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

Subhajit Ruidas
Bidhan Chandra Krishi Viswavidyalaya
Snehashis Karmakar
Bidhan Chandra Krishi Viswavidyalaya
Aloke Purkait
PalliSiksha Bhavana (Institute of Agriculture)
Arka Gangopadhyay
Bidhan Chandra Krishi Viswavidyalaya
Rounak Saha
Bidhan Chandra Krishi Viswavidyalaya
Kanad Mukherjee
Bidhan Chandra Krishi Viswavidyalaya
Prithusayak Mondal
North Bengal Agricultural University
Dipak Kumar Hazra (dipakipft@gmail.com)
Bidhan Chandra Krishi Viswavidyalaya

Research Article

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

Posted Date: March 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1464974/v1

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 extremely stable emulsifiable concentrate (EC) formulations is the bottleneck of plant growth regulator uses. Gibberellic acid (0.25 percent EC) and Brassinolide (0.15 percent EC) were synthesized using a variety of solvents (Aromatic hydrocarbon, Toluene, Dimethyl sulfoxide) and surfactants (Calcium alkylbenzene sulfonate, Nonylphenol ethoxylate-13). Emulsification, detergency, and quick wetting performance were all outstanding in laboratory adjusted phytohormone formulations (E). Highly stable oil in water emulsions with exceptional compatibility and outstanding emulsion stability have been created using secondary alcohol ethoxylates and sulfonate anionic (5:5), which are affected by the hydrophilic-lipophilic balance (HLB) value 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 percent). In brinjal (muktakeshi) and onion (sukhsagar), applications at 450, 900, 180, and 360 mL ha^{-1} considerably enhanced growth and yields over control plants. Gibberellic acid boosted brinjal yields by 37.5 percent, while Brassinolide raised onion yields by 33.9 percent. Plant growth regulator formulations that have been thoroughly developed and evaluated might be a big step toward environmentally friendly agricultural production.

Introduction

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, thrombolytic, and antioxidant properties (Nuutila et al., 2003; Vidyavati 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, 2016). 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 1.0µm 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 percent purity) and Brassinolide (90 percent 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 and NP-13. 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 Tween80.

Preparation of Gibberellic acid (0.25% EC) formulation

Purkait et al. (2019) reported a technique for making emulsifiable concentrate (EC), which was modified somewhat (Figure 1). In a graduated test tube, Gibberellic acid (0.27g w/w) was dissolved in C-IX solvent (89.73g), 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 minutes 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) were added to it and vortexed at 500 rpm for 8 minutes.

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 10oC. 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 GB standard hard water (Ca^2 109+ and Mg^2 + $0.342 gL^{-1}$) was added with constant stirring. For full emulsification, the mixture was transferred to a cylinder (100 mL) and inverted 30 times at 180^0. The cylinder was left undisturbed at room temperature (25^0C) for 1 hour 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 54^0C) according to the CIPAC technique (No. 46.3, 2000) at elevated temperatures (4, 24, and 54^0C) (2 year shelf-life at room temperature (27^0C) (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 percent aqueous solution) at 25^0C 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 milliliters of standard hard water and inverted 30 times at 180^0 for full emulsification. The cylinder was kept undisturbed for 1 minute 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^{0}C) for 14 days, and any color change, 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 percent 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 characterisation. 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 precession 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^{0}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 (Beadle, 1993; Hunt et al., 2003).

Absolute growth rate (AGR)

The variation in total dry weight of the onion crop per unit of time, represented as g day^{-1}, is the simplest indication of crop development.

Leaf area index (LAI)

It is the percentage of leaf area to ground area during a certain period of time. Its dimensions are measured in square feet per square foot, which is a dimensionless measurement.

Leaf area ratio (LAR)

The ratio of total leaf area/plant and total dry weight/plant and often expressed as $mm^2\ mg^{-1}$ or $m^2\ g^{-1}$.

Leaf weight ratio (LWR)

The ratio of total leaf dry weight per plant to total plant dry weight per plant. Its dimensions are defined by the mass per mass, which is a dimensionless quantity.

Specific leaf area (SLA)

The ratio of total leaf area/crop and total leaf dry weight/crop is mentioned as $mm^2\ mg^{-1}$ or $m^2\ g^{-1}$.

