

Homobrassinolide and chemical composition of curcuma longa L. (turmeric) rhizome

G, Rajesh and B.V. Vardhini*

Department of Botany, Telangana University, Dichpally, Nizamabad -503322, India

(Received February 16, 2021, Revised January 12, 2023, Accepted February 10, 2023)

Abstract. The effect of homobrassinolide (HBL) on the nutrient value of *Curcuma longa* L. (turmeric) rhizome grown in Nizamabad, Telangana State, India was studied. Application of homobrassinolide (HBL) as foliar spray to turmeric plants on the 20th, 40th and 60th day from sowing resulted in enhanced chemical composition of turmeric rhizome. Application of homobrassinolide (HBL) resulted in enhanced total sugars, principal ingredient curcumin, total poly phenol content, total flavonoid content, total tannin content, crude fat, crude fibre and essential oils (turmerone, zingiberene, cineole and p-cymene) present in the turmeric rhizome indicating the ability of homobrassinolide (HBL) as a potential plant growth regulator (PGR).

Keywords: cineole; crude fat; crude fiber; curcumin; P-cymene; total flavonoids; total poly phenols; total sugars; total tannins; turmerone; zingiberene

1. Introduction

Plant Growth Regulators (PGRs) are compound errand messengers that are capable of controlling the development and improvement of plants, and subsequently are likewise often represented as plant development controllers. By intrinsic limit, plants alter the dimension of PGRs specifically or by implication to adapt up with unpleasant conditions to affect growth and development (Finkelstein *et al.* 2002). Among the recently included PGRs, BRs have been the subject of sharp enthusiasm for plant scientists for their role in growth and metabolism of plants under normal and stressful environment.

Brassinosteroids (BRs) are fundamental low molecular weight PGRs (plant growth regulators) that are omnipresent through the plant kingdom (Rao *et al.* 2002, Vardhini 2020). Mitchell *et al.* (1970) first reported their presence in plants in 'Nature' depicting the growth promoting activity of *Brassica napus* L. pollen extricates at very low amounts. The research studies (Bajguz and Hayat 2009, Vardhini *et al.* 2010) on BRs clearly depict their ability to mitigate various biotic (bacteria, virus, fungi, nematodes) and abiotic (temperature, salt, drought, metal, water) in plants. The research work on BRs clearly demonstrates the positive role of BRs as potential PGRs that are non- xenobiotic to human race with the capability of reducing the risk of pesticides to mankind as well as the other biotic beings living on the surface of the earth.

Turmeric (*Curcuma longa* L.) belongs to the ginger family known as Zingiberaceae. Turmeric

*Corresponding author, Professor, E-mail: drvidyavardhini@rediffmail.com

is sterile as it is not capable of producing seeds and it is widely propagated from the rhizomes.

There is a notion that it might have been vegetatively propagated through the process of natural selection during the process of evolution from wild turmeric (*Curcuma aromatica*) which was grown in India, Sri Lanka and the Eastern Himalayas. The turmeric is often called as a wonder spice capable of acting as an antioxidant and used almost every day in India. The present research study is undertaken to give an insight on the application of homobrassinolide (HBL) on the contents of total sugars, the principal ingredient curcumin, total poly phenol content, total flavonoid content, total tannin content, crude fat, crude fibre and essential oils (turmerone, zingiberene, cineole and p-cymene) of turmeric rhizome.

2. Materials and methods

The potentiality of homobrassinolide (HBL), a potential PGR in improving the nutrient value of *Curcuma longa* L. plant popularly called as 'turmeric' which belongs to the ginger family (Zingiberaceae) is the current study theme. The underground rhizomes of *Curcuma longa* L. variety Acc-79 were purchased from Ashwini Fertilizers Ltd., Nizamabad. Homobrassinolide (HBL) was procured from Godrej Agrovet Pvt. Ltd., Hyderabad, Telangana State, India. Homobrassinolide obtained as Double comprises of 0.04% of Homobrassinolide, 4.0% of water and solvent.

Curcuma longa L. plants were grown under natural day length in Nizamabad district of Telangana State, India. The experiments were conducted in the field plots beds. Each plot bed consisted of 121 square yards in measurement. A total of 5 plot beds were prepared resulting in 605 (121 x 5) square yards of total field sizes. The distance between each bed was around two and a half feet. Care was taken to maintain 1/2 feet distance between each plant. The selected plots were mixed with manure and vermi compost. The rhizomes were sterilized by metaloxin mangozeb (250 g/250 ml concentration) by soaking for 30 minutes. After 30 minutes, the turmeric rhizomes were dried in shade for around one and a half hour and planted in the plots mentioned above. After 15 days of sowing, the saplings were exogenously treated with 70% thiophanate methyl which is a fungicide.

