Original Research Article

Effect of plant growth regulators on nutrient and quality changes in *Zingiber officinale* Rosc

ABSTRACT

Ginger is one of the oldest and renowned commercial spices well known for its medicinal and pharmaceutical value. Land degradation, availability of the quality rhizomes and diseases are some of the challenges faced in the ginger production. Since the productivity of a plant is influenced by a complex physiological process controlled by plant growth hormone balancing systems, the application of the growth regulators can have positive effect. Manipulating the PGR combinations and concentrations can lead to better quality and productivity in ginger. Hence, an experiment was conducted to study the effect of different plant growth regulators (PGRs) on nutrient and quality parameters in the ginger variety IISR Varada. Foliar spray of PGRs with 6-benzyl adenine purine (6-BAP), cycocel (Chlormequat chloride), gibberellic acid (GA) and paclobutrazol (PBZ) with five concentrations viz., 50ppm, 100ppm, 150ppm and 200ppm were applied 4th month after planting and water spray treated as control. Nutrients composition and biochemical components were observed on 5th month after planting and oleoresin was observed in the rhizomes after harvest.The results of pooled data over two years revealed that GA at 100 ppm recorded maximum nitrogen and chlorophyll content in leaves , potassium and protein in rhizomes.Regarding the oil content, maximum was noticed in treatment 100ppm Paclobutrazol followed by GA 100ppm.

Keywords: PGRs, ginger, nutrients, oleoresin.

1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) belongs to the Zingiberaceae family is mainly valued as a spice, medicine and as vegetable since the ancient days. Ginger is one of the oldest and renowned commercial spices well known for its flavor, aroma and pungency and ginger is an essential part in flavoring the food, production of alcoholic beverages in foreign countries, in perfume industry, pharmaceutical and also for the industrial use. Ginger rhizome has an important antioxidative, antitumorigenic, antimicrobial and antiviral agent [1,2,3]. Even though, India is the major producer of ginger its production falls behind demand due to the market's high demand for its nutritional and therapeutic properties. To withstand the situation, augmented production with higher quality is necessary. One of the profitable methods getting popularized nowadays is the use of suitable plant growth regulators. Plant growth regulators can internally affect the complex physiological process and thereby it can be seen in the production of ginger. The production and the quality of the ginger can be effected by the different concentrations and combinations of the plant growth regulators [31]. The possibility of the growth regulators on the ginger cultivated had not been explored well and so, the experiment was done.

Berova and Zlatev [5] studied the effects of plant growth retardant Paclobutrazol, on the physiology and yield of tomato. The effect of GA on the photosynthetic performance, growth and yield of mustard and Nigella was reported [5,6]. Benzyl amino purine and gibberellin maintain the production of chlorophyll content and antioxidant enzymes under inundation conditions in soyabean [7]. Application of cycocel, potassium chloride, salicylic acid improved the quality of ginger rhizome [8]. Cycocel 100, 500, 1000 ppm, and Ethrel 50,100,200 ppm were effective when applied three times at 15-day intervals, beginning at 70 DAP in ginger [9]. Augmentation of nutraceuticals, productivity and quality of ginger (*Zingiber officinale* Rosc.) through triacontanol application was reported [10]. Foliar application of CCC at 500 ppm showed the highest yield with good quality ginger rhizomes under Tamil Nadu conditions. Besides the suitable PGRs, since the PGRs perform in a concentration dependent manner, the concentration of PGRs also needed to be standardized. Keeping this view, the research experiment was designed with four different plant growth regulators at four different concentrations and its effect on the nutrient, biochemical and quality of produce.