Statistical analysis

All data is 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 Discussions

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 percent) and Brassinolide (0.15 percent) 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 m 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 4^0C. 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).

Table 1
Results of a single emulsifier screening for the development of EC formulations

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	Better	Best	No oil separation
	CABS	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

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-hour stability test is passed by all formulations, however only emulsion E passes the 24-hour 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.

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

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 percent EC) and Brassinolide (0.15 percent EC) were determined to be within acceptable ranges (5.0 percent). 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 minutes, 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 in all compositions (24.5^{0}C). According to the International guidelines, a formulation is regarded successful if it passes all of the physicochemical property tests.

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
Physical properties	pH range:		6.5	6.2
	Emulsion and re-emulsification stability :	0 h	Complete emulsification	Complete emulsification
		0.5h	Cream: 0mL	Cream: 0mL
		2h	Cream: 1mL Oil: no oil	Cream: 1mL Oil: no oil
		24h	Complete re-emulsification	Complete re-emulsification
		24.5h	Cream: 1mL Oil: trace	Cream:1mL Oil: trace
	Persistent foam :		Maximum: 1 mL after 1 minute	Maximum: 1 mL after 1 minute.
Storage stability	Stability at 0°C :		The volume of solid or liquid separation: 0 mL	The volume of solid or liquid separation: 0 mL

Bio-efficacy

Infield studies in 2020 (Figs. 4 and 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 percent and 105.1 percent, respectively. Brassinolide double dose-treated onion plants had a maximum of 63.5 percent 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 percent and 28.2 percent, respectively. The leaf weight ratio of Brinjal plants treated with a single dosage of Gibberellic acid was reduced by up to 52.3 percent (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 percent in Gibberellic acid double dose-treated plants. 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 percent, was seen following a second dosage of Gibberellic. In the case of onions, double-dosed Brassinolide-treated plants generated a maximum of 37.9% growth in particular leaf regions (SLA). 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 percent, respectively. As administered with twofold dosages of PGRs, onion exhibited a significant increase in AGR of up to 112 percent and 109 percent, respectively, when compared to control-treated plants.

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. Gibberellins are key growth regulators that can help xyloglucan endo transglycosylase (XET) break down the cell wall and increase cell penetration (Saptari et al., 2013).

Khan et al. (2008) found a 35.5 percent 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 percent 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., Brassinolide treated crops have increased chlorophyll content as a result of increased leaf area (1990). 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.

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 percent in yield. In contrast, a single dose of Brassinolide resulted in a 33.9 percent increase in onion output.

Conclusion

In this work, stable Gibberellic acid (0.25 percent EC) and Brassinolide (0.15 percent 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.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interests.

Acknowledgments

The authors are thankful to Agro-Chemical Formulation Laboratory and All India Network Project on Pesticide Residues, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Nadia, West Begal, India for providing the facilities for this research.

Conflicts of interest

The authors declare no conflict of interests.

Funding

Not applicable

Authors' contribution

Subajit Ruidas, Snehashis Karmakar and **Prithusayak Mondal:** Critical evaluation, technique, software, and manuscript drafting; **Arka Gangopadhyay** and **Rounak Saha:** Ideation and drafting; **Aloke Purkait**: Critical examination and expert opinion; **Dipak Kumar Hazra**: Conception, ideation, critical evaluation, supervision, and expert perspective; **Kanad Mukherjee**: Literature review and expert view. The research article was co-authored by all authors, who sent their approval to the final edition.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

