EFFECT OF LIMING AND GA_3 ON GROWTH, YIELD AND SOME NUTRIENTS CONTENT OF STEVIA

(*Stevia rebaudiana* Bert.)

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF LIMING AND GA_3 ON GROWTH, YIELD AND SOME NUTRIENTS CONTENT OF STEVIA (*Stevia rebaudiana* Bert)"** submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bonafide research work carried out by **MST. RUBA AKTER**, Registration No. **20-11150** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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The Author

Effect of liming and GA_3 on growth, yield and some nutrients content of stevia (*Stevia rebaudiana* Bertoni)

ABSTRACT

A field experiment was conducted at the experimental farm of Regional Sugercrop Research Station Thakurgaon of Bangladesh Sugarcane Research Institute (BSRI), during March 2021 to September 2021 to study the effect of lime and GA_3 on growth, yield and nutrient content of stevia. The trail was laid out in Randomized Complete Block Design (RCBD) with three replications. Factor A (lime), was used on treatment L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}. Factor B used gibberellic acid (GA_3) was H_1: control, H_2: 150 ppm, H_3: 200 ppm H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm. Data were collected on plant height (cm), number of branch $plant^{-1}$, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2), fresh weight $plant^{-1}$ (g), dry weight $plant^{-1}$ (g), fresh leaf yield $plant^{-1}$ (g), dry leaf yield $plant^{-1}$ (g), fresh leaf yield ha^{-1} (kg), dry leaf yield ha^{-1} (kg), N(%), P (%), K (%), S (%), Ca (%), Mg (%), Zn (µg g^{-1}) of stevia leaf, initial and post harvest soil analysis. Significant variations were observed on plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ in different level of lime application. Highest plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ was observed in L_3 (lime 1.0 t ha^{-1}) treatment and the lowest was in L_1 (control) treatment at 21 DAT to 147 DAT, respectively. Applying 300 ppm GA_3 was the significant effect in increasing at 21 DAT to 147 DAT, respectively and the lowest plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ was observed in control (GA_3) treatment. The highest plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2) number of primary and secondary branch $plant^{-1}$ heights was in L_3H_5 (lime 1.0 t ha^{-1} × GA_3 300 ppm) treatment and the lowest was found in L_1H_1 at all growth stages. N, K, Mg, Zn content of stevia leaf was significantly affected by different levels of lime and GA_3. The highest N, K, Mg, Zn content was observed when the plot was treated with lime 1.0 t ha^{-1} × 300 ppm GA_3 (L_3H_5) and the lowest N, K, Mg, Zn content was recorded in the control treatment. Significantly highest fresh weight $plant^{-1}$, fresh weight ha^{-1}, dry weight $plant^{-1}$, dry weight ha^{-1} was observed in L_3 treatment and lowest was observed in L_1 treatment in stevia plant. The interaction effect of lime and GA_3 in most of the combination showed significantly the highest fresh weight $plant^{-1}$, fresh weight ha^{-1}, dry weight $plant^{-1}$, dry weight ha^{-1}, fresh leaf yield ha^{-1} and dry leaf yield ha^{-1} was observed in L_3H_5 and lowest was observed in L_1H_1 treatment. Thus, the application of lime and gibberellic acid (GA_3) had positive impact on leaf yield components resulted in higher yield of study. From the result it can be recommended that lime 1.0 t ha^{-1} and GA_3 300 ppm is suitable for field cultivation of stevia production. Therefore, these findings infer that lime 1.0 t ha^{-1} and GA_3 300 ppm might help in producing more stevia leaf in Old Himalayan Piedmont Plain soil especially in Northwest of Bangladesh for environment friendly management practices.

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ABBREVIATIONS AND ACRONYMS

Cu	=	Copper
N	=	Nitrogen
P	=	Phosphorus
K	=	Potassium
%	=	Percentage
AEZ	=	Agro-ecological Zone
BSRI	=	Bangladesh Sugarcane Research Institute
Contd.	=	Continued
CRD	=	Completely Randomized Design
etc.	=	Etcetera
S	=	Sulphur
g	=	Gram
FDA	=	Food And Drug Administration
L	=	Liter
Max	=	Maximum
Min	=	Minimum
ml	=	MiliLitre
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
SD	=	Standard Deviation
^{o}C	=	Degree Celsius
t ha^{-1}	=	Ton per hectare
i.e.	=	id est = that is
Mg	=	Magnesium
mg	=	Milligram
kg	=	Kilogram
e.g.	=	Exempli gratia = for example
et al.	=	Et alia = and others
pH	=	Negative logarithm of hydrogen ion (H^{+}) concentration
Fig.	=	Figura
AAS	=	Atomic Absorption Spectrometer
*	=	5 % level of probability
WHO	=	World Health Organization
Ca	=	Calcium
Zn	=	Zinc
B	=	Boron
OM	=	Organic Matter
H	=	Hormone
L	=	Lime
GA_3	=	Gibberellic Acid
H_2O	=	water
FAO	=	Food and Agricultural Organization

CHAPTER I

INTRODUCTION

Stevia is a naturally derived, high potency sweetener that can be up to 250-300 times sweeter than sucrose, or table sugar. It is similar in sweetness intensity to many of the artificial sweeteners currently on the market (U.S. Food and Drug Administration, 2015). Indigenous populations in Paraguay and Brazil have been using the leaves of the stevia plant, *Stevia rebaudiana* (Bertoni), as a sweetener since before recorded history (Lee, 1979; Soejarto, 2002). The ancient Guarani people of Paraguay referred to stevia as "kaa he-he", which means "sweet herb" (Ranjan, 2011).Stevia (*Stevia rebaudiana* Bertoni) is native to Paraguay, Brazil, and Argentina. It is grown commercially in many parts of Argentina, Brazil, Columbia, Paraguay, China, Japan, Malaysia, South Korea, Vietnam, Israel, Australia, Kenya, and the United States. More than 150 countries have endorsed or authorized the use of high-purity steviol glycosides as sweeteners in foods and beverages. They are recognized as safe and effective by all major regulatory bodies worldwide. The first steviol glycoside introduced for use in commerce was Reb A (Ashwell 2015). It is a perennial shrub that belongs to the Asteraceae (*Compositae*) family. It is a natural sweetener commonly and variously known as sweet leaf, honey leaf, candy leaf, sweet weed or sweet herbs (David and Andrew 2002).The sweet taste of stevia is due to diterpene glycoside which is calorie-free and does not metabolize. Therefore, it is established as the sweetest plant on the earth. It is thermo-stable and can withstand a temperature range of 200ºC.