The turmeric plants were grown in the field conditions in the semi-arid tropics of Nizamabad. Homobrassinolide (HBL) was supplied as foliar spray where four different concentrations viz., 0.5 μM , 1.0 μM , 2.0 μM , and 3.0 μM were taken based on the experimental studies conducted by Vardhini and Rao (1998, 2003). Ten plants were chosen for foliar application of each concentration. Homobrassinolide (HBL) was first applied as foliar spray to twenty - day old turmeric plants. Homobrassinolide (HBL) was exogenously applied as foliar spray three times to the turmeric plants viz., on the 20th, 40th and 60th day from sowing. The control turmeric plants were treated with distilled water on the same days. The rhizome is the main consumable part of the turmeric plants and the effect of homobrassinolide (HBL) on the contents of total sugars, the principal ingredient curcumin, total poly phenol content, total flavonoid content, total tannin content, crude fat, crude fiber and essential oils (turmerone, zingiberene, cineole and p-cymene) of turmeric rhizome were analyzed in the present research study.

2.1 Homobrassinolide (HBL) and total sugars in turmeric rhizome

Two grams of turmeric rhizome was homogenized in 70% V/V ethyl alcohol. 2.5 ml of the ethyl alcohol homogenate was taken into a centrifuge tube. The centrifuge tube was kept in a boiling water bath for five minutes. The contents were cooled to room temperature. The contents were centrifuged at 4,000 rpm in a Remi- T-8 centrifuge. The residue was re-extracted with 5 ml of 70% (V/V) ethyl alcohol and was re-centrifuged for 10 minutes. This procedure was repeated three times.

The ethyl alcohol supernatants were poured into a 25ml test tube and the volume was made up to 10 ml with 70% (V/V) ethyl alcohol which was used for the determination of the total sugars present in turmeric rhizome. The total sugars in the turmeric rhizome were determined employing the procedure given by Yoshida *et al.* (1976). 5 ml of Ethyl alcohol extract was evaporated to dryness in a beaker on a water bath at 60°C temperature. The intensity of brown color developed was recorded at 630 nm in a UV Visible Spectrometer of Shimadzu UV- 1800, Japan make using a blank in terms of OD (Optical Density). A blank was prepared by using 5 ml of anthrone reagent and 1ml of 40% ethyl alcohol. The total sugars in the turmeric rhizome were estimated as D-glucose equivalents. The amount of glucose was found out from a glucose standard curve. The amount of total sugars in the turmeric rhizome was expressed as mg g⁻¹ Fr. wt.

2.2 Homobrassinolide (HBL) and curcumin in turmeric rhizome

The turmeric rhizome was collected and dried for 2 days in sunlight. The rhizome was crushed to fine powder and then passed from sieve No. 60. The collected powder was subjected to maceration with ethyl alcohol in a flask consisting of iodine for 7 days. The extract was filtered and decolorized with charcoal to get a clear liquid. The extract was then evaporated to get the semisolid mass and vacuum dried to obtain a very fine powder. The optical density (OD) was measured at 421 nm in a UV Visible Spectrometer of Shimadzu UV- 1800, Japan make.

2.3 Homobrassinolide (HBL) and total polyphenol content in turmeric rhizome

The total polyphenol content of the turmeric rhizome was estimated following Singleton *et al.* (1999) Method. 0.4 mL of the extract was mixed with 1.6 mL of 7.5% sodium carbonate solution. 2 mL of 10-fold diluted Folin-Ciocalteu reagent was added to the above solution. The final reaction mixture was incubated for 1 h in the dark. The intensity of the blue-colored complex was measured at 765 nm employing a UV Visible Spectrometer of Shimadzu UV- 1800, Japan make.

The total polyphenol content present in the turmeric rhizome was determined as gallic acid equivalents (GAEs) and was expressed as g of GAE/100 g of turmeric rhizome fresh weight.

2.4 Homobrassinolide (HBL) and total flavonoid content in turmeric rhizome

The total flavonoid content in the turmeric rhizome was determined using aluminum chloride colorimetric assay (Chang *et al.* 2002). 1 mL of the extract was mixed with 0.3 mL of 5% sodium nitrite. After 5 min, 0.3 mL of 10% aluminum chloride was also added. After 6 minutes, another 2 mL of 1 M sodium hydroxide was added followed by immediate addition of 2.4 mL of distilled water to make up the total volume of the reaction mixture to 10 mL. The color intensity of the flavonoid-aluminum complex was measured at 510 nm in Labtronics Digital Photo Calorimeter.

The total flavonoid content in the turmeric rhizome was determined as catechin equivalent (CE) and was expressed as g of CE/100 g of turmeric rhizome fresh weight.