References

1. Awatade, S. C., Saha, A., & Dhakre, D. S. (2018). Sociological status of onion growing farmers in Akola district of Maharashtra, India. International Journal of Current Microbiology and Applied Sciences, 7, 6–12.
2. Beadle, C. L. (1993). Growth analysis. In *Photosynthesis and production in a changing environment* (pp. 36–46). Springer, Dordrecht.
3. BIS Specification, 1997. Indian standard methods of test for pesticide and their formulations. IS: 6940 – 1982, Reaffirmed 21–26.
4. Brown, R., Giansiracusa, M., Kirby, A. F., Saylik, D. (2017). U.S. Patent No. 9,781,921. Washington, DC: U.S. Patent and Trademark Office.
5. Camara, M. C., Vandenberghe, L. P., Rodrigues, C., de Oliveira, J., Faulds, C., Bertrand, E., & Soccol, C. R. (2018). Current advances in Gibberellic acid (GA3) production, patented technologies and potential applications. Planta, 248, 1049–1062.
6. CIPAC MT12. Flash Point. In: Dobrat W, Martijn A, ed. CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 1995. p. 1.
7. CIPAC MT 36.3. Emulsion stability and re-emulsification. In: Dobrat W, Martijn A, eds. CIPAC handbook K. Physicochemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 2003. p. 137
8. CIPAC MT 47.2. Persistent foaming. In: Dobrat W, Martijn A, eds. CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 1995. p. 152–3.
9. CIPAC MT 75.3. Determination of pH.In: Dobrat W, Martijn A, eds. CIPAC handbook J. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 2000. p. 131.
10. CIPAC MT 46.3. Accelerated storage procedure. In: Dobrat W, Martijn A, eds. CIPAC handbook J. Physicochemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd.; 2000. p. 128.
11. CIPAC 1985. 221.b/EC/M/3. Handbook 1C, Collaborative International Pesticides Analytical Council, Harpenden, UK; p. 2028–31.
12. Clouse, S. D., & Sasse, J. M. (1998). Brassinosteroids: Essential regulators of plant growth and development. Annual review of plant biology, 49, 427–451.
13. Drinkwater, W. O., & Janes, B. E. (1955). Effects of irrigation and soil moisture on maturity, yield and storage of two onion hybrids. In *Proceedings of the American Society for Horticultural Science*, 66, 267– 279.
14. Gasic, S., Brkic, D., & Tomasevic, A. (2011). Oil dispersion with abamectin as active ingredient. Pestic Fitomed, 26, 409–413.
15. Hunt, R., Thomas, B., Murphy, D. J., & Murray, D. (2003). Growth analysis, individual plants. Encyclopedia of applied plant sciences, 2, 579–588.

16. Inada, S., & Shimmen, T. (2000). Regulation of elongation growth by gibberellin in root segments of Lemna minor. Plant Cell Physiology, 41, 932–939.
17. Iwahari, S., Tominaga, S., & Higuchi, S. (1990). Retardation of abscission of citrus leaf and fruitlet explants by Brassinolide. Plant Growth Regulation, 9, 119–125.
18. Khan, N. A., & Ansari, H. R. (2008). Effect of Gibberellic acid spray during ontogeny of mustard on growth, nutrient uptake and yield characteristics. Journal of Agronomy and Crop Science, 181, 61–63.
19. Khripach, S., Zhabinskii, V., & Groot, A. (2000). Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI century. Annals of Botany, 86, 441–447.
20. Knowles, A. (2009). Global trends in pesticide formulation technology: The development of safer formulations in China. Outlooks on Pest Management, 20, 5.
21. Krishna, P. (2003). Brassinosteroid- mediated stress response. Journal of Plant Growth Regulation, 22, 289–297.
22. Kumari, S., Bakshi, P., Sharma, A., Wali, V. K., Jasrotia, A., & Kour, S. (2018). Use of Plant Growth Regulators for Improving Fruit Production in Sub Tropical Crops. International Journal of Current Microbiology and Applied Sciences, 7, 659–668.
23. Mallick, J. R., Dash, S. & Patnaik, H. P. (2018). Efficacy of Biorational and eco-friendly control strategies in brinjal against Epilachna beetle, jassids and whiteflies. Journal of Entomology and Zoology Studies, 7, 203–235.
24. Mitra, J., Shrivastava, S. L. & Rao, P. S. (2012). Onion dehydration: a review. Journal of food science and technology, **49**, 267–277.
25. Mollavali, M., Bolandnazar, S. A. Schwarz, D., Rohn, S., Riehle, P., & ZaareNahandi, F. (2016). Flavonol glucoside and antioxidant enzyme biosynthesis affected by mycorrhizal fungi in various cultivars of onion (*Allium cepa* L.). Journal of agricultural and food chemistry, 64, 71–77.
26. Naidu, C. V., & Swamy, P. M. (1995). Effect of Gibberellic acid on growth, biomass production and associated physiological parameters in some selected tree species. Indian Journal of Plant Physiology, 38, 15–17.
27. Nuutila, A. M., Puupponen-Pimiä, R., Aarni, M., & Oksman-Caldentey, K. M. (2003). Comparison of antioxidant activity of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food chemistry, 81, 485–493.
28. Olalusi, A. (2014). Hot air drying and quality of red and white varieties of onion (*Allium cepa*). Journal of agricultural chemistry and environment, 3, 13–19.
29. Purkait, A., Biswas, S., Saha, S., Hazra, D. K., Roy, K., Biswas, P. K., Ghosh, S. K., & Kole, R. K. (2019). Formulation of plant based insecticides, their bio-efficacy evaluation and chemical characterization. Crop Protection, 125, 104907.
30. Ramraj, V. M., Vyas, B. N., Godrej, N. B., Mistry, K. B., Swami, B. N. & Singh, N. (1997). Effects of 28-homoBrassinolide on yields of wheat, rice, groundnut, mustard, potato and cotton. The journal of agricultural science, 128, 405–413.

