crops, fruit trees, vegetable crops, decorative crops, grapevines, and turf, can benefit from biostimulants (Basile et al. 2020; Rouphael et al. 2017, 2018, 2020). It can be non-organic and organic compounds and/or microorganisms that are sprayed as a foliar spray, utilized as granules, solutions, or powders in the soil, or combined with fertilizer, micronutrients, and compost (Colla and Rouphael 2018; Kocira et al. 2018; Malik et al. 2021). The Solanaceae family’s Solanum melongena (L.), sometimes known as brinjal, is native to India. This widely eaten vegetable has the potential to be used as a pickling ingredient (Mallick et al. 2018). The Amaryllidaceae family’s Allium cepa (L), or “Queen of the Kitchen”, is one of Asia’s top culinary spice vegetables (Mollavali et al. 2016; Awatade et al. 2018). Onions are high in vitamin C and B6, as well as minerals including phosphorus, iron, potassium, and magnesium (Mitra et al. 2012; Olalusi 2014). Onions contain anti-inflammatory, thrombotic, and antioxidant properties (Nuuutila et al. 2003; Vidhyavati et al. 2010; Mitra et al. 2012). Continuous eggplant farming can result in a rise in autotoxins, which stifle plant growth, diminish resistance, impede development, and lower quality output. Onions are very vulnerable to water deficiency stress due to their shallow root architecture (Drinkwater and Janes 1955; Rao et al. 2000). Onion crops can be nurtured with biostimulants to produce high-quality yields.

Biostimulants are used in modern agriculture (Kumari et al. 2018) to boost the yield of brinjal and onions while also improving their quality. Plant growth regulators such as GA, NAA, IAA, IBA, 2,4-D, 2,4,5-T, TIBA, Brassinolide, and Ethephon are commonly used to promote flowering, fruit set, fruit size, fruit quality, and yields. Gibberellic acid (GA), a diterpenoid carboxylic acid that belongs to the gibberellin family, is a natural plant growth regulator that regulates a variety of developmental events in plants (Camara et al. 2018; Shani et al. 2013). Brassinolide is a steroidal plant hormone that promotes growth (Clouse and Sasse 1998; Khripach et al. 2000) with larger physiological effects (Khripach et al. 2000; Vardhini et al. 2012).

Agricultural biostimulants are now accessible in dust, wettable powder, and solution forms, but not in emulsifiable concentrate (EC) forms, according to data collected from the Central Insecticides Board & Registration Committee, Government of India. After dilution in water for spraying, an emulsifiable concentrate formulation is a homogeneous combination (solution) of active chemicals, solvents, and surface-active agents (surfactant) that generates a stable emulsion (Brown et al. 2017). When diluted with water in the spray tank, ECs create a spontaneous emulsion with emulsion droplets ranging from 0.1 to 1.0 µm in size. Selecting one or more surfactants based on their capacity to emulsify the solvent system, including the active component, into water can result in a spontaneous emulsion. A physically stable emulsion is generated by combining water-soluble and oil-soluble surfactant components at the water/solvent interface. When sprayed on the crop, the dilute emulsion ensures a consistent and precise delivery of active chemicals, which is critical for successful pest control.

In light of this, the current study was conducted to produce an emulsifiable concentrate (EC) formulation of Gibberellic acid and Brassinolide and to investigate their effects on brinjal and onion development and yield metrics.

Materials and methods

Chemicals

Sigma-Aldrich, India, provided both Gibberellic acid (90% purity) and Brassinolide (90% purity). Sunflower oil, soybean oil, and mustard oil were purchased at a nearby market in Nadia, West Bengal, India. India Glycols provided organic solvents such as C-IX and emulsifiers such as CABS (Calcium alkylbenzene sulfonate, 70% pure) and NP-13 (Nonylphenol ethoxylate-13, 99% pure). Rankem, India, provided toluene and dimethyl sulfoxide (DMSO). Merck India provided methanol, methyl oleate, calcium chloride, and magnesium chloride. Other emulsifiers were obtained from SD Fine Chem Ltd. in Mumbai, including Span 60, Span 80, Tween 60, and Tween 80.

Preparation of gibberellic acid (0.25% EC) formulation

Purkait et al. (2019) reported a technique for making emulsifiable concentrate (EC), which was modified somewhat (Fig. 1). In a graduated test tube, Gibberellic acid (0.27 g w/w) was dissolved in C-IX solvent (89.73 g), and 10 g of emulsifier mix (5 g CABS and 5 g NP-13) was added while stirring. The entire mixture was then vortexed for 10 min at 500 rpm to produce a homogeneous, translucent formulation.