Stevia is gaining significant popularity in different parts of the world and is expected to be a major source of high-potency sweeteners (Khan et al. 2012). The leaves of this popular plant are sweet and ideal for people who are conscious of sugar and carbohydrate intake. With zero calories, the plant is being recognized as a great replacement for sugar and other sweeteners (Ashwell 2015). This attraction is due to the fact that stevia is plant-based, has zero calories, and has a sweet flavor that is 50–350 times sweeter than sugar, making it a great option for usage in food and beverage items with lower sugar and calorie content (Samuel et al. 2018). Despite the fact that the safety of stevia has been affirmed by several food regulatory and safety authorities around the world, insufficient education about stevia's safety and benefits, including continuing concern with regard to safety, deters health professionals and consumers from recommending or using stevia (Priscilla et al. 2018). Stevia is an antibacterial,

anticandidal, antifungal, antiviral, cardiotonic, diuretic, hypoglycemic as well as a vasodilator. It lowers blood pressure, heartburn cavities and contains depression activity. Dry leaves of this plant are 30-40 times sweeter than sugar with zero calories (Yadav et al. 2011, Beemnet et al. 2014). It is reported that steviosides have insulinotropic effects in pancreatic beta cells because it increases insulin secretion and thereby decreases blood glucose level (Piovan et al. 2018). It can be extracted and used as alternative non-caloric, natural sweeteners which can save people from diabetes and may possibly receive greater focus in the future. It is used in the treatment of diabetes and obesity by suppressing appetite and reducing the urge for sweets. Recent research has shown that consuming stevia in its raw form, fresh or dried helps to solve several health problems such as diabetes, allergies, digestive problems, anxiety, and high blood pressure (Cuervo et al. 2012). Besides these benefits, stevia also contains vitamin C, calcium, beta-carotene, niacin, iron, magnesium, potassium, proteins and fiber. Changes in leaf yield and accumulation patterns of stevioside have been observed in response to different environmental conditions. Nutritional variations provide leads for developing strategies to increase stevia productivity under different agro-climatic conditions (Pal et al. 2015).

Limes are materials containing carbonates, oxides or hydroxide required to apply on acidic soil to raise soil pH and neutralizes toxic elements in the soil. Liming materials include $CaCO_3$, $Ca(OH)_2$, CaO and others, which vary according to their neutralizing value and degree of fineness (TSO, 2010). Soil reaction is expressed in terms of pH indicating whether the soil is acidic, alkaline or neutral. Soil pH measures the molar activity (concentration) of hydrogen ions in the soil solution (Moody and Cong, 2008). Soil pH helps to identify the kinds of chemical reactions that are likely taking place in the soil. It affects nutrient availability and toxicity, microbial activity, and root growth. Most plants grow well at a pH range of 5.5 to 6.5 and liming is aimed to increase the pH to this range. Liming is a management practice to reduce the soil acidity and therefore one of the soil fertility management practices (AGRA. 2009). When lime is added to acid soils that contain high $Al3^+$ and H^+ concentrations, it dissociates into Ca_2^+ and OH^- ions. The hydroxyl ions will react with hydrogen and $Al3^+$ ions forming $Al3^+$ hydroxide and water, thereby increase soil pH in the soil solution. Soil pH increased significantly from 5.03 in the plots without lime to 6.72 at the lime rate of 3750 kg $CaCO_3$ ha^{-1} (Buni.A. 2014).

Rajmani et al., 2019 reported that plant growth regulators, Gibberellins are the most widely used and proven growth substances in Horticultural crops. Generally GA_3 influences a range

of developmental process in plants life like stem elongation, germination, breaking dormancy, flowering, sex expression, enzyme induction and leaf and flower senescence. Looking to the mode of action of the application of GA_3 in early stage enhances the growth of the plant.

Gibberellic acid (GA_3) is a phytohormone that is needed in small quantities at low concentration to accelerate plant growth and development by inducing metabolic activities and regulating nitrogen utilization (Sure *et al.,* 2012). So, favorable condition may be induced by applying growth regulator exogenously in proper concentration at a proper time in a specific crop by GA_3. It is such a plant growth regulator, which can manipulate a variety of growth and development phenomena in various crops. It is the most important growth regulator, which breaks seed dormancy, promotes germination, inter nodal length, hypocotyls growth and cell division in cambial zone and increases the size of leaves. GA_3 stimulates hydrolytic enzymes that are needed for the degradation of the cells surrounding the radicle and thus speeds germination by promoting seedling elongation growth of cereal seeds (Rood *et al.,* 1990). The present study was planned to evaluate the effect of different levels of liming and Gibberellic Acid (GA_3) on growth, yield and nutrient elements content of stevia but no such comparative study of liming has so far been reported till now therefore, the present study has been undertaken with the following objectives:

1. To study the effect of exogenous appilication of liming on growth, yield and nutrient elements content of stevia.
2. To study the effect of exogenous appilication of GA_3 on growth, yield and nutrient elements content of stevia, and
3. To find out the combined effects of liming and GA_3 on stevia production.