31. Rao, D. S., Srinivasa, S., Krishnasami, R., Chandrasek, G. M. (2000). Storage Stable Pesticide Formulations Containing Azadirachtin, Patent No: US 6811790 B1
32. Sairam, R. K. (1994). Effects of homoBrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. Plant Growth Regulation, 14, 173–181.
33. Saptari, R. T., & Dewi, K. (2013). Effect of borax andgibberellic acid on the growth and development of red chilli (Capsicum annuum L. “gelora”). The Third Basic Science International Conference, 4(1), 1–3.
34. Shani, E., Weinstain, R., Zhang, Y., Castillejo, C., Kaiserli, E., Chory, J., Tsien, R. Y.& Estelle, M. (2013). Gibberellins accumulate in the elongating endodermal cells of arabidopsis root. Proceedings of the National Academy of Sciences, 110, 4834–4839.
35. Vardhini, B. V. (2012). Effect of Brassinolide on certain enzymes of sorghum grown in saline soils of Karaikal. Journal of Phytology, 4, 30–33.
36. Vidyavati, H. G., Manjunatha, H., Hemavathy, J., Srinivasan, K. (2010). Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats. Journal of food science and technology<bi>,</bi> **l47**, 55–60.
37. Wu, X. M., Feng, J. G., & Ma. C. (2013). Pesticide preparation processing experiment. Chemical industry press, Beijing, pp. 15–18.