Preparation of brassinolide (0.15% EC) formulation

Brassinolide (0.17 g w/w) was dissolved in DMSO (9.83 g) as the co-solvent, then amalgamated in toluene to make the EC formulation (80 g). To make it a clear, homogeneous solution, 10 g of appropriate emulsifiers (5 g CABS and 5 g NP-13) was added to it and vortexed at 500 rpm for 8 min.
**Physicochemical characterization**

The generated formulations’ physicochemical properties were investigated using the Collaborative International Pesticides Analytical Council (CIPAC 1985) and Indian Standard (IS) guidelines (BIS 1997).

**Cold test**

A thermometer-equipped stopper was used to seal a 50 mL EC formulation into a 600 mL glass bottle. After being put in cold water, the formulation was cooled to 10 °C. With a minimum opening of the stopper, a little seeding crystal of the target technical was introduced to it and gently swirled for one hour. Any turbidity, separated oily substance, or particles in the formulation were examined.

**Emulsion stability**

2 mL of the EC formulation was put in a 250 mL beaker, and 100 mL of China GB (Guo Biao) (standard hard water [standard hard water (342 ppm) was prepared by addition of 0.304 g calcium chloride (CaCl₂) and 0.139 g hydrated magnesium chloride (MgC₁₂, 6H₂O) in 1 L of double distilled water] was added with constant stirring. For full emulsification, the mixture was transferred to a cylinder (100 mL) and inverted 30 times at 180°. The cylinder was left undisturbed at room temperature (25 °C) for 1 h before being checked for the formation of a creamy layer on top or sediments on the bottom (CIPAC MT 36.3 2003).

**Flashpoint/flammability**

Abel’s flashpoint was used to assess the EC formulation’s flashpoint (Scavini, IP0170-110). The formulation was placed in the cup, and the external flame was directed at intervals, recording the temperature of the formulation’s ignition (CIPAC MT12 1995).

**Storage stability**

Accelerated storage stability tests were conducted for 14 days at elevated temperatures (4, 24, and 54 °C) according to the CIPAC technique (No. 46.3, 2000) at elevated temperatures (4, 24, and 54 °C) (2 year shelf-life at room temperature (27 °C) (CIPAC MT46.3, 2000). Color change, phase separation, creamy layers, and sedimentation were investigated after 14 days in the studied EC formulations.

**pH**

A precalibrated pH meter (Systronics, Model 335; Gujarat, India) was used to measure the pH of created formulations (1% aqueous solution) at 25 °C using pH 5.0, 7.0, and 10.2 as reference buffer solutions (CIPAC MT 75.3 2000).

**Persistent foam**

In a cylinder, two milliliters of EC formulation were combined with 98 ml of standard hard water and inverted 30 times at 180 ° for full emulsification. The cylinder was kept undisturbed for 1 min before measuring the foam volume (CIPAC MT 47.2 1995).

**Storage stability**

The test was carried out in triplicates to look for any physical or chemical changes that happened during storage at elevated temperatures (4, 25, and 54 °C) for 14 days, and any color change (developed formulation’s yellow or brown color to any other color), phase separation, or creamy layers were visually evaluated.
Quantification of gibberellic acid and brassinolide

Waters HPLC linked to an API 3000 tandem mass spectrometer (ABSciex, Concord, ON, Canada) equipped with an electrospray (ESI) source in negative mode were used to quantify Gibberellic acid and Brassinolide levels in the created EC formulations. The quantification was performed on a C-18 reversed-phase column with a mobile phase of methanol/water (50:50, v/v) and 0.2% formic acid at a flow rate of 1.0 mL min⁻¹. SRM transitions for Gibberellic acid and Brassinolide were 345 > 239, 301 and 481.4 > 445.5, 463.4 for Gibberellic acid and Brassinolide, respectively. For Gibberellic acid and Brassinolide, good linearity was discovered within the ranges of 0.05–10 g mL⁻¹, and their quantification limits based on signal-to-noise ratio were 0.05 g mL⁻¹ and 0.03 g mL⁻¹, respectively.

Evaluation of growths and yields of brinjal and onion

The two best formulations for in-vivo bioassay were chosen based on physicochemical characterization. Efficacy on growth parameters of Solanum melongena (L.) cv. Muktakeshi and Allium ascalonium (L.) cv. Sukhsagar was examined in the field at the District Seed Farm (C-Block) of BCKV, Kalyani, Nadia, West Bengal (India) between January and March 2020. The research used a Randomized Block Design (RBD) with three replicates, six treatments, and a control group. Five plant samples (whole plants) were randomly plucked for laboratory examination after the first spray (Stage-I) from each plot, and the process was repeated 20 days later as a second count (Stage-II) and third count (Stage-III), respectively. In a precension weight machine (Mettler Toledo, Model 204), the fresh weight of leaf, shoot, root, and whole plant was recorded, and samples were dried in the oven dryer at 57 °C for 3 days in a brown paper bag. The leaf, branch, root, and entire plant samples were weighed again to determine their dry weight. In triplicates, the leaf areas of brinjal and onion were graphically measured.