CHAPTER II

REVIEW OF LITERATURE

Stevia is a perennial shrub that belongs to the Compositae family. Native to South America, it is now cultivated in many regions of the world including Asia, Europe and North America (Lemus-Mondaca, Vega-Galvez, Zura-Bravo, and Ah-Hen, 2012). At present, more than 200 species of stevia are present around the world, but Stevia rebaudiana is the only one with a sweet taste (Shivanna, Naika, Khanum, and Kaul, 2013). Stevia is also known as honey leaf, candy leaf or sweet leaf, and its sweet taste is due to the presence of steviol glycosides, having 100–300 times the sweetness of sucrose (LemusMondaca, Vega-Gálvez, Zura-Bravo, and Ah-Hen, 2012). Apart from the sweet glycosides, stevia is also a good source of vitamins, minerals, essential amino acids, fatty acids, and other heath beneficial bioactive compounds including non-glycosidiclabdanediterpenes, flavonoids, phenolic compounds, crude fiber, phytosterols, chlorogenic acids, triterpenes and hydrocarbons (Wolwer-Rieck, 2012). In many countries, stevia is widely used as a sugar substitute in foods, beverages and medicine, and commercial products have been formulated from stevia derivatives (Abbas Momtazi-Borojeni, Esmaeili, Abdollahi, and Sahebkar, 2017). Interestingly, the leaves of stevia have superior functional and sensory properties than various other highpotency sweeteners, and is likely to become a major source of highpotency sweetener for the growing natural food market in the future (Goyal, Samsher, and Goyal, 2010). Besides its industrial applications, many studies have shown that stevia possesses various health benefitsincluding anti-diabetic, anti-obesity, anti-tumour, anti-hypertensive, anti-microbial, anti-caries and antioxidant properties (Abbas MomtaziBorojeni et al., 2017; Ruiz-Ruiz, Moguel-Ordonez, and Segura-Campos, 2015). In addition, various studies have reported that steviol glycosides of stevia leaves are not teratogenic, carcinogenic and mutagenic, and cause no sub-acute or acute toxicity (Abbas Momtazi-Borojeni et al., 2017).

2.1. Sweet and Bitter Tastes

Individual compounds that elicit basic tastes are referred to as tastants. In addition to sugars, sweet tastants include a variety of chemical compounds such as sugar alcohols, glycosides (including steviol glycosides), amino acids and proteins (Bachmanov, 2014). For bitter taste, more than 550 chemically diverse compounds have been identified (Wiener, 2011). Some commonly identifiable bitter tastants (and where they are found) include quinine (tonic water), caffeine (coffee), epicatechin (tea), tetralone (hops) and naringin (grapefruit) (Reed, 2010).

Taste perception can change when multiple taste stimuli are presented together in a food or beverage rather than when presented alone. This is referred to as a binary taste interaction (Keast, 2003). On a practical level, binary taste interactions are important in the development and modification of foods and beverages made with steviol glycosides. Sweet and bitter tastes found in steviol glycosides interact such that the presence of one suppresses the other (Hellfritsch, 2012).

2.2. Position of Stevia in plant kingdom

Taxonomic position from kingdom to Species (http://website[1], 2010)

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Compositae
Genus	*Stevia*
Species	*Stevia rebaudiana* (Bertoni)

2.3. Origin of stevia

Stevia rebaudiana Bert. is one of 154 members of the genus Stevia and one of only two that produce sweet steviol glycosides (Robinson, 1930; Soejarto*et al.*,1982; Soejarto*et al.*,1983). It is native to the valley of the Rio Monday in highlands of Paraguay, between 25 and 26 degrees south latitude, where it grows in sandy soils near streams (Katayama *et al.*, 1976).

2.4. Benefits of Stevia as a Natural Flavor

The benefits of using stevia natural flavors are multiple. Steviol glycosides have a hydrophobic component (steviol) and hydrophilic components (mainly glucose) and can modify the flavor profile of foods and beverages. For instance, in a 30% reduced sugar version of a cola flavored carbonated soft drink, stevia natural flavor enhanced spice notes, balanced citrus/sugar flavors and provided a more syrupy mouthfeel (Pure Circle, 2016). Inclusion of stevia natural flavors have been shown to positively benefit numerous product types such as lemon-lime soft drinks, apple juice, alcoholic cocktails, flavored beers, lemon flavored tea and flavored yogurt (Pure Circle, 2016).

Other benefits revolve around stevia's inherent sugar-like profile. By including stevia natural flavor, the sweetness profile can be improved to allow for up to a 20% reduction in added sweetener. Stevia flavors can also modify the profile of stevia based sweeteners already in the formulation. Lastly, if artificial sweeteners are included in the formulation, stevia natural flavor can round out those flavor profiles to make them more "sugar-like". It does this by masking off notes such as metallic and bitter and optimizing the time related (temporal) aspects such as onset and duration of sweetness.

2.5. Propagation of stevia and plant density

Stevia can be grown from seed or stem cuttings. Due to low seed fertility and extremely low germination rates, the ability to multiply the stevia plant is constrained (Tadhani et al. 2006, Yang et al. 1981). Seed does not produce homogenous population. On the other hand, vegetative propagation is limited by the low number of individuals that can be obtained from a single plant (Yang et al. 1981, Sivaram and Mukundan 2003). In addition, due to the low primary growth, the seedling is not able to compete with weeds (Jain et al. 2009). Keeping

these difficulties in consideration, propagation through tissue culture could be suitable as an alternative method to obtain a sufficient number of plants with a homogeneous population within a short period of time (Ibrahim et al. 2008). There are various reports of in vitro propagation of Stevia using different explants (Akita et al. 1994, Hossain et al. 2005, Salim et al. 2006, Uddin et al. 2006). Ghuari et al. (2009) reported micro propagation from the apical meristem and nodal segment. Some of the reports clearly support the possibility of propagating *S. rebaudiana* by tissue culture techniques (Razak et al. 2014, Uddin et al. 2006, Pande and Gupta, 2013). In vitro clonal propagation of stevia was carried by using leaf, nodal, inter nodal segment and shoot tip as explant (Das et al. 2006, Preethi et al. 2011, Uddin et al. 2006, Rao et al. 2012,Anbazhagan et al. 2010, Giridhar et al. 2010). Stevia is grown in the following season in the same field after uprooting the mother plant. Stevia stem cutting has the potential to become an important method of stevia plant multiplication (Castaneda-Saucedo et al. 2020).

The most cultural system involved transplanted seedlings and so densities less than the optimum are usually recommended to save costs. Densities of 80,000 -100,000 plants per hectare on row spacing of 45-65 cm are generally recommended with densities up to 160,000 suggested for higher yield (Prasann and Pankaj 2015).