Figures

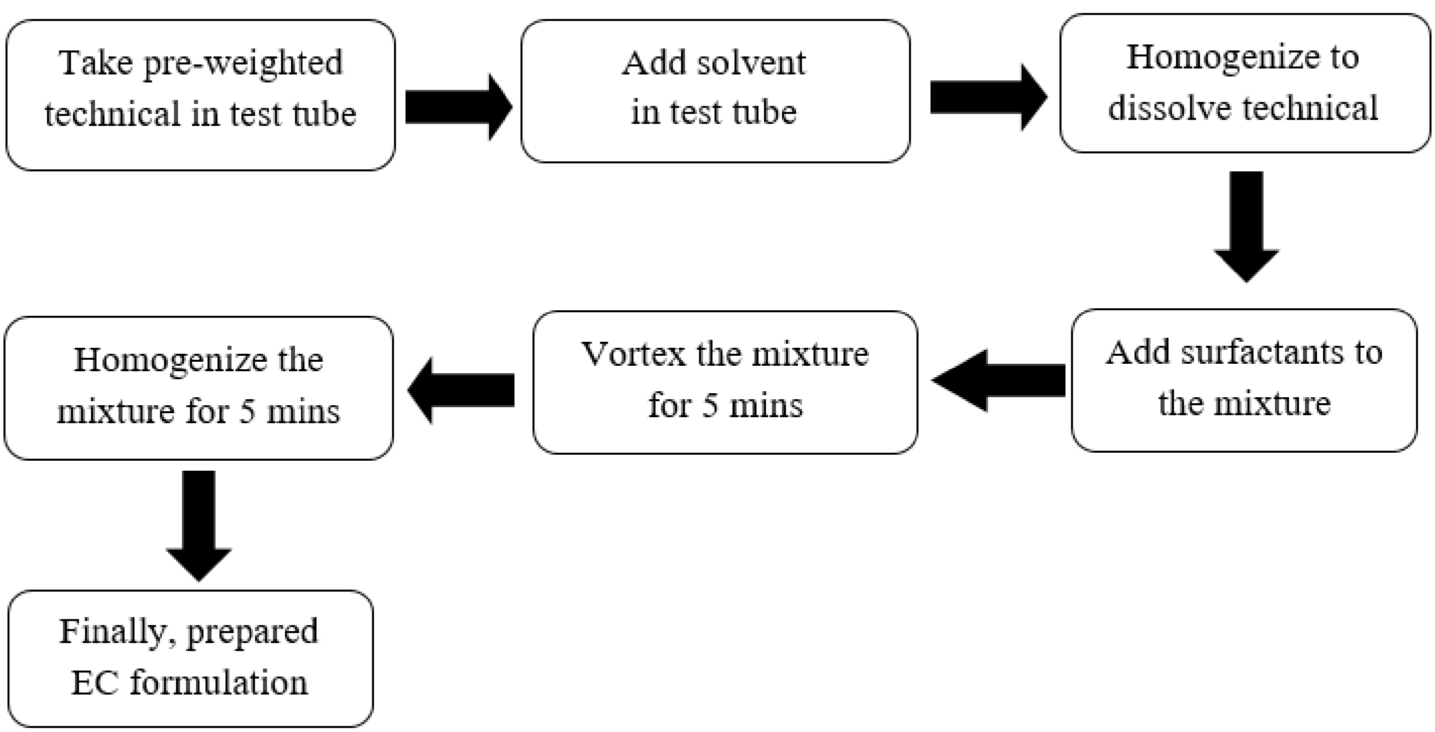
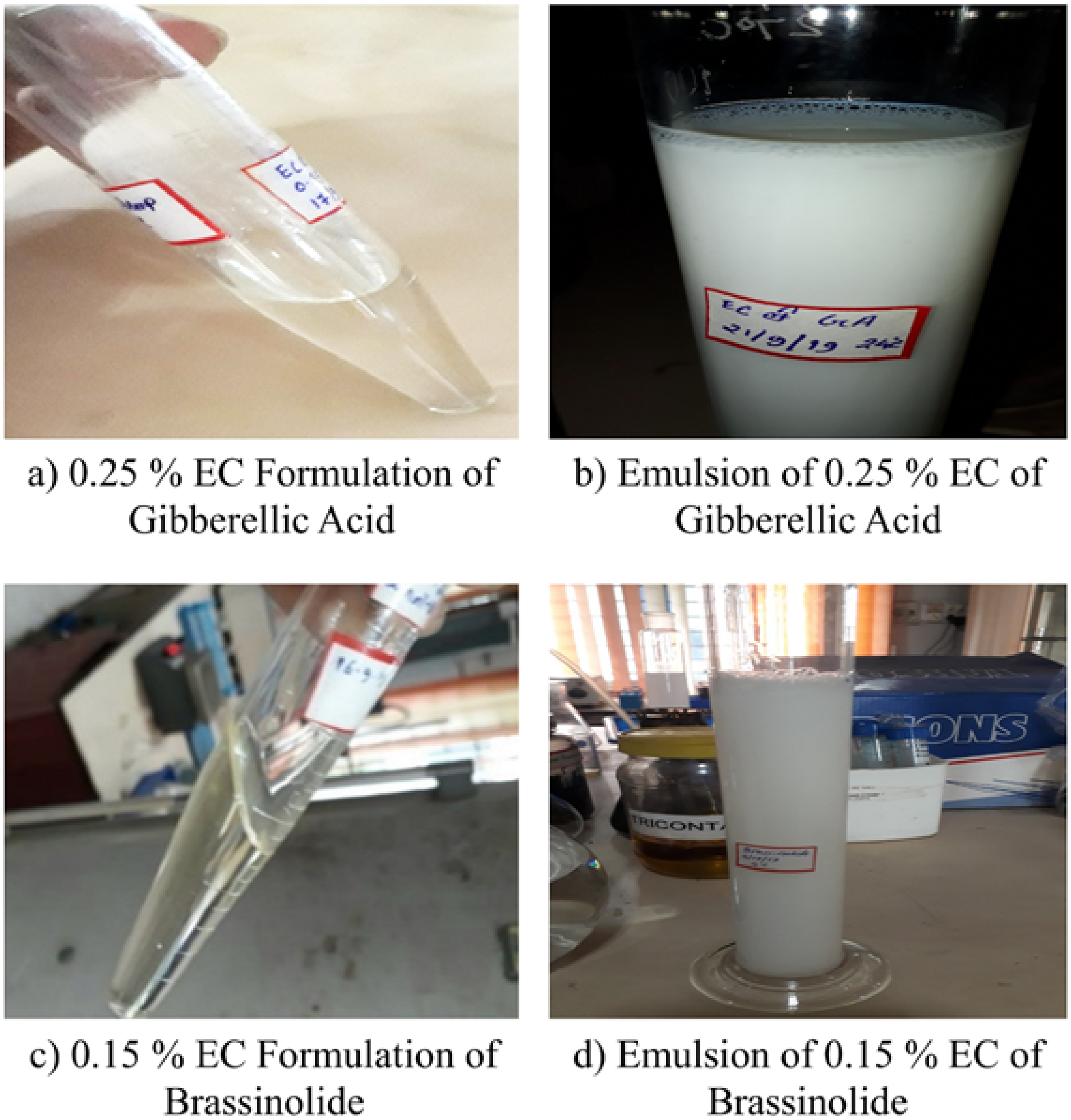