2.5 Homobrassinolide (HBL) and the total tannin content in turmeric rhizome

The total tannin content in the turmeric rhizome was estimated using the Folin-Ciocalteu method adopted by Afroz *et al.* (2014) employing tannic acid as a standard. 0.1 mL of the solution containing 1 mg of the extract was mixed with 7.5 mL of distilled water, and 0.5 mL of Folin-Ciocalteu reagent was added. Further, 1 mL of 35% sodium carbonate and 0.9 mL of distilled water were added. The solution was mixed and then incubated for 30 min. The intensity of the developed blue-colored complex was measured at 725 nm in a UV Visible Spectrometer of Shimadzu UV- 1800, Japan make. The results were expressed as g of tannic acid equivalent per 100 g of turmeric rhizome fresh weight.

2.6 Homobrassinolide (HBL) and crude fat in turmeric rhizome

The crude fat content in the rhizome of turmeric plants was determined using the Soxhlet Extraction Method using triplicate samples as described in AOAC Method (AOAC, 2005). 2 grams of pre-dried turmeric rhizome was weighed in a thimble and placed in the Soxhlet apparatus. A pre-weighed round bottom flask was taken and 100 ml of hexane was added. The assembly was set-up and extracted for 16 h. The thimble was removed after extraction and the solvent was distilled off. Latter, the flask was dried in a hot air oven to remove solvent residue. It was cooled in a desiccator, and the mass of the extracted crude fat present in the turmeric rhizome was determined and expressed in percentage.

2.7 Homobrassinolide (HBL) on crude fiber in turmeric rhizome

The amount of crude fiber in turmeric rhizome was determined using triplicate samples by AOAC Method (AOAC, 2005). Crude fiber determination was based on the loss on ignition of dried residue remaining after digestion of sample with 1.25% sulphuric acid and 1.25% sodium hydroxide solutions under specific conditions. About 2 g of the defatted turmeric rhizome was taken in conical flask. The rhizome was digested by boiling in 200 ml of sulphuric acid (1.25%) for 30 min. After digestion, the rhizome was filtered through linen placed in fluted funnel and residue was washed thoroughly with boiling water to remove acid. Later, the residue was transferred to a conical flask and digested with 200 ml of boiling sodium hydroxide (1.25%) in the same manner. The rhizome was filtered through a pre-weighed Gooch crucible and washed thoroughly to remove the alkali completely. The residue was finally washed with alcohol.

The Gooch crucible was dried in a hot air oven for 2 h, cooled in a desiccator and then weighed. Later, the residue was ignited in muffle furnace maintained at 550°C for 1 h. The loss in weight after igniting was determined and reported as crude fiber which was expressed in percentage.

2.8 Homobrassinolide (HBL) and essential oils in the turmeric rhizome

The essential oils (turmerone, zingiberene, cineole and p-cymene) from turmeric rhizome were extracted by hydro-distillation in a Clevenger's apparatus following the method of Guenther (1972). Fresh rhizomes of turmeric were thoroughly washed to remove soil particles. They were peeled and sliced. Sliced rhizomes (100 g) were mixed with distilled water and heated in a flask 6–10 h and the condensed vapour was separated throughout an auto-oil/water separator. The oil

present at the upper most layers were collected in the eppendorf tube. The oil yield percentage in rhizomes was calculated by following method and expressed in terms of g/fr.wt.

Rhizome Oil % Yield (v/fr.wt) = Volume of essential oil (ml) /wt. of raw rhizomes x 100.

3. Results

Exogenous application of homobrassinolide (HBL) to turmeric plants improved the total sugars in the turmeric rhizome (Table 1.) gradually as the concentrations increased from 0.5 μ M HBL to 3.0 μ M HBL. The levels of total sugars in the turmeric rhizome treated with 0.5 μ M HBL increased around 36.53%, 1.0 μ M HBL increased around 80.08%, 2.0 μ M HBL increased around 100.25% and 3.0 μ M HBL increased around 100.69% when compared with untreated controls.

Application of HBL resulted in increased curcumin content in turmeric rhizome (Table 1). HBL (0.5 μ M, 1.0 μ M, and 2.0 μ M and 3.0 μ M) stimulated the curcumin content in turmeric rhizome to a great extent. The observation was showed that the curcumin content was increased by 36% for 0.5 μ M HBL application, 47.92% for 1.0 μ M HBL application, 65.56% for 2.0 μ M HBL application and 80.26% for 3.0 μ M HBL application over with controls.

Exogenous application of HBL to turmeric plants improved the total polyphenol content in the turmeric rhizome (Table 1). Treatment of HBL at 0.5 μ M, 1.0 μ M, and 2.0 μ M and 3.0 μ M concentrations resulted in enhanced total polyphenol content around 54.66% for 0.5 μ M HBL treatment, 55.56% for 1.0 μ M HBL treatment, 57.11% for 2.0 μ M HBL treatment and 58.95% for 3.0 μ M HBL treatment compared with control plants treated with water.