2. MATERIAL AND METHODS

2.1 Plant Materials and treatments

The experiment was conducted during 2020-2022 at the ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India (Longitude 75.780411° E, latitude 11.258753°N). The experiment was set up in polyhouse conditions with average temperature, light intensity, and the humidity 20-30 ºC, 200 to 800 k lux and 60 -70g/m^3. The variety IISR Varada was used in this experiment which is a high yielding and good quality variety with an average yield of 22.6 tonnes per hectare and also tolerant to diseases. Poly bags size of 40×40×10 cm were filled with potting mixture consisting of soil, sand and farmyard manure in 2:1:1 proportion and planted the ginger rhizomes having the average weight of 25g in these bags. Nutrients were provided as per package of practice recommended by ICAR-IISR, Kozhikode. The experiment was laid out in factorial CRD with three replications. Physicochemical properties of the soil were: texture-sandy loam, pH-4.48, E.C-506.3 µs/cm, available N, P and K 170.8 ppm, 4.11ppm and 245ppm, respectively. The following four PGRs viz., 6 –benzyl adenine purine, cycocel (chlormequat chloride), gibberllic acid and paclobutrazol at four different concentrations 50ppm, 100ppm, 150ppm and 200ppm in PGRs were sprayed at 90 and 120 days after planting. Spraying of water was considered as control. Each treatment was replicated three times. The plants were kept free from weeds and watered when required.

The stock solution of each plant growth regulator was prepared by initially dissolving in a surfactant and then diluting it to appropriate concentrations. Each growth regulator has particular surfactant such as BAP in 1N NaOH (freshly prepared), Cycocel in water, Paclobutrazol in ethanol or methyl alcohol and GA in alcohol. The prepared solution in various concentrations was sprayed to the plant manually using a spray machine in the morning.

2.2 Estimation of chlorophyll content

Chlorophyll ‘a’ and ‘b’ as well as total chlorophyll content from selected leaves (1 g) were extracted with 80% acetone and quantified according to Arnon’s method [11]. Spectrum absorption was measured at 645, 663 and 652 nm and the chlorophyll contents were expressed as mg g-1 of fresh.

The formula for calculation of total chlorophyll content was

$$\text{Total chlorophyll} = \frac{\text{OD652} \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where,

V=final volume of the chlorophyll extract
W= fresh weight of the tissue extracted

2.3 Estimation of nutrient content in leaves and rhizomes

For the nutrient uptake studies, leaves and rhizomes were oven-dried at 60°C and powdered using mixer grinder. The nitrogen (N) uptake was assessed using the Kjeldahl method [12]. For the estimation of the P, one-gram powdered plant sample were digested using a mixture of nitric acid (HNO_3) and hydrochloric acid (HCl 60%) with a ratio of 9:4 (v: v) and assessed using spectrophotometer at 660nm [13]. Potassium (K) was estimated using an atomic absorption spectrophotometer (Varian AA 240FS) [14].

2.4 Estimation of protein content in rhizome

Total soluble protein in leaves and rhizomes were estimated by using the method of Lowry. [15] by using BSA as standard. Fresh leaves (100 mg) were added in test tubes having a 10 ml phosphate buffer. The content was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected and made up to 10 ml. 1 ml of the supernatant was pipette out to a test tube and 5 ml of alkaline copper tartrate reagent and 0.5 ml of folin reagent were added. The color intensity was measured at 660 nm in spectrophotometer and the amount of soluble protein present in the sample was calculated by using bovine serum albumin as standard and expressed as mg g-1 fresh weight.

2.5 Estimation of oleoresin content

Quality parameters such as essential oil, oleoresin content, fiber content and dry recovery percentage were estimated using standard procedures. Rhizome samples were dried and powdered and oleoresin estimation was done using ASTA [16] method. Ten gram of sample was weighed and packed in cotton wool and placed in glass column (18 × 500 mm) with stopcock. To this, 50 ml of acetone was added and kept to stand overnight. The filtrate extracted through the non-absorbent cotton was collected in a pre-weighed 100ml beaker and column was washed with acetone. The extracts in the beaker were evaporated to dryness and weighed to determine the percentage of oleoresin. The amount of oleoresin was estimated gravimetrically.