Analysis of growth parameters

Crop growth analysis is a comprehensive method for analyzing crops grown in natural or semi-natural environments. It examines the processes within crops using primary data such as weights, areas, volumes, and plant components. The simplest indicator of agricultural development is absolute growth rate (AGR), which is the variation in total dry weight of the crop per unit of time, expressed in g day⁻¹. The ratio of leaf area to ground area over time is known as the leaf area index (LAI). Its dimensions are expressed in square feet per square foot, a dimensionless measurement. The leaf area ratio (LAR) is the ratio of total leaf area/plant to total dry weight/plant and is frequently represented in mm² mg⁻¹ or m² g⁻¹. The leaf weight ratio is the ratio of total leaf dry weight per plant to total plant dry weight per plant (LWR).

Statistical analysis

All data are the average of three independent replicates' standard deviations (SD). Using the SPSS16.0 version, differences between treatments were assessed using variance and Duncan's Multiple Range Test (DMRT) with a 5% impact (P 0.05).

Results and discussion

EC is still one of the most popular formulation types (Knowles 2009) and offers a number of benefits, including ease of manufacture, less equipment required, good stability, increased active ingredient ingestion capacity, outstanding biological activity, and convenience of administration (Wu et al. 2013). The authors opted to produce Gibberellic acid (0.25%) and Brassinolide (0.15%)
EC formulations (Fig. 2) using appropriate emulsifier blends because of the numerous benefits of EC formulations. Due to the right selection of two separate emulsifiers to thoroughly emulsify the active components in water, Gibberellic acid and Brassinolide ECs created water in oil emulsions (0.1–1.0 μm droplet sizes) in the spray tank. The spray mixture was created by the inhomogeneous and appropriate distribution of PGRs on the target vegetable leaf surfaces when it was sprayed.

**Establishment of the right solvent system**

The dissolution capacities of Gibberellic acid and Brassinolide in various solvent systems (organic solvents: toluene, DMSO, and C-IX, as well as vegetable oils: soybean oil, sunflower oil, and mustard oil) were investigated, and the results revealed that C-IX and DMSO are the two best carrier solvents for Gibberellic acid and Brassinolide, respectively, as both solutions remained unchanged for 7 days at 4 °C. The active components in the produced Gibberellic acid and Brassinolide formulations were entirely soluble in carrier solvents and compatible with additional compounds. If the technical requirements are solid in nature, they must first be dissolved in appropriate solvents. The organic solvents utilized in the solubilization and dilution of Gibberellic acid and Brassinolide were crucial. The use of C-IX, which has excellent compatibility and solvency power, as well as regulated evaporation and low polarity, produced dense milky emulsions with water. As an alternative to hazardous solvents like N-Methyl Pyrrolidone, DMSO was employed to manufacture the Brassinolide formulation as an enhanced solubilizer and diluents (NMP).

**Determination of emulsifier**

The proper selection of emulsifiers is critical to the long-term performance of EC formulation under varying circumstances. Gibberellic acid and Brassinolide were blended with individual emulsifiers to minimize surface and interfacial tension in our EC formulation creation process, and the emulsifiers were chosen after a series of experiments.

**Selection of single emulsifier**

After cold storage, emulsifying agents such NP-13, Tween-60, Tween-80, Span-60, Span-80, and CABS were tested for any oil separation or creamy layer. When Tween-60 and Tween-80 were employed as emulsifiers, Gibberellic acid and Brassinolide emulsions generated some cream layers, whereas Span-60 and Span-80 resulted in a very thick cream layer on top of the emulsions. As a consequence of the thorough study of the data, NP-13 and CABS were chosen as the best individual emulsifiers (Table 1).