Figure 1

A flow diagram of the preparation of an emulsifiable concentrate formulation of plant growth regulators



a) 0.25 % EC Formulation of Gibberellic Acid

b) Emulsion of 0.25 % EC of Gibberellic Acid

c) 0.15 % EC Formulation of Brassinolide

d) Emulsion of 0.15 % EC of Brassinolide

Figure 2

Emulsifiable concentrate (EC) formulations and emulsions of Gibberellic Acid and Brassinolide

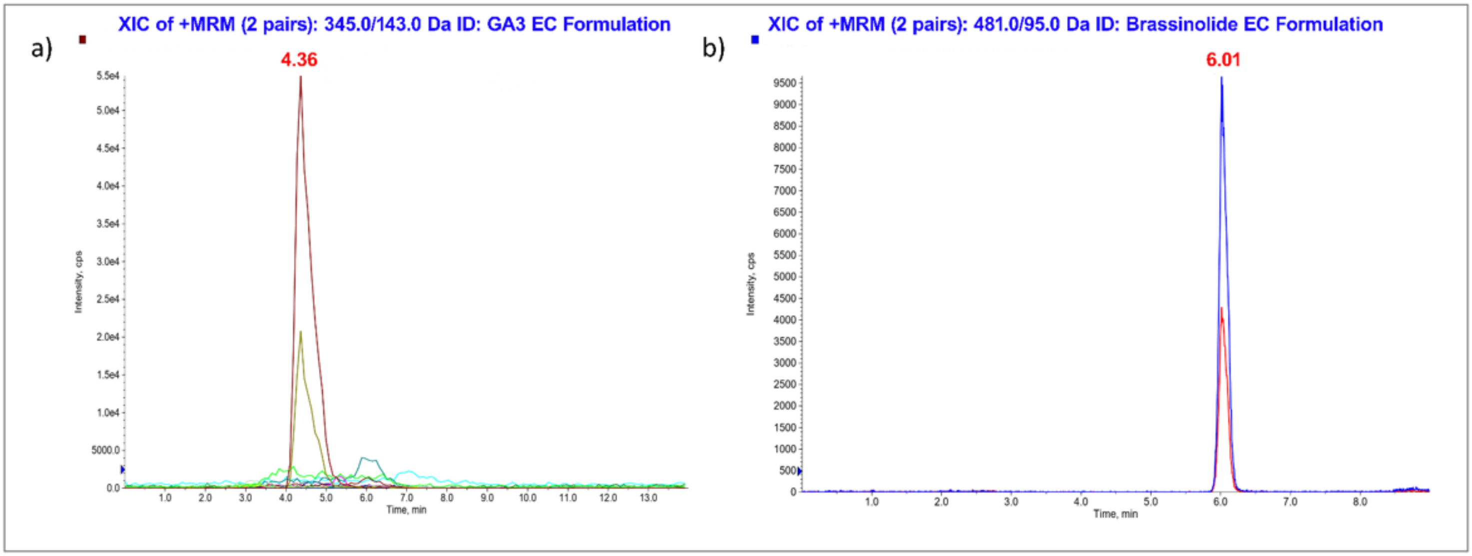


Figure 3