Yield of oleoresin on dry weight basis was calculated using the formula.

Oleoresin (%) = [Weight of residue (g) / weight of sample (g)] *100

2.6 Estimation of Essential oil content

Essential oil content on fresh weight basis was determined by steam distillation of freshly harvested rhizomes using Clevenger apparatus, Clevenger's [17] method. The prepared sample was accurately weighed and transfers in to a flask. Then about 500ml of water was

added and the flask was assembled in a Clevenger trap. The flask was heated with stirring and the distillation rate was maintained. Distill until two consecutive readings taken at one hour intervals show no change of oil volume in the trap. Cool to the room temperature, allow the stand until the oil layer is clear and read the volume of oil collected.

$$\text{Volume of the oil \%} = \frac{\text{Volume of oil (ml)}}{\text{Weight of the sample (g)}} \times 100$$

2.7 Statistical analysis

The experiments were performed in a factorial completely randomized design with three replications. The SAS 9.3 statistical analysis package's General Linear Models (GLM) tool was used to do an analysis of variance on all data (Cary, North Carolina-based SAS Institute) Duncan's new multi-product line.

3. RESULTS AND DISCUSSION

In the present study, foliar application of growth regulators, it significantly affected the nutrient, biochemical and quality traits of ginger.

3.1 Leaf Chlorophyll content

The foliar spray of growth regulator gibberllic acid significantly increased the chlorophyll content in leaves at 180 DAP than other PGRs (Fig.1). Among the interaction mean, Gibberllic acid (GA) at 100ppm showed 1.40 mean values for total chlorophyll content in the pooled year data followed by Cycocel at 150ppm. The minimum chlorophyll content was recorded in control treatment.GA has reported to increase the leaf chlorophyll content in Gladiolus (El-Naggar [18]. GA significantly increased the total chlorophyll contents in *Mentha piperita*. Gibberellic acid could induce the cell division among the leaves and thereby increase the surface area and increased its total gibberellin content in potato plants [18]. GA increased chlorophyll concentration per leaf while also increasing leaf area, resulting in a drop in chlorophyll per unit area and darker leaves than GA untreated leaves [20]. The treatment of GA in soyabean leads to increase in chlorophyll content and thereby increase the rate of photosynthesis [10]. The increased chlorophyll content, net photosynthetic rate may also be due to the increased potassium content in the leaves since the potassium can affect the respiration, photosynthesis, leaf NPK content, chlorophyll development, water content of leaves, carbon dioxide (CO_2) assimilation and carbon movement [21] . In present study, GA influenced the synthesis of chlorophyll irrespective of all the concentrations.