**Determination of compound emulsifiers**

The effects of different emulsifier combinations have been studied. The total emulsifiers’ dose is set at 10% by weight. Table 2 summarizes the findings, indicating that all emulsifiers are incompatible with the oil phase and that stability performance varies. The 2-h stability test is passed by all formulations, however only emulsion E passes the 24-h stability test. After the accelerated storage stability test, only the combination of NP-13 and CABS met all of the requirements. The created EC formulations achieved the best stability in the emulsifier ratio (NP13: CABS = 5:5), according to the results.

| Name of technical | Emulsifier | Blooming properties | Emulsifying properties | Cold storage stability |
|-------------------|------------|---------------------|-----------------------|------------------------|
| Gibberellic acid | Tween-60 | Good | Not good | Separate oil |
| | Tween-80 | Poor | Not good | Separate oil |
| | Span-60 | Poor | Good | Separate oil |
| | Span-80 | Poor | Good | Separate oil |
| | NP-13<sup>a</sup> | Better | Best | No oil separation |
| | CABS<sup>b</sup> | Better | Best | No oil separation |
| Brassinolide | Tween-60 | Poor | Not good | Separate oil |
| | Tween-80 | Poor | Not good | Separate oil |
| | Span-60 | Poor | Not good | Separate oil |
| | Span-80 | Good | Not good | Separate oil |
| | NP-13 | Best | Best | No oil separation |
| | CABS | Best | Best | No oil separation |

<sup>a</sup>Nonylphenol ethoxylate (NP-13)

<sup>b</sup>Calcium salt of alkylbenzene sulfonate (CABS)
To create long-term stable delivery systems, the authors used a combination of surfactant techniques, one non-ionic and the other anionic. CABS is an oil-soluble anionic emulsifier that was employed in a triangle screening strategy to improve stability, blooming, and electrostatic stabilization. Non-ionic emulsifiers, on the other hand, provide steric stabilization and hence increase emulsion stability in water. NP-13 (nonylphenol ethoxylate) is a good example of a non-ionic surfactant that has superior connections at the oil/water interface, resulting in exceptional stability even under harsh conditions.

**Quality assurance of the developed formulations**

After rapid storage, the content fluctuations of Gibberellic acid (0.25% EC) and Brassinolide (0.15% EC) were determined to be within acceptable ranges (5.0%). The LC–MS/MS approach is adequate for assessing two PGRs in the created EC formulations, as evidenced by the acceptable recovery percent (95.5–102.4) of targeted biostimulants. For gibberellic acid and brassinolide, the retention periods (RT) were 4.36 and 6.01 min, respectively (Fig. 3).

Proper formulation and good distribution are the important criteria for the performances of various formulations. Increased penetration of active chemicals into target plant leaf enhances formulation efficacy while reducing non-target organism effects (Gasic et al. 2011). Both formulations passed with acceptable limits for several physicochemical characteristics such as blooming, emulsion stability, re-emulsification, persistent foam, pH, flash point, cold storage, and accelerated storage, according to the results (Table 3). When dispensed in standard hard water, both formulations bloomed beautifully. Both formulations were stable for 14 days at high temperatures, with great results in persistent foam, emulsion stability, and cold tests. After increased temperature storage (ATS), the active component content fluctuation of each formulation was within the permitted range (5%). The flammable temperature was above the flashpoint.

**Table 2** Optimization of emulsifiers for development of phytohormone EC formulations

| Name of formulation | Test sample | Ratios of emulsifier | Heat storage (54 ± 2) °C | Cold storage (0 ± 2) °C |
|---------------------|-------------|----------------------|--------------------------|------------------------|
| Gibberellic acid 0.25% EC | A | NP-13:CABS = 6:4 | Oil separating, 2.3% | Light deposition |
|                     | B | NP-13:CABS = 4:6 | Oil separating, 4.1% | Deposition |
|                     | C | NP-13:CABS = 7:3 | Oil separating, 1.6% | Light deposition |
|                     | D | NP-13:CABS = 3:7 | Oil separating, 2.5% | Deposition |
|                     | E | NP-13:CABS = 5:5 | Up to standard | No deposition |
| Brassinolide 0.15% EC | A | NP-13:CABS = 6:4 | Oil separating, 2.3% | Light deposition |
|                     | B | NP-13:CABS = 4:6 | Oil separating, 4.1% | Deposition |
|                     | C | NP-13:CABS = 7:3 | Oil separating, 1.6% | Light deposition |
|                     | D | NP-13:CABS = 3:7 | Oil separating, 2.5% | Deposition |
|                     | E | NP-13:CABS = 5:5 | Up to standard | No deposition |

**Fig. 3** Chromatograms of Gibberellic acid (a) and Brassinolide (b) EC formulations
in all compositions (24.5 °C). According to the International guidelines, a formulation is regarded successful if it passes all of the physicochemical property tests.

**Bio-efficacy**

Infield studies in 2020 (Figs. 4, 5) evaluated the effects of developed EC formulations on brinjal and onion growth and yields. Pre-treatment (PT) growth of brinjal and onion was uniform and increased considerably after treatments compared to control.