2.6. Cultural practices of stevia

Planting densities ranging from 40,000 to 400,000 plants ha^{-1} have been tried in experiments conducted in Japan (Katayama *et al.,* 1976). Leaf yield increased with increasing density up to 83,000 and 111,000 plants ha^{-1} for the first year of production. The concentration of stevioside in the leaves of stevia increases when the plants are grown under long days (Metvier and Viana, 1979). Since glycoside synthesis is reduced at or just before flowering, delaying flowering with long days allows more time for glycoside accumulation. It follows that stevia production would be best situated in a long day environment where vegetative period is longer and steviol glycoside yields will be higher.

Fertility requirements for stevia grown as an annual crop are moderate. Results from Japan demonstrate that, at the point of maximum dry matter accumulation, stevia plants consist of 1.4% N, 0.3% P, and 2.4% K (Katayama *et al.,* 1976). In Ontario total biomass production of

7500 kg ha $^{-1}$ are possible and of that total, 26% would be roots, 35% stems, and 39% leaves (R. Beyaert pers. comm.). Based on the composition observed by Katayama, 1976 such biomass would require approximately 105 kg N, 23 kg P and 180 kg Kfrom both soil and fertilizer. The actual rates of application will vary according to soil type and production environment, and need to be optimized for each specific situation.

Two fungal diseases, *Septoriasteviae* and *Sclerotiniasclerotiorum*, have been reported in stevia grown in Canada (Lovering and Reeleder, 1996; Chang *et al.*, 1997). *Septoria* disease was characterized by depressed, angular, shiny olive gray lesions, sometimes surrounded by a chlorotic halo, that rapidly coalesce. *Sclerotinia* disease was characterized by brown lesions on the stem, near the soil line, followed by wilting and eventually by the complete collapse of affected individuals. No means of controlling these diseases have yet been published. Since stevia is very slow to establish and does not compete well with weeds, herbicides or other means will be essential to control weed growth to produce ample yield and a clean crop. The herbicide trifluralin appears to be well tolerated by stevia (Katamaya, 1979).

Stevia is harvested just prior to flowering when steviol glycoside content in the leaves is at its maximum (Sumida, 1980, Xiang, 1983). Following harvest the whole plant is dried and the leaves separated from the stems for further processing (Murai, 1988). The stems have very low concentrations of sweet glycosides and are removed to minimize processing costs (Brandle and Rosa, 1992). Drying stevia under artificial conditions is affected by a number of factors including loading rate, temperature, and ambient air conditions (Van Hooren and Lester, 1992). The effect of drying conditions on glycoside levels or processing quality of the leaves has not been investigated.

2.7. Seed production and quality of stevia

Given stevia's daylength requirements, seed production in the Northern hemisphere would be best situated between 20 and 30EN latitude. The crop could be transplanted in February or March and seed collected in late summer. Flowering under these conditions should occur between 54-104 d following transplanting, depending on the daylength sensitivity of the cultivars used for seed production (Katayama *et al.*, 1979). One-thousand seed weights for stevia seed usually range between 0.15 and 0.30 g and, depending on plant density, seed yields of up to 8.1 kg ha^{-1} are possible (Carniero, 1990). Seed germination is often poor and

rates less than 50% are common (Miyazaki and Wantenabe, 1974). Given the aforementioned conditions, seed produced on one ha could be enough to supply transplants for up to 200 ha of leaf production. Seed viability and yield are affected by growing conditions during pollination and seed filling. Excessive rainfall during pollination can affect both seed yield and germination (Carneiro, 1990, Shuping and Shizhen, 1995). Seed is best stored at 0EC, but even under low temperature conditions germination will still decline 50% over three years (Shuping and Shizhen, 1995). Sealing of storage containers or using lower temperatures did not prevent the decrease in germination over time.

2.8. Cultivar development of stevia

A variety of plant breeding procedures have been used to improve leaf yield and rebaudiosideA concentration in the leaves. Based on cultivar descriptions from Japan, China and Korea and our own work, it appears that sufficient genetic variability exists to make significant genetic gains in leaf yield, rebaudioside A content and the ratio of rebaudioside A to stevioside (Brandle and Rosa, 1992; Lee *et al.*, 1979 and 1982; Shizhen, 1995; Shyu*et al.*, 1994 and Morita, 1987). Brandle and Rosa, (1992) found that the heritability of stevioside content to be high (83%), based on calculations from a group of half-sib families. Heritabilities for leaf yield (75%) and leaf to stem ratio (83%) were also substantial indicating that selection would be effective. Total sweet glycoside concentration in some lines from China was reported to be as high as 20.5%, and a rebaudioside A to stevioside ratio of 9:1 was disclosed in the Japanese patent literature (Shizhen, 1995 and Morita, 1987). Two breeding methods reported by the latter authors were: phenotypic mass selection and, recurrent selection for phenotype where selected plants are intercrossed before another round of selection. Some cultivars such as the high rebaudioside A selection from Japan, and Suweon 2 and 11 from Korea are based on the selection of single plant and because of self-incompatibility they can only be reproduced vegetatively, which limits their utility.

Nakamura and Tamura, 1985 studied a population of 300 random individuals and found that total glycoside concentrations at the seedling and harvest stages were not correlated suggesting that early selection for total glycosides would not be effective. However, the proportion of individual glycosides relative to the total was correlated between seedlings and mature plants making early selection for glycoside composition possible. The authors also

observed a wide range of variation in the four main glycosides and found that dulcoside A and stevioside, and rebaudioside A and C, were positively correlated with each other. Stevioside and rebaudioside A, and dulcoside and rebaudioside C, were negatively correlated with each other. These correlations can be partially explained by the biosynthetic relationships between the individual glycosides because stevioside is the substrate for the synthesis of rebaudioside A, plants high in rebaudioside A will probably be low in stevioside (Shibata *et al.*,1991).