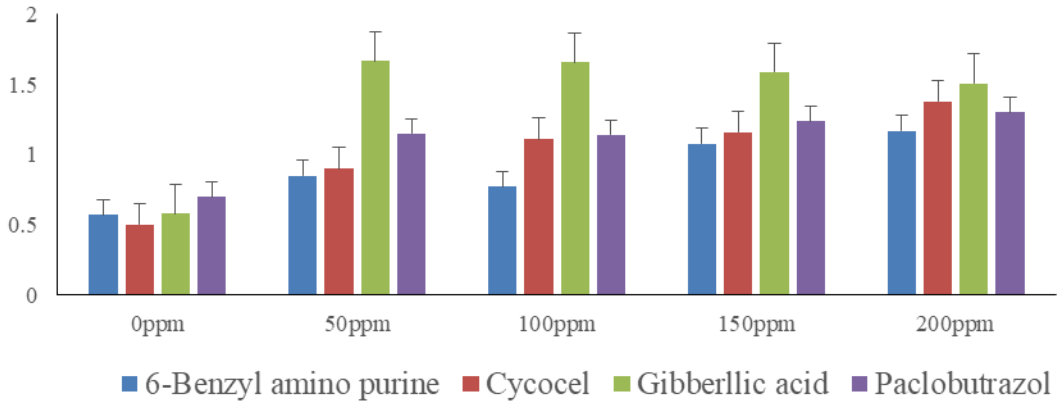

Fig. 1 Effect of PGRs on the chlorophyll content of the leaves

3.2 N, P and K content of leaf and rhizomes

Foliar application of the different PGRs significantly affected the N, P, K content of leaf and rhizome. From the results, higher N was reported in Cycocel at 100ppm (Table 1), and maximum K was reported in GA at 100ppm (Table 2), but the P content in the leaves was found to be insignificant (Data not shown). In rhizomes, higher K content observed in GA treated plants at 100ppm (Table 4), higher N content in GA at 150ppm (Table 3) and higher P in GA treated plants at 50ppm (Table 5). The control plants significantly lower content of N, P and K was observed both in rhizomes and leaves. Nitrogen content in the leaves was found to be increased with the treatment of Cycocel and found that the Cycocel treated plants contained more N, P, Ca and Mg but less K in tomato plants. The maximum K content in leaves due to the application of GA alone or in combination with potassium nitrate in *Solanum lycopersicum* [22]. Foliar application of GA improved the leaf potassium content by 17.65% in Indian Mustard [23]. Leaf nitrogen and phosphorus content was found to increase due to the application of GA_3 and KNO_3 was reported in *Cucumis sativus* plants [24]. The maximum N, P and K content in the rhizomes in GA treated plants influences the protein content in the rhizomes and increasing its nutritive [25]. In other report phosphorus was increased in the sweet pepper fruit due to the application of GA [26]. In present study also showed the positive relation in the application of exogenous growth regulators in the content of major nutrient in the plant leaves and rhizomes.

3.3 Protein content in rhizome

Among the four PGRs applied the GA at 100ppm (69.04) sprayed plant rhizome registered significantly higher protein. The increased protein content in rhizome might be due to the increased Nitrogen content in the rhizomes leading to the production of maximum amino acids biosynthesis and thereby overall increase in the protein content [27,28,29,30]. Enhancing effect of the protein content in the rhizomes may be due to the direct role of Nitrogen in their biosynthesis [32]. Biosynthesis of certain hormones (gibberellins, auxins, and cytokinins) involved in protein synthesis, and the formation of the ribosome structure is influenced by the N content [25]. In present study, all PGRs showed significant influence on rhizome protein content (Fig. 2).

3.4 Oleoresin and essential oil content

The essential and oleoresin content in ginger determines the quality of a ginger. The treatments with the different growth regulators could influence the quality of ginger, since it acts on the biochemical pathways. Polled data showed that the maximum quantity of essential oil was reported in paclobutrazol (2.03) at a concentration of 100ppm (2.14) followed by GA treated plants. The oleoresin content in the ginger found to decrease due to the PGRs application. There was 82% increase from the normal value in the essential oil content. According to Farooqi [32] 200ppm of kinetin resulted in the biomass production and thereby rises in essential oil in Mint (*Mentha arvensis*). The obtained results substantiate that by paclobutrazol (growth retardant) reduce the vegetative growth and thus leads to the major portioning of biomass. As a result of increase in the number of leaves with the application of 100 mg/ L of gibberllic acid (GA) higher essential oil content was reported in sage (*Salvia officinalis*) [34]. Maximum oleoresin content in the GA applied plants may be due to the increase in the number of leaves. Both the quality and quantity of the essential oil and oleoresin are influenced by the application of plant growth regulators. The increased nitrogen content in leaves could enhance oil content and yield in aromatic plants as the amount of biomass yields per unit area, leaf area development and the photosynthetic rate by the high nitrogen content [35].