Exogenous application of HBL to turmeric plants improved the total flavonoid content in the turmeric rhizome (Table 2). Application of HBL resulted in increased total flavonoid content in all four treated concentrations viz., 0.5 μ M, 1.0 μ M, 2.0 μ M and 3.0 μ M. The total flavonoid content improved around 28.81% for 0.5 μ M HBL treatment, 21.16% for 1.0 μ M HBL treatment, 52.46% for 2.0 μ M HBL treatment and 68.37% for 3.0 μ M HBL treatment when compared with controls.

Exogenous application of HBL to turmeric plants improved the total tannin content in the turmeric rhizome (Table 2) and it was observed that as the concentrations increased from 0.5 μ M HBL to 3.0 μ M HBL, the total tannin contents were also found increased. The observations showed that the total for tannin content rose to 98.31% in 0.5 μ M HBL application, 117.45% in 1.0 μ M HBL application, 140.94% in 2.0 μ M HBL application and 172.26% in 3.0 μ M HBL application over untreated - controls.

Exogenous application of HBL to turmeric plants improved the crude fat content in the turmeric rhizome (Table 2) and it was observed that supplementation of HBL (0.5 μ M, 1.0 μ M, and 2.0 μ M and 3.0 μ M) to turmeric plants resulted in increased crude fat content. It was observed that application of 0.5 μ M HBL increased crude fat content around 47.87%, 1.0 μ M HBL increased around 48.42%, 2.0 μ M HBL increased around 49.99% and 3.0 μ M HBL increased around 51.90% when compared to control plants.

Exogenous application of HBL to turmeric plants improved the crude fiber in the turmeric rhizome (Table 2). Application of 3.0 μ M H B L concentration caused an increase of n e a r l y 59.94% crude fiber content in the rhizome of turmeric plants. The remaining treatments of HBL (0.5 μ M, 1.0 μ M and 2.0 μ M) also markedly enhanced the crude fiber content over controls by 22.35% (0.5 μ M HBL), 25.9% (1.0 μ M HBL) and 30.05% (2.0 μ M HBL).

Table 1 Effect of homobrassinolide on total sugars, curcumin and total phenols in turmeric rhizome

Treatments	Total Sugars (mg.g ⁻¹ fr.wt)*	Curcumin Content (%)*	Total Polyphenol Content (g of GAE/100 g)*
Control	300.60±7.93	1.47 ± 0.00	7.36 ± 1.2
0.5 µM HBL	337.13 ±7.32	2.48 ± 0.005	7.42 ± 0.9
1.0 µM HBL	381.40 ±6.11	3.26 ± 0.005	7.55 ± 1.1
2.0 µM HBL	426.08 ±6.47	4.46 ± 0.005	7.76 ± 0.12
3.0 µM HBL	470.21±6.40	5.46 ± 0.005	8.01± 1.1

HBL = Homobrassinolide

GAE = Gallic acid

Equivalent

*Mean ± SE (n = 5); The Mean if followed by the same alphabet in a column was found not significantly different at p=0.05 according to Post Hoc test

Table 2 Effect of homobrassinolide on total flavonoids, total tannins, crude fat and crude fibre in turmeric rhizome

Treatments	Total Flavonoid Content (g of CE/100 g)*	Total Tannin Content (g of tannic acid)*	Crude Fat Content (%)*	Crude Fiber (%)*
Control	0.43 ± 0.01	0.87 ± 0.09	6.82 ± 0.011	4.25 ± 0.002
0.5 µM HBL	0.67 ± 0.34	1.13 ± 0.12	7.02 ± 0.004	5.26 ± 0.012
1.0 µM HBL	0.91 ± 0.19	1.35 ± 0.05	7.10 ± 0.006	6.11 ± 0.008
2.0 µM HBL	1.22 ± 0.05	1.62 ± 0.03	7.33 ± 0.015	7.06± 0.006
3.0 µM HBL	1.59 ± 0.07	1.98 ± 0.21	7.61 ± 0.005	7.98± 0.002

HBL = Homobrassinolide

CE = Catechin

Equivalent

*Mean ± SE (n = 5); The Mean if followed by the same alphabet in a column was found not significantly different at p=0.05 according to Post Hoc test

Exogenous spraying of HBL resulted in increments in all four essential oils present in turmeric rhizome viz., turmerone, zingiberene, cineole and p-cymene. Exogenous application of HBL to turmeric plants substantially improved turmerone content in the turmeric rhizome (Table 3). Among all the concentrations attempted under field conditions, 3.0 µM concentration of HBL was found most efficient in causing maximum increments in turmerone content in turmeric rhizome wherein 47.58% of increase was observed of the same. Similarly 0.5 µM HBL, 1.0 µM HBL and 2.0 µM HBL concentrations also accounted for good increments amounting to 17.55%, 23.4% and 31.59% respectively.