2.9. Chemical properties of stevia

Total chemical composition of stevia is still unavailable, a range of stevia species has been studied by biochemists and bio-technologists for its chemical constituents and out of 110 species, only 18 were found having this features (Soejarto et al. 1982). Diterpeneglycosides, stevioside and rebaudioside are responsible for its high sweetening potential of leaves. The bio-sweeteners of stevia leaves, called steviol glycosides, are isolated and identified as stevioside, steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside (Geuns 2003). According to Shibata et al. (1995) report, all of these diterpenoid glycosides encompass an identical chemical backbone structure (steviol) but have bit different in the carbohydrate residues at C13 and C19 positions. The chemical backbone of stevioside and its derivatives are shown in Figure 2.1. The percentage of components was also studied and those were stevioside 5-10% of total dry weight, rebaudioside A 2-4%, rebaudioside C 1-2% and dulcoside A 0.4- 0.7% (Wood et al. 1955). The structural formulation of stevioside derivatives and its sweetness fold compared to sucrose is studied well (Crammer and Ikan 1986, Geuns 2003) which are enlisted in the. Along with sweetness, stevia has some bitter aftertaste due to the presence of some essential oils, tannins and flavonoids and it was noticed that stevioside and rebaudioside A is responsible to some extent for the aftertaste, albeit the role of rebaudioside A is considerably less than of stevioside (Phillips 1987).

Stevioside has been extracted and its products were also prepared such as (Ngowatana 1997) extracted stevioside as white fine powder form which is highly hygroscopic. More to the point of stevioside, some other compounds were also identified in stevia plants, like 80-85% water, ascorbic acid, beta carotene, riboflavin, thiamine, gibberellic acid, indole-3-acetonitrile, isoquercitrin, kaempferol, stigmasterol, xanthophylls, umbelliferone, chlorogenic acid, caffeic acid, chromium, cobalt, magnesium, iron, potassium and phosphorus (Sharma et al. 2006).

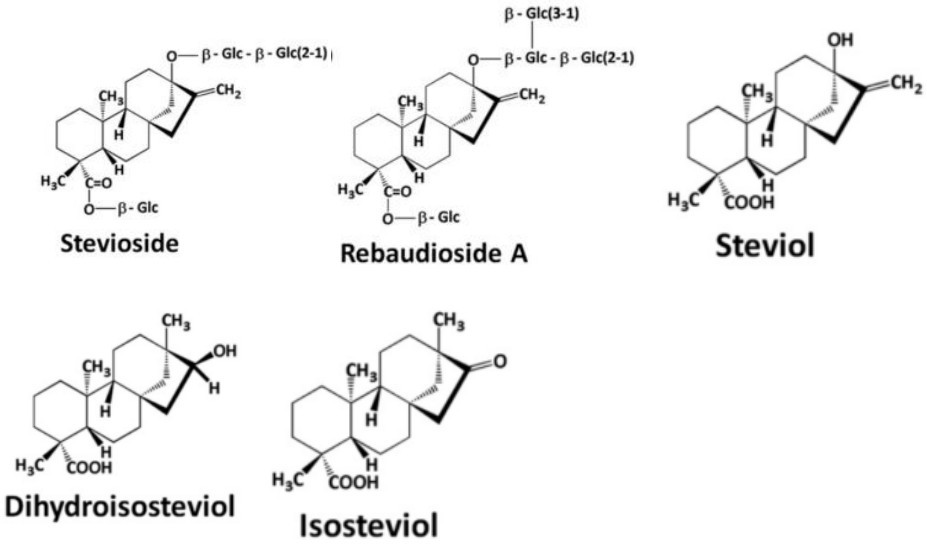

Figure 2.1. Chemical backbone of stevioside, the main compound found in the leaf of stevia and some other interrelated compounds.

2.10. Health benefits of stevia

2.10.1. Effect on diabetes mellitus

Diabetes mellitus is a metabolic disorder characterised by hyper glycaemia as a result of insulin resistance, a defect in insulin secretion or both (WHO, 2017). People with diabetes are

Table 2.1. Structural derivatives of stevioside and its related compounds and sweetness fold than sugar (Crammer and Ikan 1986, Geuns 2003).

Compound	R1 chain	R2 chain	Fold change of sweetness
Stevioside	β- Glc	β- Glc- β-Glc (2→1)	300
Steviolbioside	H	β- Glc- β-Glc (2→1)	100-125
Rebaudioside A	β- Glc	β- Glc- β-Glc (2→1) | β- Glc- (3→1)	250-450
Rebaudioside B	H	β- Glc- β-Glc (2→1) | β- Glc- (3→1)	300-350
Rebaudioside C	β- Glc	β- Glc- α-Rha (2→1) | β- Glc- (3→1	50-120
Rebaudioside D	β- Glc- β-Glc (2→1)	β- Glc- β-Glc (2→1) | β- Glc- (3→1)	250-450
Rebaudioside E	β- Glc- β-Glc (2→1)	β- Glc- β-Glc (2→1)	150-300
Dulcoside A	β- Glc	β- Glc- α-Rha (2→1)	50-120