A review of the data found that single dosages of Gibberellic acid and Brassinolide EC enhanced the leaf area index (LAI) of brinjal by up to 109.1% and 105.1%, respectively. Brassinolide double dose-treated onion plants had a maximum of 63.5% rise in leaf area index, according to research (LAI). The leaf area ratio (LAR) was found to be similar in both types of plants. Over the control treatment, Gibberellic acid double dose-treated brinjal plants and Brassinolide single dose-treated onion plants demonstrated the greatest increases in leaf area ratio (LAR), 33.3% and 28.2%, respectively. In both brinjal and onion, the increases in LAI and LAR could be due to an increase in leaf area rather than leaf dry weight, as it has been shown previously that application of 28-homobrassinolide in *Vigna radiata* (Hayat et al. 2010) and GA3 in blueberry (Zang et al. 2016) can increase leaf area, plant biomass, and plant height. The leaf weight ratio of Brinjal plants treated with a single dosage of Gibberellic acid was reduced by up to 52.3% (LWR). In the case of onions, a single dosage of Gibberellic acid and Brassinolide greatly increased LWR decrease. The highest drop in leaf weight ratio (LWR) was 61.6% in Gibberellic acid double dose-treated plants. This abrupt decline in LWR is similar to Ali et al (2021)’s earlier work in strawberry, in which he indicated that the biomass distribution pattern in plants owing to exogenous application of 24-epibrassinolide was shoot > roots > leaves. He also speculated that the drop in LWR may be due to decreasing N2 concentration in leaves. The administration of these two growth regulators resulted in an excessive increase in specific leaf area (SLA) for both brinjal and onion plants. However, the largest increase in SLA, 21.2%, was seen following a second dosage of Gibberellic acid. In the case of onions, double-dosed Brassinolide-treated plants generated a maximum of 37.9% growth in particular leaf regions (SLA). SLA supplementation followed by a rise in SLA might be due to its involvement in regulating photosynthesis and source-sink connections (Otite et al. 2019). The rapid rise in SLA was linked to an increase in leaf area and a decrease in LWR. Similarly, brinjal plants treated with twofold dosages of Gibberellic acid and Brassinolide showed substantial increases in absolute growth rate (AGR) of up to 55.9% and 53.9%, respectively. As administered with twofold dosages of PGRs, onion exhibited a significant increase in AGR of up to 112% and 109%, respectively, when compared to control-treated plants. Greater photosynthetic CO2 fixation, increased cell membrane permeability, and better enzymatic activities were thought to cause dry matter buildup in Gibberellic acid-treated plants, which might promote mineral salt absorption and use as well as photosynthesize transportation (Miceli et al. 2019; Vetrano et al. 2020).

### Table 3  Comparison of the physicochemical parameters of Gibberellic acid (0.25% EC) and Brassinolide (0.15% EC)

| Parameters                        | Gibberellic acid 0.25% EC | Brassinolide 0.15% EC |
|-----------------------------------|---------------------------|-----------------------|
| **Description**                   | Yellow liquid without sediments | Brown liquid, free without sediments |
| **Active ingredient**             | Identity tests            | The technical complies with an identity test | The technical complies with an identity test |
| **Active ingredient content**     | 2.7 g/l at 20 ± 2 °C      | 1.6 g/l at 20 ± 2 °C  |
| **pH range**                      | 6.5                       | 6.2                   |
| **Emulsion and re-emulsification stability** | 0 h | Complete emulsification | Complete emulsification |
|                                  | 0.5 h                     | Cream: 0 mL           | Cream: 0 mL           |
|                                  | 2 h                       | Cream: 1 mL           | Cream: 1 mL           |
|                                  |                           | Oil: no oil           | Oil: no oil           |
|                                  | 24 h                      | Complete re-emulsification | Complete re-emulsification |
|                                  | 24.5 h                    | Cream: 1 mL           | Cream: 1 mL           |
|                                  |                           | Oil: trace            | Oil: trace            |
| **Persistent foam**              | Maximum:                  | Maximum:              | Maximum:              |
|                                  | 1 mL after 1 min          | 1 mL after 1 min      | 1 mL after 1 min      |
| **Storage stability**            | Stability at 0 °C         | The volume of solid or liquid separation: 0 mL | The volume of solid or liquid separation: 0 mL |
The brinjal and onion growth effectiveness in supervised field tests using EC formulations was positive. At stage III, Gibberellic (450 and 900 mL ha\(^{-1}\)) acid and Brassinolide (180 and 360 mL ha\(^{-1}\)) EC formulations produced the greatest leaf (area index, area ratio, specific area) and absolute growth rate when compared to the control. Growth stimulation by GA and BR in this study might be attributed to a higher rate of carboxylation, which boosted carbon absorption and hence helped increase total biomass and leaf area (Miceli et al. 2019; Vetrano et al. 2020; Zulkarnaini et al. 2019). Furthermore, as previously documented, brassinolide spraying can considerably increase the deposition of dry matter in leaves and stems (Otie et al. 2019). It has been shown that BR promotes elongation by altering cell wall extensibility and producing cell wall relaxing qualities (Zulkarnaini et al. 2019). On the other hand, gibberellins are key growth regulators that can help xyloglucan endo(trans)glycosylase (XET) break down the cell wall and increase cell penetration (Saptari and Dewi 2013).