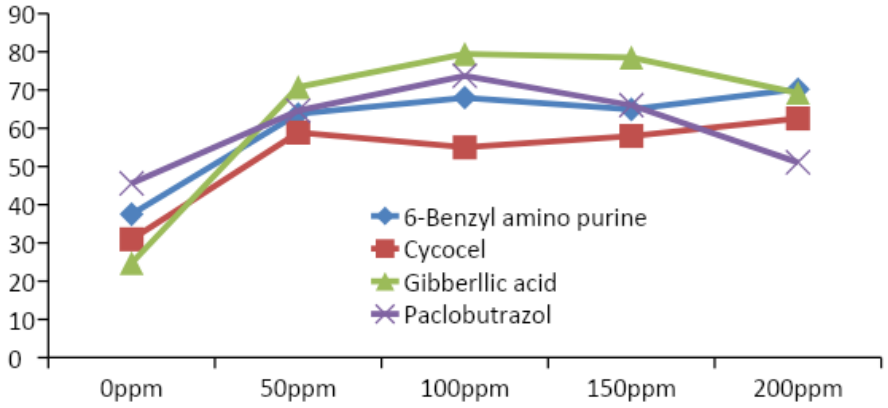

Fig. 2 Effect of PGRs on the protein content of the rhizomes

Table 1. Effect of different concentrations of plant growth regulators on the N content of leaves

Source/ concentration	0	50ppm	100ppm	150ppm	200ppm	Mean
BAP	1.27	2.34	2.41	2.55	2.11	2.13
Cycocel	1.18	2.49	2.97	3.01	2.36	2.40
GA	1.09	2.43	2.57	2.19	2.05	2.06
PCA	1.15	2.36	2.63	2.15	2.51	2.16
Mean	1.17	2.40	2.64	2.47	2.25	
(P = .05)						
Hormone (H)	0.06					
Concentration (C)	0.08					
H*C	0.22					

Table 2. Effect of different concentrations of plant growth regulators on the K content of leaves

Source/ concentration	0	50ppm	100ppm	150ppm	200ppm	Mean of hormones
BAP	1.20	1.90	2.10	1.97	1.70	1.77
Cycocel	1.21	1.62	1.80	1.59	1.68	1.58
GA	1.32	2.22	1.91	1.79	1.90	1.82
PCA	1.41	1.70	1.72	1.99	1.82	1.72
Mean	1.31	1.86	1.88	1.83	1.77	
(*P* = .05)						
Hormone (H)	0.02					
Concentration (C)	0.04					
H*C	0.08					

Table 3. Effect of different concentrations of plant growth regulators on the N content of rhizomes

Source/ concentration	0	50ppm	100ppm	150ppm	200ppm	Mean
BAP	0.21	1.44	1.52	2.55	2.11	1.56
Cycocel	0.14	1.55	2.57	2.19	2.05	1.70
GA	0.32	1.38	2.97	3.01	2.36	2.00
PCA	0.25	1.34	2.63	2.15	2.51	1.77
Mean	0.23	1.42	2.42	2.47	2.25	
(*P* = .05)						
Hormone (H)	0.02					
Concentration (C)	0.13					
H*C	0.23					

Table 4. Effect of different concentrations of plant growth regulators on the K content of rhizomes

Source/ concentration	0	50ppm	100ppm	150ppm	200ppm	Mean
BAP	1.25	2.06	1.85	2.07	1.94	1.83
Cycocel	1.02	1.89	1.72	1.89	2.32	1.76
GA	1.09	2.31	2.51	1.92	1.97	1.96
PCA	1.13	1.98	2.36	1.87	1.94	1.85
Mean	1.12	2.06	2.11	1.93	2.04	
(*P* = .05)						
Hormone (H)	0.03					

Concentration (C)	0.02
H*C	0.05

Table 5. Effect of different concentrations of plant growth regulators on the P content of rhizomes

Source/ concentration	0	50ppm	100ppm	150ppm	200ppm	Mean
BAP	0.14	0.32	0.30	0.31	0.28	0.27
Cycocel	0.08	0.30	0.29	0.29	0.29	0.25
GA	0.07	1.13	0.96	1.12	0.26	0.70
PCA	0.11	0.31	0.32	0.32	0.32	0.27
Mean	0.1	0.51	0.46	0.51	0.28	
(*P* = .05)						
Hormone (H)	0.01					
Concentration (C)	0.01					
H*C	0.02					