at increased risk of acute- and long-term health complications including ketoacidosis or a non-ketotic hyperosmolar state, retinopathy, nephropathy, neuropathy, as well as cardiovascular, cerebrovascular and peripheral vascular diseases (Alberti&Zimmet, 1998). Therefore, it comes as no surprise that early diagnosis and effective management of diabetes should be top priority. In a previous study, stevioside administration showed a dose-dependent effect in lowering the glucose level of type-1 and type-2 diabetic rat models, as compared to the controls, while also reducing the rise in blood glucose during glucose-tolerance testing in non-diabetic rats. The regulation of blood glucose levels by stevioside was not only through the enhancement of insulin secretion in a dose-dependent manner and thereby increasing the glucose utilisation alone, but also due to the decrease in the gene expression of phosphoenol pyruvate carboxykinase (PEPCK) - a rate-limiting enzyme for gluconeogenesis expressed mainly in the liver - in a dose-dependent manner (Chen et al., 2005). Similar results were shown in a 2013 study, in which rats pre-fed with stevia leaves powder prior to being injected with streptozotocin (STZ) - a known diabetogen - showed less severe diabetic symptoms such as polyphagia and weight loss while their hyperglycaemia was less elevated when compared to the untreated diabetic rats. In this study, it was clear that the stevia leaves powder and its polyphenol extract enhances the secretion of insulin from the pancreatic islet β-cells in rats with type-1 diabetes, and increased cellular insulin sensitivity and improved glucose tolerance in type-2 diabetic rats (Shivanna et al., 2013). Another possible mechanism by which stevia can reduce blood glucose level is the inhibition of the activities of α-amylase and α-glucosidase, important enzymes used in the digestion of dietary carbohydrates, and thus can be helpful in the management of blood glucose level in diabetic patients. Recently, the extract of stevia leaves inhibited the activities of α-amylase and α-glucosidase enzymes in vitro (Carrera Lanestosa, Coral-Martínez, Ruíz-Ciau, Moguel-Ordoñez, &Segura Campos, 2020; Zaidan et al., 2019). Furthermore, a study in 2017 has also successfully shown that crystals derived from stevia leaves possess anti-diabetic properties since treatment of diabetic rats with these crystals at a concentration of 500 mg/kg resulted in a decrease in the body weight and the blood glucose level. Additionally, the histopathological study showed that the crystals also exhibited a protective effect on the pancreas by restoring, to a small extent, its structural

damage (Das et al., 2017). Interestingly, a novel phenylethanoid glycoside, namely steviophethanoside (4-hydroxyphenyl ethyl-8-O-[α-l-arabinopyranosyl-(1→6)]-β-d-glucopyranoside), and four phenylethanolyl glycosides have been isolated from stevia leaves. Besides poor toxicity, steviophethanoside showed a significant stimulatory effect on rat INS-1 islet β-cells, and thus may be a safe hypoglycemic compound (He et al., 2019). However, additional studies are needed to confirm the hypoglycemic potential of steviophethanoside and its mechanism of action in diabetes. Moreover, future studies should also focus on isolating other bioactive compounds from stevia, investigating any synergistic effect(s) that might contribute towards and justify the observed hypoglycemic potential. In a similar study, rats with STZ-induced diabetes were treated with an aqueous extract of stevia leaves. After 8 weeks of treatment, results showed that, compared to the control rats, the diabetic rats treated with the stevia extract showed a significant decrease in both the random and fasting blood glucose as well as in the glycosylated haemoglobin (HbA1c), while insulin and liver glycogen levels improved significantly (Ahmad & Ahmad, 2018). In another, more recent study, steviolglucuronide, a metabolite of steviol glycosides showed stimulation of insulin secretion from isolated pancreatic islets in a dose- and glucosedependent manner. The authors argued that steviolglucuronide might be the major metabolite after oral consumption of stevia glycosides (Gu et al., 2019). In a study on the effects of stevia amongst other sweeteners on satiety, food intake, postprandial glucose and insulin levels in human subjects which is already previously mentioned, it was found that those who were treated with a stevia preload showed a significant decrease in both the postprandial glucose and postprandial insulin levels when compared to the subjects that were treated with either an aspartame or sucrose preload (Anton et al., 2010). In another study, diabetic patients were divided into two groups: one group served as a control and the other group was given stevia leaf powder. The results showed that the consumption of stevia leaf powder for 60 days significantly decreased both fasting- and postprandial-blood glucose levels in the intervention group compared to the control group (Ritu&Nandini, 2016). Opposite results were shown by only a few recent studies in which no effect of stevia on postprandial blood glucose was observed (Ahmad et al., 2018; Farhat et al., 2019; Samakkarnthai, Payanundana, Sathavarodom, Siriwan, & Boonyavarakul, 2018). However, these human studies are limited in numbers, making it

impossible to make conclusive statements about the hypoglycemic potential of stevia. Overall, based on the in vivo and clinical studies, it is likely that stevia can be regarded as a promising new therapy for the management of diabetes which could result in a reduction in complications associated with this pathology. However, to confirm these results, more indepth mechanistic studies are warranted to establish the clinical potential of stevia and possible underlying mechanism in diabetes management.

2.10.2. Effect on obesity

Obesity is a major health problem, affecting adults and children alike. Although the aetiology of obesity can be multifactorial; an increased caloric intake due to the consumption of sugar-rich foods and beverages has been found to be one of the main causes of obesity (Ashwell, 2015; TeMorenga, Mallard, & Mann, 2012). Therefore, it goes with reasoning that a substitution of sugar-rich foods and beverages with those sweetened with a non-nutritive sweetener would result in a decrease in the total caloric intake, and therefore a reduction in body weight (Ashwell, 2015). Stevia can fit this role as a non-nutritive sweetener which can take the place of other, more calorie-dense sweeteners such as sucrose. This is because high-purity stevia leaf extracts are considered to have zero calories while providing a taste which is 100–300 times as sweet as that of sucrose (Ashwell, 2015). Indeed, this theory has translated well in practice, wherein a 2016 study found a significant decrease in body weight, total cholesterol, triglycerides and low-density lipoproteins and an increase in highdensity lipoprotein in rats which were exposed to stevia in their diet instead of sucrose as compared to the sucrose-exposed control rats (Elnaga, Massoud, Yousef, & Mohamed, 2016). In a more recent study, published in 2018, diabetic rats which had been administered with an aqueous extract of stevia leaves for 8 weeks showed an improved caloric management as they reduced the feed and water intake when compared to the controls, leading to a decreased body weight (Ahmad & Ahmad, 2018). These promising results, however, have failed to materialise when translated in human subjects as multiple randomised controlled trials have failed to describe a change in body weight between those subjected to stevia and the controls (Lohner, Toews, &Meerpohl, 2017). However, in a 2010 study on the effect of taking stevia and other sweeteners on food intake and satiety amongst other parameters found that those individuals who took a preload containing stevia before a meal, consumed 300 calories less than those

who took a sucrose-containing preload. Moreover, the self-reported hunger and satiety levels between those individuals who took the stevia-preload and those who took the sucrose-preload did not differ, even though the former had consumed less calories (Anton et al., 2010). Similar findings were shown by a recent study in which stevia-preload decreased self-reported hunger and desire-to-eat in healthy subjects compared to water (Farhat, Berset, & Moore, 2019). In another recent study, cookies containing stevia leaf powder were found to reduce the hunger in those who ate them when compared to the control cookies made from 100% wheat flour (Ahmad, Khan, Johnson, Alam, & Din, 2018). Although foods and beverages can be sweetened with artificial sweeteners that are also non-caloric, they have been proven to cause weight gain, bladder cancer and brain tumours amongst other hazards in animal studies (> Gupta et al., 2013). Therefore, it can be argued that the use of a natural, safe sweetener such as stevia remains the preferred option, which indeed has received much attention and acceptance of both the scientific community and consumers.