Khan and Ansari (2008) found a 35.5% increase in leaf area and leaf area index following GA treatments in mustard, indicating that the plant had a good chance to collect more sun rays and create drier matter, resulting in a 27.1% increase in yield. Brassinolide boosted crop yields by modifying plant metabolisms and safeguarding crops from various environmental challenges, according to Krishna (2003).
Brassinolide can stimulate photosynthesis by increasing leaf water balance, according to Sairam (1994). As revealed by Iwahari et al. (1990) Brassinolide treated crops have increased chlorophyll content as a result of increased leaf area. Gibberellic acid, according to Inada et al. (2000), can cause cell elongation and cell division, which are responsible for the creation of larger leaf regions. Because meristems enhance photosynthetic surface area and vegetative development, the leaf area of Brassinolide-treated Indian beans increased (Ramrajet et al. 1997). Gibberellic acid boosted biomass output, according to Naidu et al. (1995), perhaps owing to an increase in leaf area. In our trials, treated plots had greater leaf area index, leaf area ratio, specific leaf area, and yields than control plots. The highest number of branches per plant was observed with GA single dose (8.94) and BR double dose (8.37), while the lowest number was found with control (6.19) (Fig. 6). The increased number of branches was most likely owing to enhanced rates of photosynthesis and photosynthetic supply for maximum, which led to apical dominance. These results are consistent with those of Dubey et al. (2013), and Meena and Dhaka (2015).

All of the created EC formulations of these two biostimulants greatly increased the yields of both vegetables. In brinjal, a single dosage of Gibberellic acid, as well as a double dose, resulted in the highest crop output, with a maximum increase of 37.5% in yield. In contrast, a single dose of Brassinolide resulted in a 33.9% increase in onion output. Increased cellular metabolic activities through increased nutrients and water uptake, improved enzymatic activities, and increased protein synthesis could explain...
the increase in yield potential in brinjal and onion after treatment with bioformulations (GA and or BR), as it was in the previous study on maize (Miceli et al. 2019; Otie et al. 2019; Vetrano et al. 2020).

Conclusion

In this work, stable Gibberellic acid (0.25% EC) and Brassinolide (0.15% EC) formulations were created by stringent optimization techniques. The finest emulsifiable concentrate (EC) formulations with acceptable physicochemical properties were made with a blend of nonylphenol ethoxylate (NP-13) and a calcium salt of alkylbenzene sulfonate (CABS) in defined ratios. The created formulations were found to be extremely successful in boosting plant growth in brinjal and onion, resulting in enhanced dry matter production, or yield. This comprehensive development of EC biostimulant formulations might be a big step toward more sustainable agriculture.