Exogenous application of HBL to turmeric plants improved zingiberene content in the turmeric rhizome as the concentrations increased from 0.5 µM HBL to 3.0 µM HBL (Table 3). 3.0 µM HBL concentration was found most efficient in causing maximum increments in zingiberene content in turmeric rhizome wherein it accounted for 40.92 % increase followed by 0.5 µM HBL, 1.0 µM HBL and 2.0 µM HBL accounting around 16.17%, 20.46 % and 28.05% respectively.

Table 3 Effect of homobrassinolide on the essential oils in turmeric rhizome

Treatments	Turmerone Content (%)	Zingiberene Content (%)*	Cineole Content (%)*	p-Cymene Content (%)*
Control	0.39 ± 0.01	0.33 ± 0.02	0.81 ± 0.01	0.54 ± 0.01
0.5 µM HBL	0.45 ± 0.05	0.49 ± 0.06	0.25 ± 0.04	0.65 ± 0.04
1.0 µM HBL	0.60 ± 0.11	0.62 ± 0.10	0.47 ± 0.02	0.79 ± 0.02
2.0 µM HBL	0.81 ± 0.02	0.85 ± 0.04	0.69 ± 0.01	0.90 ± 0.01
3.0 µM HBL	1.22 ± 0.01	1.24 ± 0.03	0.97 ± 0.01	1.11 ± 0.01

HBL = Homobrassinolide

*Mean ± SE ($n = 5$); The Mean if followed by the same alphabet in a column was found not significantly different at $p=0.05$ according to Post Hoc test

Exogenous application of HBL to turmeric plants improved the p-cymene content in the turmeric rhizome (Table 3). Application of 3.0 µM HBL concentration caused an increase of 59.94% in p-cymene content. The remaining treatments of HBL (0.5 µM, 1.0 µM and 2.0 µM) also markedly enhanced the p-cymene content over controls by 35.1% (0.5 µM HBL), 42.66% (1.0 µM HBL) and 48.6% (2.0 µM HBL).

4. Discussion

4.1 Homobrassinolide (HBL) and total sugars in turmeric rhizome

Exogenous application of HBL to turmeric plants improved the total sugars in the turmeric rhizome (Table 2). Schilling *et al.* (1991) reported that application of HBL increased the sucrose content in sugar beets grown under drought stress. Mousavi *et al.* (2009) reported that 24-EBL (24-epibrassinolide) alleviated the negative effects of water stress in Colza (*Brassica napus* L.) and increased the tolerance of plants to combat water stress by increasing the contents of osmolytes (proline, sugars and ions). Further, foliar application of 28-homobrassinolide and 24-epibrassinolide (Vardhini *et al.* 2012) and brassinolide (Vardhini *et al.* 2014) resulted in enhanced carbohydrate fractions (reducing and starch) in the storage roots of radish exhibiting the capability of BRs (brassinosteroids) in enhancing the carbohydrates in plants.

4.2 Homobrassinolide (HBL) and curcumin in turmeric rhizome

Application of HBL resulted in increased curcumin content in turmeric rhizome and all the different concentrations of HBL like 0.5 µM, 1.0 µM, 2.0 µM and 3.0 µM were capable of the same. Earlier researchers reported that PGRs are capable of increasing the curcumin content in turmeric. Supplementation of 10 ppm and 20 ppm of kinetin or 50 ppm or 100ppm of NAA showed increased contents of curcumin compared to untreated controls (Lynrah *et al.* 2002). Application of two plant growth regulators like 6-benzylaminopurine (6-BA) and α -naphthalene acetic acid (NAA) increased the accumulation of curcumin and other curcuminoids in *Curcuma aromatic* (Wu *et al.* 2015) which is in tune with the present research where application of HBL substantially increased the main secondary metabolite, curcumin in the turmeric rhizome.

4.3 Homobrassinolide (HBL) and total polyphenol content in turmeric rhizome

Foliar application of HBL (0.5 μ M, 1.0 μ M, 2.0 μ M and 3.0 μ M) resulted in raised total polyphenol content in turmeric rhizome. Plant growth regulators (PGRs) such as gibberellic acid (GA3), indole-3-acetic acid (IAA) and kinetin were found to effectively increase the total phenol content and antioxidant system in lentils (Giannakoula *et al.* 2015). Methyl jasmonate, another PGR was also capable of increasing the total soluble phenols in *Stevia rebaudiana* Bertoni plants growing in a hydroponic system over other PGRs like spermidine, salicylic acid, and paclobutrazol (Lucho *et al.* 2018) which is similar to the present study where homobrassinolide, a potential plant growth regulator increased the total phenol contents in turmeric rhizome.