2.10.3. Effect on hypertension

Hypertension is often referred to as a "silent killer" because, although it is a major risk factor for the development of sethreatening or disabling conditions (such as congestive heart failure, myocardial infarction, aneurysm, left ventricular hypertrophy, atrial fibrillation, peripheral vascular disease, stroke, hypertensive nephropathy and hypertensive retinopathy), mild to moderate hypertension can remain asymptomatic for many years (Forouzanfar et al., 2016; Oparil et al., 2018). Both hypertension and its related disabling conditions could be reduced if it is detected early and treated effectively through anti-hypertensive medications and adequate lifestyle changes (Oparil et al., 2018). In a study conducted by Chan et al. on 106 hypertensive women aged between 28 and 75 years who were administered 0.25 g stevioside three-times daily found a reduction in both the systolic and diastolic blood pressures by 14 mmHg and 14.3 mmHg respectively, after just 7 days. This hypotensive effect persisted throughout the entire duration of the study (1 year) with no side effects or significant changes in blood parameters (such as glucose and lipid levels) being reported. As a result, Chan et al. suggested the use of oral stevioside as an alternative or supplementary treatment for

hypertension (Chan et al., 2000). A 2015 meta-analysis of nine randomised clinical trials involving a total of 756 participants found a reduction in the blood pressure of patients treated with stevioside, yet the small extent of this effect as well as the substantial heterogeneity of the results obtained do not allow a robust conclusion to be made. This is likely a result of inadequate sample sizes and therefore the authors recommend the need for further clinical trials (Onakpoya & Heneghan, 2015). In a more recent systematic review and meta-analysis including data from seven randomised controlled trails with 403 participants, steviol glycosides showed a significant decrease in the systolic blood pressure compared with placebo, while no significant effect on diastolic blood pressure was observed (Bundgaard Anker, Rafiq, & Jeppesen, 2019).

2.10.4. Antimicrobial effect

Multiple studies have high lighted the antimicrobial activity of different extracts prepared from stevia leaves. In one of these studies, the crude water leaf extract of stevia was found to be ineffective against all tested strains of bacteria and fungi. In contrast, the crude leaf extract of stevia in methanol and chloroform were both found to possess antibacterial properties against all the bacteria tested i.e. Escherichia coli, Streptococcus mutans, Bacillus subtilis and Staphylococcus aureus. On the other hand, their anti-fungal properties were found to be more restricted, with the chloroform extract being active only against one fungus (Sclerotonia minor) out of the six fungi (Aspergillusniger, Curvularialunata, Sclerotonia minor, Rhizopus, Alternaria alternate and Microsporiumgypsium) it was tested on. Similarly, the methanol extract was found to be active against only two (Sclerotonia minor and Curvularia) out of the six fungi that were evaluated (Debnath, 2008). Similar to the aforementioned study, the aqueous extract of stevia leaves was also found to lack anti-bacterial properties but did show anti-fungal and anti-yeast properties in a study carried out by Jayaraman et al. The acetone, chloroform and ethyl acetate extracts all exhibited antimicrobial properties against a variety of microorganisms as follows: Aeromonashydrophila, Candida albicans, Salmonella typhii,Vibrio cholera, Cryptococcus neoformans, Trichophytonmentagrophytes, Epidermophyton, E. coli, B. subtilis and S. aureus (Jayaraman et al., 2008). The anti-bacterial properties of the extracts were quantified by measuring the ‘zone of inhibition’ whereas the

anti-fungal and anti-yeast properties were quantified by measuring the 'mycelial growth'. In a more recent study by Abdel-Fattah et al., wild stevia extracts (aqueous, ethanolic and alcoholic) showed potential antimicrobial activities against four pathogenic bacteria such as Enterococcus facium, Pseudomonas aeruginosa, Bacillus cereus and Klebsiellaponeumoniae, when compared to a commercial antibiotic (Chloramphenicol). Among the three tested extracts of stevia, alcoholic extract showed higher antibacterial property (Abdel-Fattah, Badr, Seif, Ali, & Hassan, 2018). A possible application of the antimicrobial and antioxidant properties of stevia (as elaborated later) is the use of its extracts as a preservative of salmon paste and other seafood products (Ortiz-Viedma et al., 2017). Other studies have also confirmed the antimicrobial property of stevia extracts against different species of bacteria. For instance, the inhibition of bacterial growth by the stevia extracts was found to be dose-dependent against all the species tested (Abou-Arab & Abu-Salem, 2010; Puri& Sharma, 2011; Singh, Garg, Yadav, Beg, & Sharma, 2012).

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2.11. Effects of lime on soil and plant

2.11.1. Acid soils

Soil acidity is a condition where there is a preponderance of hydrogen and aluminum ions such that a pH value of less than 7 is created (Ocampo, 2000). Generally a pH of 7.0 indicates neutrality, higher value indicate alkalinity, while lower values indicate acidity. But for practical purposes a soil with a pH below 6.6 commonly called acid soils. These soils have low cation exchange capacity (CEC), low base saturation and low organic matter content (Biswas and Mukherjee, 1991). The term is usually applied to the surface soil. Nutrient availability depends on soil pH and are categorized on the basis of pH named as alkaline, neutral and acidic having pH range more than 7.4, 6.6 to 7.4 and less than 6.6 respectively (Hausenbuiller, 1972). Most plant nutrients are available around neutral soil having pH 6.6 to 7.4. The descriptive terms are used for the ranges of pH are as follows; for pH less than 4.0 there is free acid due to the oxidation of sulfides to sulfates in acid sulfate soil; pH less than 5.50, occurrence of exchangeable Al and Mn from the dissolution of Al, Mn and pH greater then 7.80, there is occurrence of free $CaCO_3$ in upland soils (Ocampo, 2000).