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Declarations

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References

Ali MM, Anwar R, Malik AU, Khan AS, Ahmad S, Hussain Z, Chen F (2021) Plant growth and fruit quality response of strawberry
is improved after exogenous application of 24-epibrassinolide. J Plant Growth Regul 41:1786–1799
Awatake SC, Saha A, Dhakre DS (2018) Sociological status of onion growing farmers in Akola district of Maharashtra, India. Int J Curr Microbiol Appl Sci 7:6–12
Basile B, Rouphael Y, Colla G, Soppelsa S, Andreotti C (2020) Appraisal of emerging crop management opportunities in fruit trees, grapes and berry crops facilitated by the application of biostimulants. Sci Hortic 267:109330
Beadle CL (1993) Growth analysis. In: Photosynthesis and production in a changing environment. Springer, Dordrecht, pp 36–46
BIS Specification (1997) Indian standard methods of test for pesticide and their formulations. IS: 6940-1982, Reaffirmed 21-26
Brown R, Giangiravusa M, Kirby AF, Sayluk D (2017) U.S. Patent No. 9,781,921. U.S. Patent and Trademark Office, Washington, DC
Camara MC, Vandenbergh LP, Rodrigues C, de Oliveira J, Faulds C, Bertrand E, Soccol CR (2018) Current advances in Gibberellic acid (GA3) production, patented technologies and potential applications. Planta 248:1049–1062
CIPAC MT 47.2 (1995) Persistent foaming. In: Dobrat W, Martijn A (eds) CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Collaborative International Pesticides Analytical Council Ltd., Harpenden, pp 152–153
CIPAC MT 75.3 (2000a) Determination of pH. In: Dobrat W, Martijn A (eds) CIPAC handbook J. Physico-chemical methods for technical and formulated pesticides. Collaborative International Pesticides Analytical Council Ltd., Harpenden, p 128
CIPAC MT 36.3 (2003) Emulsion stability and re-emulsification. In: Dobrat W, Martijn A (eds) CIPAC handbook K. Physico-chemical methods for technical and formulated pesticides. Collaborative International Pesticides Analytical Council Ltd., Harpenden, p 137
CIPAC MT 46.3 (2000b) Accelerated storage procedure. In: Dobrat W, Martijn A (eds) CIPAC handbook K. Physico-chemical methods for technical and formulated pesticides. Collaborative International Pesticides Analytical Council Ltd., Harpenden, p 131
CIPAC MT 36.3 (2003) Emulsion stability and re-emulsification. In: Dobrat W, Martijn A (eds) CIPAC handbook K. Physico-chemical methods for technical and formulated pesticides. Collaborative International Pesticides Analytical Council Ltd., Harpenden, p 137
CIPAC MT 12 (1995) Flash point. In: Dobrat W, Martijn A (eds) CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Collaborative International Pesticides Analytical Council Ltd., Harpenden, p 1
CIPAC MT 36.3 (2003) Emulsion stability and re-emulsification. In: Dobrat W, Martijn A (eds) CIPAC handbook K. Physico-chemical methods for technical and formulated pesticides. Collaborative International Pesticides Analytical Council Ltd., Harpenden, p 128
Clouse SD, Sasse JM (1998) Brassinosteroids: essential regulators of plant growth and development. Annu Rev Plant Biol 49:427–451
Colla G, Rouphael Y (2015) Biostimulants in horticulture. Sci Hortic 166–1–2
Drinkwater WO, Janes BE (1955) Effects of irrigation and soil moisture on maturity, yield and storage of onion hybrids. Proc Am Soc Hortic Sci 66:267–279
Dubey GD, Parmar AS, Kanwer HS, Verma SC, Mehta DK (2013) Effect of micronutrients on plant growth and fruit yield parameters of bell pepper (Capsicum annum L.) grown under mid hill conditions of Himachal Pradesh. Veg Sci 40(1):107–108
Gasic S, Brkic D, Tomasevic A (2011) Oil dispersion with abamectin as active ingredient. Pestic Fitomed 26:409–413
Hayat S, Hasan SA, Yusra M, Hayat Q, Ahmad M (2010) Effect of 28 homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in Vigna radiata. Environ Exp Bot 69:105–112
Hunt R, Thomas B, Murphy DJ, Murray D (2003) Growth analysis, individual plants. Encyclopedia Appl Plant Sci 2:579–588
Inada S, Shimmen T (2000) Regulation of elongation growth by gibberellin in root segments of Lemma minor. Plant Cell Physiol 41:932–939
Iwahari S, Tominaga S, Higuchi S (1990) Retardation of abscession of citrus leaf and fruitlet explants by brassinolide. Plant Growth Regul 9:119–125
Khan NA, Ansari HR (2008) Effect of gibberellic acid spray during ontogeny of mustard on growth, nutrient uptake and yield characteristics. J Agron Crop Sci 181:61–63
Khrapsh V, Zhabinskii V, Groot A (2000) Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. Ann Bot 86:441–447
Knowles A (2009) Global trends in pesticide formulation technology: the development of safer formulations in China. Outlooks Pest Manag 20:5
Kocira A, Swieca M, Kocira S, Zlotek U, Jakubczyk A (2018) Enhancement of yield, nutritional and ’nutraceutical properties of two common bean cultivars following the application of seaweed extract (Ecklonia maxima). Saud J Biol Sci 25:563–571
Krishna P (2003) Brassinosteroid-mediated stress response. J Plant Growth Regul 22:289–297
Kumari S, Bakshi P, Sharma A, Wali VK, Jasrotia A, Kour S (2018) Use of plant growth regulators for improving fruit production in sub tropical crops. Int J Curr Microbiol Appl Sci 7:659–668
Malik A, Mor VS, Tokas J, Punia H, Malik S, Malik K et al (2021) Biostimulant-treated seedlings under sustainable agriculture: a global perspective facing climate change. Agronomy-Basel 11:24
Mallick JR, Dash S, Patnaik HP (2018) Efficacy of biorrhicular and eco-friendly control strategies in brinjal against Epilachna beetle, jassids and whiteflies. J Entomol Zool Stud 7:203–235
Menna SS, Dhaka RS (2015) Effect of plant growth regulators on growth & yield of brinjal under semi-arid conditions of Rajasthan. Ann Agric Res 24(3):516–521
Miciel A, Moncada A, Sabatino L, Vetran F (2019) Effect of gibberellic acid on growth, yield, and quality of leaf lettuce and rocket grown in a floating system. Agron 9:382
Mitra J, Shrivastava SL, Rao PS (2012) Onion dehydration: a review. J Food Sci Technol 49:267–277
Mollavali M, Bolandnazar SA, Schwarz D, Rohn S, Riehle P, Zaare-Nahandi F (2016) Flavonol glucoside and antioxidant enzyme biosynthesis affected by mycorrhizal fungi in various cultivars of onion (Allium cepa L.). J Agric Food Chem 64:71–77
Naidu CV, Swamy PM (1995) Effect of gibberellic acid on growth, biomass production and associated physiological parameters in some selected tree species. Indian J Plant Physiol 38:15–17
Nuttila AM, Puupponen-Pimiä R, Aarni M, Oksman-Caldentey KM (2003) Comparison of antioxidant activity of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem 81:485–493
Olalusi A (2014) Hot air drying and quality of red and white varieties of onion (Allium cepa). J Agric Chem Environ 3:13–19
Otie V, Ping A, Udo I, Eneji E (2019) Brassinolide effects on maize (Zea mays L.) growth and yield under waterlogged conditions. J Plant Nutri 42:945–969
Purkait A, Biswas S, Saha S, Hazra DK, Roy K, Biswas PK, Ghosh SK, Kole RK (2019) Formulation of plant based insecticides, their bio-efficacy evaluation and chemical characterization. Crop Prot 125:104907
Ramraj VM, Vyas BN, Godrej NB, Mistry KB, Swami BN, Singh N (1997) Effects of 28-homoBrassinolide on yields of wheat, rice, groundnut, mustard, potato and cotton. J Agric Sci 128:405–413
Rao DS, Srihivas SA, Krishnasami R, Chandrasek GM (2000) Storage stable pesticide formulations containing Azadirachtin. Patent No: US 6811790 B1
Rouphael Y, Colla G (2018) Synergistic biostimulatory action: designing the Next generation of plant biostimulants for sustainable agriculture. Front Plant Sci 9:1–24
Rouphael Y, Colla G, Graziani G, Ritiemi A, Cardarelli M, De Pascale S (2017) Phenolic composition, antioxidant activity and mineral
profile in two seed-propagated artichoke cultivars as affected by microbial inoculants and planting time. Food Chem 234:10–19