Chromatograms of Gibberellic acid (a) and Brassinolide (b) EC formulations

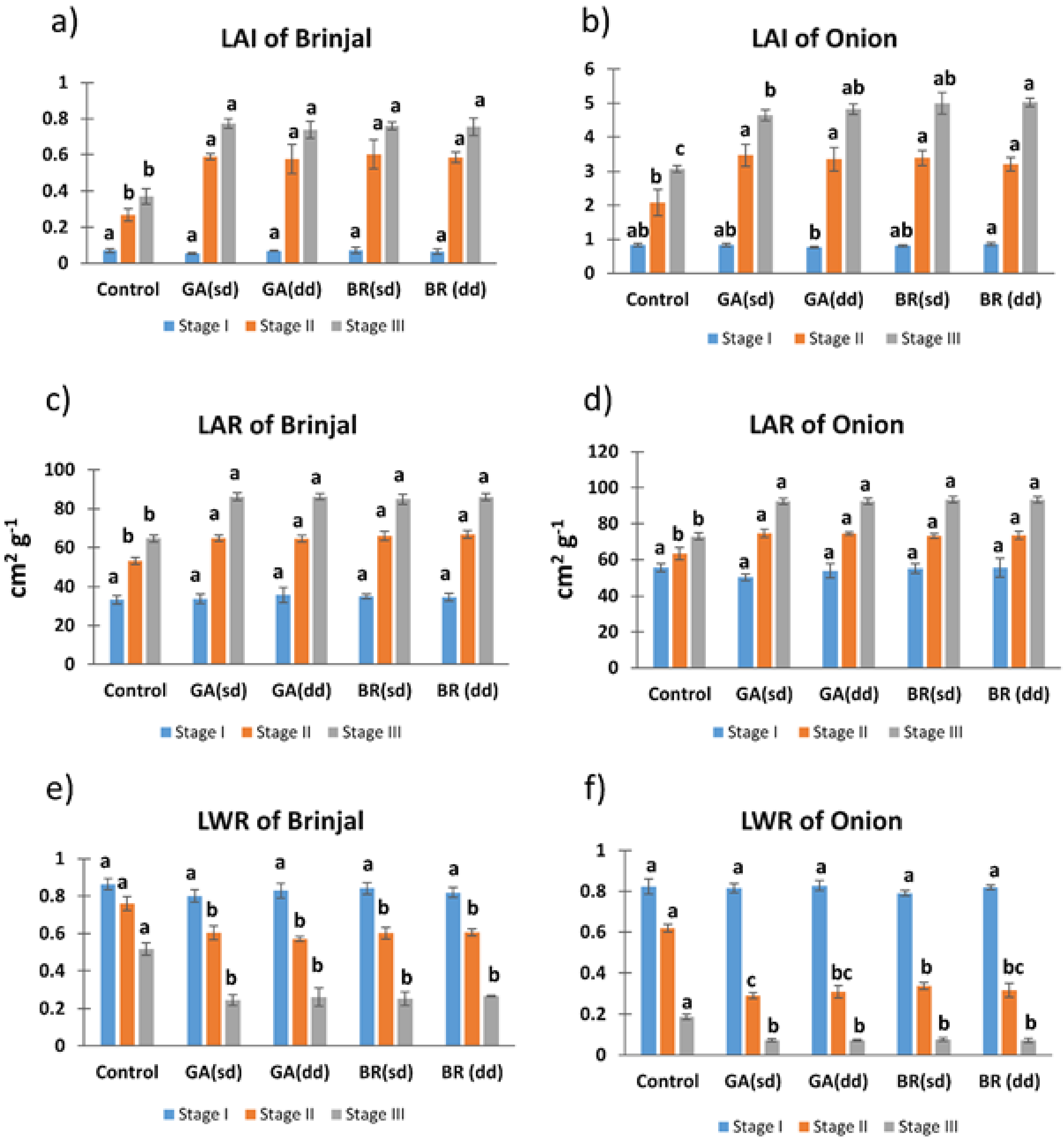


Figure 4

Applications of Gibberellic Acid and Brassinolide Emulsifiable Concentrate (EC) formulations and their interactions on the leaf area index (LAI), leaf area ratio (LAR), and leaf weight ratio (LWR) of Brinjal and Onion plants; All values are the mean of three independent replications SD; error bars represent SD.