4.4 Homobrassinolide (HBL) and total flavonoid and tannin content in turmeric rhizome

Foliar treatment of all four concentrations of HBL enhanced the total flavonoid content in turmeric rhizome like other PGRs. Methyl jasmonate increased total flavonoids in *Stevia rebaudiana* Bertoni plants growing in a hydroponic system over other PGRs like spermidine, salicylic acid, and paclobutrazol (Lucho *et al.* 2018). The total flavonoid content and antioxidant activities were substantially increased in methyl jasmonate and salicylic acid treated sweet potato (*Ipomoea batatas* L.) plants (Ghasemzadeh *et al.* 2016) just like HBL treatment increased total flavonoids in turmeric rhizome. Białyzyk *et al.* (1998) studied that exogenous application of 10^{-4} mol/L kinetin increased the total tannin content in tomato seedlings over 56% to 70% with respect to the untreated control though other PGRs like indole -3- acetic acid and gibberellins played no significant role. Seeds of *Balanites aegyptiaca* soaked in gibberellic acid solutions (0, 50, 100, 150, ppm) and indole 3-acetic acid solutions (1000, 2000, 3000 ppm) for 14 hours showed enhanced germination percentage, alkaloids, tannins and saponins contents (Mostafa and Alhamd 2011) clearly depicting that PGRs have the potential ability to increase the total tannin of plants.

4.5 Homobrassinolide (HBL) and crude fat and crude fiber in the turmeric rhizome

The present research study revealed that HBL treatment substantially increased the crude fat and crude fiber in the turmeric rhizome with respect to controls. Du *et al.* (2017) reported that application of different PGRs like salicylic acid, 1-naphthaleneacetic acid, gibberellic acid, 6-benzylaminopurine, 24 - epi-brassinolide positively increased the lipids and polyunsaturated fatty acids in *Chlorella pyrenoidosa* ZF strain. Gaveliené *et al.* (2013) observed that treatment of auxin physiological analogues 1-[2- chloroethoxycarbonylmethyl] - 4 naphthalenesulfonic acid, and 1 - [2- di methyl amino ethoxy carbonyl methyl] naphthalene chlormethylate substantially increased the crude fat yield in cvs. 'Hornet', 'Sunday' and 'Libea' varieties of rapeseed (*Brassica napus*).

Exogenous application of PGRs like gibberellic acid and naphthalene acetic acid to *Abelmoschus esculentus* (Linn.) Moench. (okra, bhindi or ladies' fingers) showed enhanced and improved of fibre quality of the vegetable (Fathima and Balasubramanian 2006). Soybean seeds treated with biologically active substances like lignohumate B (a mixture of humic acids and fulvic acids); lexin (a mixture of humic acids and fulvic acids enriched with auxins) and a synthetic analogue of natural epibrassinolide exhibited significant increases in crude fibre (Procházka *et al.* 2017).

4.6 Homobrassinolide (HBL) and essential oils in the turmeric rhizome (turmerone,

zingiberene, cineole, p-cymene)

Application of HBL in different concentrations like 0.5 μ M, 1.0 μ M, 2.0 μ M and 3.0 μ M increased the contents of the essential oils like Turmerone, Zingiberene, Cineole, p- Cymene in turmeric rhizome. In earlier studies, Salicylic acid and Putrescine) increased the oil quality of two canola cultivars cvv. Rainbow and Dunkeld canola (*Brassica napus* L.) subjected to drought stress (Faizanullah *et al.* 2012). Exogenous application 0.5, 1.5 and 2.5ppm of 24-epibrassinolide resulted in enhanced essential oil and menthol content in peppermint (*Mentha piperita* L.) and 2.5 ppm of 24-epibrassinolide was found most effective compared to the other treatments (Çoban and Baydar 2016). Povh and Ono (2006) observed in sage (*Salvia officinalis*) when treated with 100 mg L⁻¹ of gibberellic acid (GA) had more essential oil content compared to the untreated control plants. Recently, Ibrahim *et al.* (2019) reported that PGRs viz., benzyladenine and naphthalene acetic acid enhanced the production of volatile organic compounds (cismuuro-la-4(141)5-diene, γ -cadinene, γ -muurolene and prenyl acetate) in the flowers of two modern rose varieties, Hybrid Tea and Floribunda.

5. Conclusions

Thus, the research study gives an insight that the treatment of HBL resulted in increased contents of total sugars and the principal ingredient, curcumin. The chemical composition of turmeric rhizome in terms of total poly phenol content, total flavonoid content, total tannin content, crude fat as well as crude fiber were also found increased on foliar application of HBL. Even, the essential oils in the turmeric rhizome viz., turmerone, zingiberene, cineole and p-cymene were found significantly increased by HBL application to turmeric plants. The crop plants grown for their nutritive value are being improved through various techniques such as plant breeding, genetic engineering and application of PGRs. Thus, the present study clearly reveals that the application of HBL to turmeric plants as exogenous spray was very effective in improving the nutritive value of crop plants on par with other PGRs.