Rouphael Y, Kyriacou MC, Petropoulos SA, De Pascale S, Colla G (2018) Improving vegetable quality in controlled environments. Sci Hortic 234:275–289

Rouphael Y, Lucini L, Miras-Moreno B, Colla G, Bonini P, Cardarelli M (2020) Metabolomic responses of maize shoots and roots elicited by combinational seed treatments with microbial and non-microbial biostimulants. Front Microbiol 11:664

Sairam RK (1994) Effects of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. Plant Growth Regul 14:173–181

Saptari RT, Dewi K (2013) Effect of borax and gibberellic acid on the growth and development of red chilli (Capsicum annuum L. “gelora”). In: The third basic science international conference, vol 4, no 1, pp 1–3

Shani E, Weinstain R, Zhang Y, Castillejo C, Kaiserli E, Chory J, Tsien RY, Estelle M (2013) Gibberellins accumulate in the elongating endodermal cells of arabidopsis root. Proc Natl Acad Sci 110:4834–4839

Vardhini BV (2012) Effect of brassinolide on certain enzymes of sorghum grown in saline soils of Karaikal. J Phytol 4:30–33

Vetrano F, Moncada A, Miceli A (2020) Use of gibberellic acid to increase the salt tolerance of leaf lettuce and rocket grown in a floating system. Agronomy 10:505

Vidyavati HG, Manjunatha H, Hemavathy J, Srinivasan K (2010) Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats. J Food Sci Technol 47:55–60

Wu XM, Feng JG, Ma C (2013) Pesticide preparation processing experiment. Chemical Industry Press, Beijing, pp 15–18

Zang YX, Chun JJ, Zhang LL, Hong SB, Zheng WW, Xu K (2016) Effect of gibberellic acid application on plant growth attributes, return bloom, and fruit quality of rabbiteye blueberry. Sci Hortic 200:13–18

Zulkarnaini ZM, Zaharah SS, Mohamed MTM, Jaafar HZ (2019) Effect brassinolide application on growth and physiological changes in two cultivars of fig (Ficus carica L.). Pertanika J Trop Agric Sci 42:333–346

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