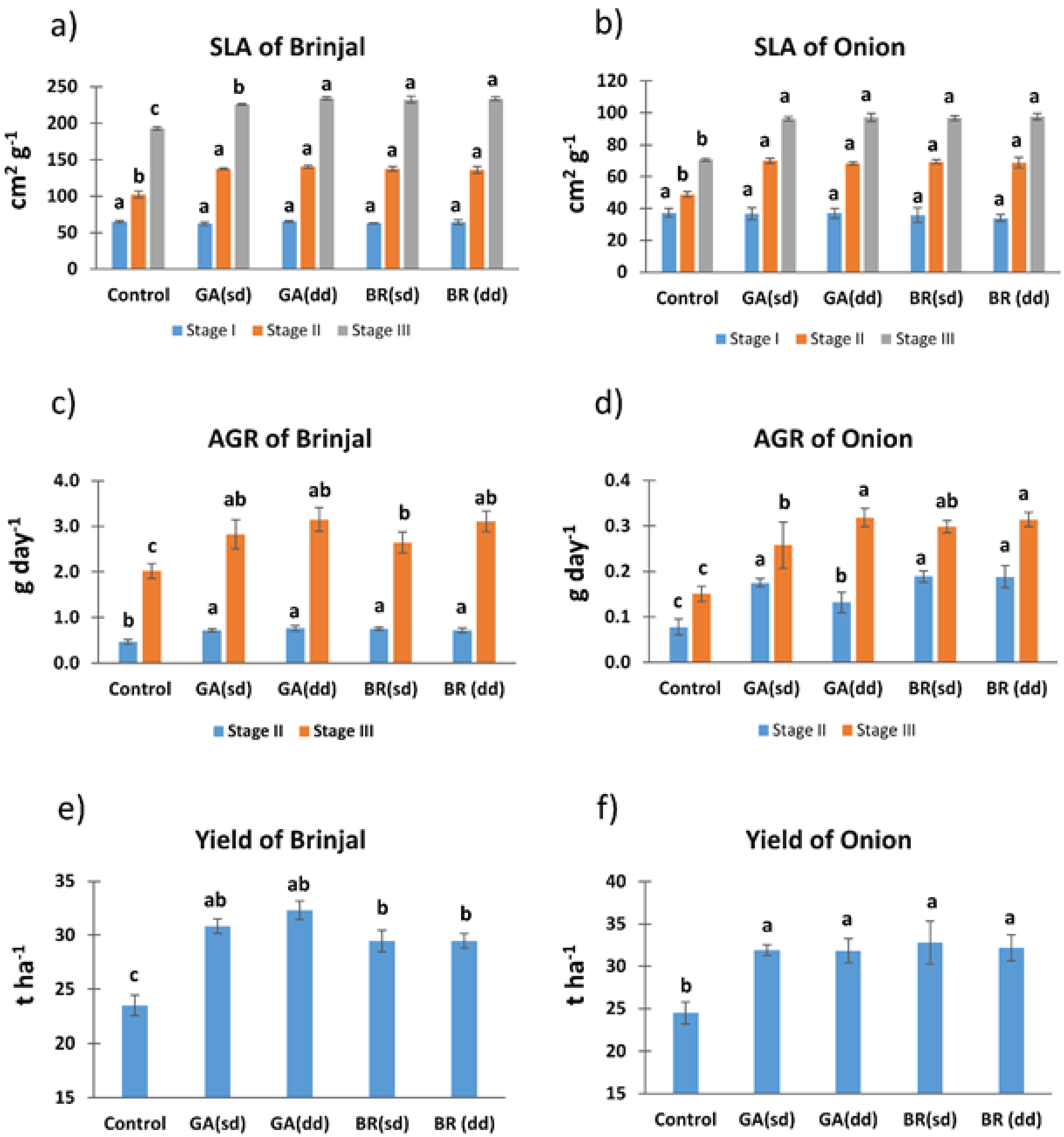


Figure 5

Applications of Gibberellic acid and Brassinolide Emulsifiable Concentrate (EC) formulations and their interactions on the specific leaf area (SLA), absolute growth rate (AGR), and yield of Brinjal and Onion plants; All values are the mean of three independent replications SD; error bars represent SD.