4. Conclusion

It is obvious from the experiment that the application of 6-benzyl adenine purine (6-BAP), cycocel (Chlormequat chloride), gibberellic acid (GA) and paclobutrazol (PBZ), at four concentrations 50ppm, 100ppm,150ppm and 200ppm leads to various changes in the nutrient, biochemical content in leaves as well as in quality of rhizomes . Among the growth regulators, application of GA at 100ppm found to beneficial since it enhanced nitrogen and chlorophyll content in leaves , potassium content and protein content in rhizomes. Application of paclobutrazol augmented the oil content in ginger.

References

1. Akoachere JF, Ndip RN, Chenwi EB, Ndip LM, Njock TE, Anong DN. Antibacterial effect of Zingiber officinale and Garcinia kola on respiratory tract pathogens. East Afr Med J. 2002;79(11):588–592.
2. Ippoushi K, Azuma K, Ito H, Horie H, Higashio H. [6]-Gingerol inhibits nitric oxide synthesis in activated J774. 1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. Life sciences. 2003;73(26):3427–3437.
3. Borrelli F, Capasso R, Pinto A, Izzo AA. Inhibitory effect of ginger (*Zingiber officinale*) on rat ileal motility in vitro. Life sciences. 2004:74(23):2889–2896.
4. Berova M, Zlatev Z. Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). Plant Growth Regulation. 2000;30:117–123.
5. Akter A, Ali E, Islam MMZ, Karim R, Razzaque AHM. Effect of GA_3 on growth and yield of mustard. Int J Sustain Crop Prod. 2007;2:16–20.

6. Shah AS, Khan GM, Badshah A, Shah SU, Shah KU, Mirza SA, Khan, KA. Nigella sativa provides protection against metabolic syndrome. African Journal of Biotechnology. 2012;11(48):10919–10925.
7. Damanik RI, Manurung D, Bayu ES, Rahmawati N. Response of some soybean (*Glycine max* L. Merrill) varieties on flooded condition with application of Benzyl Amino Purine (BAP) and Salicylic Acid (SA) in the R3 phase. In IOP Conference Series: Earth and Environmental Science. 2020. 454(1): 012162).
8. Sengupta DK, Maity TK, Dasgupta B. Effect of growth regulators on growth and rhizome production of ginger (*Zingiber officinale* Rosc.) in the hilly region of Darjeeling district. Journal of Crop and Weed. 2008;4(2):10–13.
9. Jayachandran BK, Sethumadhavan P. Vegetative growth of ginger (*Zingiber officinale* R) as influenced by Cycocel, Ethrel and Kinetin. 1979.
10. Singh M, Khan MMA, Moinuddin, Naeem M. Augmentation of nutraceuticals, productivity and quality of ginger (*Zingiber officinale* Rosc.) through triacontanol application. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology. 2012;146(1):106–113.
11. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology. 1949; 24(1): p.1.
12. Nelson DW, Sommers LE. Determination of total nitrogen in plant material 1. Agronomy journal. 1973; 65(1):109–112.
13. Jackson WA, Flesher D, Hageman RH. Nitrate uptake by dark-grown corn seedlings: some characteristics of apparent induction. Plant Physiology. 1973;51(1):120–127.
14. Thomas RL, Sheard RW, Moyer JR. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion 1. Agronomy Journal. 1967; 59(3):240–243.
15. Lowry OH, Rose brough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J. Biol. Chem. 1951; 193:265–275.
16. ASTA. Official analytical methods of the American Spice Trade Association. 2nd ed. New Jersey: American Spice Trade Association, Inc. 1978; 38–41.
17. Clevenger JH. Apparatus for the determination of volatile oil. J Am Pharm Assoc. 1928;17:346.
18. El-Naggar AH. Effect of potassium and gibberellic acid on vegetative growth, flowering, corms and cormels production of gladiolus plants in the sandy deserts soil. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, Egypt. 1999.
19. Kavina J, Gopi R, Panneerselvam R. Plant growth regulators and fungicides alters growth and biochemical contents in *Mentha piperita* Linn. International Journal of Environmental Sciences. 2011;1(7): 2096–2108.
20. Wheeler AW, Humphries EC. Effect of gibberellic acid on growth, gibberellin content, and chlorophyll content of leaves of potato (*Solanum tuberosum*). Journal of Experimental Botany. 1963;14(1):132–136.
21. Sangakkara R, Amarasekera P, Stamp P. Growth, yields, and nitrogen-use efficiency of maize (Zea mays L.) and mungbean (*Vigna radiata* L. Wilczek) as affected by potassium fertilizer in tropical South Asia. Communications in soil science and plant analysis. 2011;42(7):832–843.