References

- Afroz, R., Tanvir, E.M., Islam, V., Alam, Gan, S.H. and Khalil, I. (2014), "Potential antioxidant and antibacterial properties of a popular jujube fruit: apple kul (*Zizyphus mauritiana*)", *J. Food Biochemistry*, **38**(6), 592-601. <https://doi.org/10.1111/jfbc.12100>.
- AOAC (2005), Ash of Flour (Direct Method). Method 923.03. *Official Methods of Analysis, 18th Ed.*, AOAC International Publisher, Gaithersburg, USA.
- Bajguz, A. and Hayat, S. (2009), "Effects of brassinosteroids on the plant responses to environmental stresses: a review", *Plant Physiol. Biochem.*, **47**, 1-8. <https://doi.org/10.1016/j.plaphy.2008.10.002>.
- Białczyk, J., Lechowski, Z. and Libik, A. (1998), "Regulation of tannin synthesis in leaves of tomato seedlings by phytohormones and plant growth inhibitors", *J. Plant Diseases Protection*, **105**(5), 496-503. <https://www.jstor.org/stable/43386546>.
- Chang, C., Yang, M., Wen, H. and Chern, J. (2002), "Estimation of total flavonoid content in propolis by two complementary colorimetric methods", *J. Food Drug Anal.*, **10**, 178-182. <https://doi.org/10.38212/2224-6614.2748>.

- Çoban, O. and Baydar, N.G. (2016), “Brassinosteroid modifies growth and essential oil production in peppermint (*Mentha piperita* L.)”, *J. Plant Growth Regulation*, **36**, 43-49. <https://doi.org/10.1007/s00344-016-9614-1>.
- Du, H., Ahmed, F., Lin, B., Li, Z., Huang, Y., Sun, G., Ding, H., Wang, C., Meng, C. and Gao, Z. (2017), “The effects of plant growth regulators on cell growth, protein, carotenoid, PUFAs and lipid production of *Chlorella pyrenoidosa* ZF strain”, *Energies*, **10**: 1696. doi:10.3390/en10111696.
- Faizan, U., Asgari, B. and Asia, N. (2012), “Effects of plant growth regulators on growth and oil quality of canola (*Brassica napus*) under drought stress”. *Pak. J. Bot.*, **44**(6), 1873-1880.
- Fathima, M. and Balasubramanian, A. (2006), “Effect of plant growth regulators on the quality of bast fibres in *Abelmoschus esculentus* (Linn.) Moench”, *Acta Bot. Croat.*, **65**(1), 101-112.
- Finkelstein, R.R., Gampala, S.S.L. and Rock, C.D. (2002), “Abscisic acid signaling in seeds and seedlings”, *The Plant Cell*, **14**, 15-45. <https://doi.org/10.1105/tpc.010441>.
- Gavelienė, V., Novickienė, L. and Pakalniškytė, L. (2013), “Effect of auxin physiological analogues on rapeseed (*Brassica napus*) cold hardening, seed yield and quality”, *J. Plant Res.*, **126**(2), 283-292. <https://doi.org/10.1007/s10265-012-0525-3>.
- Ghasemzadeh, A., Talei, D., Jaafar, H.Z.E., Juraimi, A.S., Mohamed, M.T.M., Puteh, A. and Mohd Halim, R.A. (2016), “Plant-growth regulators alter phytochemical constituents and pharmaceutical quality in Sweet potato (*Ipomoea batatas* L.)”, *BMC Complementary and Alternative Medicine*, **16**, 152. <https://doi.org/10.1186/s12906-016-1113-1>.
- Giannakoula, A. E., Ilias, I.F., Dragišić Maksimović, J.J., Maksimović, V.M. and Živanović, B.D. (2015), “Effects of plant growth regulators on growth, yield, and phenolic profile of lentil plants”, *AGRIS*, **28**(1), 46-53. <https://doi.org/10.1016/j.jfca.2012.06.005>.
- Guenther, E. (1972), *The essential oils*. Krieger Publishing Company, New York.
- Lucho, S., do Amaral, M., López-Orenes, A., Kleinowski, A., do Amarante, L., Ferrer, M., Calderón, A. and Braga, E. (2018), “Plant growth regulators as potential elicitors to increase the contents of phenolic compounds and antioxidant capacity in stevia plants”, *Sugar Tech.*, <https://doi.org/10.1007/s12355-018-0673-4>.
- Lynrah, P.G., Chakraborty, B.K. and Chandra, K. (2002), “Effect of CCC, Kinetin, NAA and KNO₃ on yield and curcumin content of turmeric”, *Indian J. Plant Physiol.*, **7**(1), 94-95.
- Ibrahim, M., Agarwal, M., Yang, J.H., Abdulhussein, H., Du, X., Hardy, G. and Ren, Y. (2019), “Plant growth regulators improve the production of volatile organic compounds in two rose varieties”, *Plants*, **8**, 35. <https://doi.org/10.3390/plants8020035>.
- Mitchell, J.W., Mandava, N., Worley, J.F., Plimmer, J.R. and Smith, M.V. (1970), “Brassins-a new family of plant hormones from rape pollen”, *Nature*, **225**, 1065-1066. <https://doi.org/10.1038/2251065a0>.
- Mostafa, G.G. and Alhamd, M.F.A. (2011), “Effect of gibberellic acid and indole -3- acetic acid on improving growth and accumulation of phytochemical composition in *Balanites aegyptiaca* plants”, *Am. J. Plant Physiol.*, **6**, 36-43. <https://doi.org/10.3923/ajpp.2011.36.43>.
- Mousavi, E.A., Kalantari, K.M. and Jafari, S.R. (2009), “Change of some osmolytes accumulation in water-stressed colza (*Brassica napus* L.) as affected by 24-epibrassinolide”, *Iranian J. Sci. Tech. T. A – Sci.*, **33**, 1-11.
- Povh, J.A. and Ono, E.O. (2006), “Rendimento do óleo essencial de *Salvia officinalis* L. sob ação de reguladores vegetais”, *Acta Sci. Biol. Sci.*, **28**(3), 189-193. <https://doi.org/10.4025/actascibiolsci.v28i3.209>.
- Procházka, P., Štranc, P., Pazderů, K., Štranc, J. and Vostřel, J. (2017), “Effects of biologically active substances used in soybean seed treatment on oil, protein and fibre content of harvested seeds”, *Plant Soil Environ.*, **63**. <https://doi.org/10.17221/702/2017-PSE>.
- Rao, S.S.R., Vardhini, B.V., Sujatha, E. and Anuradha, S. (2002), “Brassinosteroids a new class of phytohormones”, *Curr. Sci.*, **82**, 1239-1245.
- Schilling, G, Schiller, C. and Otto, S. (1991), “Influence of brassinosteroids on organ relation and enzyme activities of sugar beet plants”, *Brassinosteroids- Chemistry, Bioactivity and Application. ACS Symposium Series*, American Chemical Society, Washington, USA.

- Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999), "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent", *Method. Enzymol.*, **299**, 152-178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
- Vardhini, B.V. (2020), "PGRs as chemical agents to ameliorate diverse environmental stresses in plants – A Review of the Past Decade". *Protective Chemical Agents in the Amelioration of Plant Abiotic Stress – Biochemical and Molecular Perspectives*. John Wiley and Sons Ltd., Chichester, UK.
- Vardhini, B.V. and Rao, S.S.R. (1998), "Effect of brassinosteroids on growth, metabolite content and yield of *Arachis hypogaea*", *Phytochemistry*, **48**, 927-930. [https://doi.org/10.1016/S0031-9422\(97\)00710-3](https://doi.org/10.1016/S0031-9422(97)00710-3).
- Vardhini, B.V. and Rao, S.S.R. (2003), "Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum", *Plant Growth Regul.*, **41**, 25-31. <https://doi.org/10.1023/A:1027303518467>.
- Vardhini, B.V., Anuradha, S., Sujata, E. and Rao, S.S.R. (2010), "Role of Brassinosteroids in alleviating various abiotic and biotic stresses-A Review", *Plant Stress*, **4**, 55-61.
- Vardhini, B.V., Sujatha, E. and Rao, S.S.R. (2012), "Studies on the effect of brassinosteroids on the qualitative changes in the storage roots of radish", *Bulgarian J. Agricult. Sci.*, **18**, 63-69.
- Vardhini, B.V., Sujatha, E. and Rao, S.S.R. (2014), "Brassinolide improves nutrient value in storage roots of *Raphanus sativus* L. var Pusa chetki long", *Recent Res. Sci. Technol.*, **6**(2), 12-15.
- Wu, K., Zhang, X., Sun, S. and Wang, X. (2015), "Factors affecting the accumulation of curcumin in microrhizomes of *Curcuma aromatica* Salisb", *Biomed Res. Int.*, **2015**, 740794. <https://doi.org/10.1155/2015/740794>.
- Yoshida, S., Forno, D.A., Cock, J.H. and Gomez. (1976), *Laboratory physiological studies of rice*. InternationalRice Research Institute (IRRI), Laguna, Phillipines.