22. Kazemi M. Effect of gibberellic acid and potassium nitrate spray on vegetative growth and reproductive characteristics of tomato. *Journal of Biological and Environmental Sciences. 2014; 8*(22):1–9.
23. Islam S, Mohammad F. Plant growth regulators modulate photosynthetic efficiency, antioxidant system, root cell viability and nutrient acquisition to promote growth, yield and quality of Indian mustard. Acta Physiol Plant. 2022;44:132.
24. Pal P, Yadav K, Kumar K, Singhm N. Effect of gibberellic acid and potassium foliar sprays on productivity and physiological and biochemical parameters of parthenocarpic cucumber (*Cucumis sativus*) cv. 'seven star F1. J. Horticultural Res. 2016;24(1):93–100.
25. Jones Jr, JB, Wolf B, Mills HA. Plant analysis handbook. A practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Publishing, Inc. 1991.
26. Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA. Plant hormones and plant growth regulators in plant tissue culture. In Vitro Cell Dev. Biol. Plant. 1996;32:272–289.
27. Knowles NR, Ries SK. Rapid growth and apparent total nitrogen increases in rice and corn plants following applications of triacontanol. Plant Physiol. 1981;68:127.
28. Ries SK. Regulation of plant growth with triacontanol CRC. Crit Rev Plant Sci. 1985;2:239–285.
29. Muthuchelian K, Velayutham M, Nedunchezhian N. Ameliorating effect of triacontanol on acidic mist-treated Erythrina variegata seedlings changes in growth and photosynthetic activities. Plant Sci. 2003;165:1253–12.
30. Naeem M, Khan MMA, Moinuddin, Siddiqui MH. Triacontanol stimulates nitrogen-fixation, enzyme activities, photosynthesis, crop productivity and quality of hyacinth bean (*Lablab purpureus* L.). Sci Hort. 2009;121: 389–396.
31. Rusmin, D., Suhartanto, M.R., Ilyas, S., Manohara, D. and Widajati, E., 2015. Production and quality improvement of ginger seed rhizome by paclobutrazol applications. *International Journal of Sciences: Basic and Applied Research*, *21*, pp.132-146.
32. Taiz L, Zeiger E, Møller IM, Murphy A. Plant physiology and development (No. Ed. 6). Sinauer Associates Incorporated. 2015.
33. Farooqi AHA, Khan A, Sharma S. Effect of kinetin and chlormequat chloride on growth, leaf abscission and essential oil yield in *Mentha arvensis*. Indian Perfumer. 2003;47(4): 359–363.
34. Povh JA, Ono EO. Rendimento do óleo essencial de *Salvia officinalis* L. sob ação de reguladores vegetais. Acta Sci Biol Sci. 2006;28(3):189–193.
35. Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. Regulation of essential oil production in plants. Plant Growth Regul. 2001;34:3–21.