While the effective exchange sites of acid soils are encountered by Ca^{2+}, Mg^{2+}, Al^{3+} and even H^{+}, the dominant cations associated with sol acidity is exchangeable Al. This form releases H^{+} upon hydrolysis in its nonnumeric or polymeric form. Hydrogen ions produced by organic matter decomposition are unstable in mineral soils. These react with silicate clays releasing exchangeable Al (autolysis) and siliceous acid. Exchangeable hydrogen is found in small amount in mineral soils. In soils having high organic matter hydrogen is associated with the -COOH group.

Sources of acidity in soils are natural conditions, as well as artificially by the continuous use of acid forming fertilizers. Acid igneous material, leaching of nitrate nitrogen and basic cations from soil, removal of basic cations by crops, continuous application of acid forming fertilizer like urea, DAP, ammonium sulfate organic manures, and oxidation of pyrite mineral are the major causes of developing acidity in soils. Rao *et al. (*1982) reported that the NH_3 reacts with soil CO_2 and produces carbonic acid and increases acidity of soil. Acid precipitation (pH<5.0) on farms near highly industrial area can be significant on soils with low CEC, low organic matter content and clay content (acid rian deposition). Lipa clay loam soil of UPBL having pH 6 decreased to 5.30 in a span of 11 years (Samonte*et al*., 1965) and Lipa clay loan soil of another field of UPBL having pH 6.00 decreases to 4.70 in two decades (Samonte and Ocampo, 1977). In Bangladesh most of the topsoil in the cultivated/deforested areas of the hills, terraces and other floodplains are acidified to variable extent (Shaheed, 1995).

2.11.2. Soil Acidity and plants

Culleton*et al.* (1993) reported that crops differ in their sensitivity to pH. In addition, the optimum use of fertilizers containing nitrogen and phosphorus is obtained when the soil pH is between 6.2 and 7.2. The availability of some trace elements, especially manganese and boron is decreased when pH is above seven.

Explanati**o**n**s**of poor plantgrowthon acid soils have included Al^{3+} toxicity, Mn^{2+} toxicity, lowN supply (mainly NH_4^{+}-N rather than NO_3^{-}-N), P deficiency(Foy, 1984), Mo deficiency (particularly in legumes), and toxic c**o**ncentrati**o**ns of phenolic acids.The hydrogen (H^{+}) i**o**n itself has been c**o**nsidered as the proximalcause of poor growth relatively rarely in seeming

deference.The poor growth observed in lettuce, tomato and Bermudagrass when grown in soluti**o**ns **o**flow pH was the result ofalow Ca supply.

Culleton*et al.* (1993) reported in a publication that the pH for maximum availability of nutrients for crop use is not the same for all nutrients. The pH values at which the availability of the individual nutrient is maximal: generally pH values in the range 6.5-7.0 is taken to be the optimum value for soil pH.

2.11.3. Effect of lime on acid soil

In the world, fertilization and liming are the most important soil management practice to sustain high yield. Through which soil quality is improved to a high level to meet the requirement for high yields. In other way it is tailoring the soil to meet plant needs (Ocumpo and Samonte, 1989). Liming reduces soil acidity by decreasing the concentration of hydrogen ions and increasing the concentration of hydroxyl ions thus reduces the solubility of aluminum, iron, and manganese in soil. It increases the availability and plant uptake of phosphorus, calcium, magnesium and molybdenum. Acid weathering of primary and secondary minerals is reduced by the decreased concentration of H^+ ions. The pH dependent anion exchange capacity decreases; forcing previously adsorbed anions such as SO_4^{2-} into the solution.

Liming promotes the decomposition of organic matter by making condition more favorable for the growth of microorganisms. The bacteria that fixed nitrogen from the air both non-symbiotically and in the nodules of legumes are specially stimulated by the application of lime. The successful growth of most soil microorganisms depends upon lime that satisfactory biological activities cannot be expected if calcium and magnesium levels are low. Lime increases the Ca and Mg status in soils, which act as cementing material for soil aggregate. It is suggested that in the long term, liming will increase crop yield, organic matter returns, soil organic matter content and thus soil aggregation (Haynes and Naidu, 1998).

2.11.4. Lime and its effect on an acid soil

Lime is powdered limestone rock containing calcium and/or magnesium. It is basic, that is, it can neutralize acidity or sourness of the soil. Lime application or liming corrects soil acidity, neutralizes toxic effects of excessive amounts of aluminum, iron, and manganese, improves availability of some plant nutrients, particularly calcium, magnesium and phosphorus, promotes desirable microbiological activities and improves the physical conditions (structure) of the soil.

The main function of liming in soils is to correct soil acidity. In addition to reducing Al and Mn to sub-toxic levels, liming alters the capacity of the soils to retain cations and anions and changes the availability of most plants nutrients (Jackson, 1962).

As pH drops beyond this preferred pH range, crop yield also drops, declining increasingly steeply as soil pH decreases further. Growth and yield decrease as soil acidity increases because some element like active aluminum, iron and manganese may increased to toxic levels, calcium and magnesium may become deficient, availability of phosphors and molybdenum are decreased while desirable soil microbial activities are reduce such detrimental effects can restricts the root and for growth of plants. In acid sulphate soil, after amending 10 t ha^{-1} of lime the pH of soil increased from 4.62 to 8.19 in the first week of application and then gradually decreased to 7.39 (Murali, 1976). But in another experiment, the pH of soil steeply increased during the first 20 days after liming, then slightly increased and finally slightly decreased with time until the end of 120 days of experimentation. Contrasting Malaysian acid sulphate soil, the pH increased from 4.1 to 6.4 by the application of 10 t ha^{-1} of ground limestone up to 120 days and then decreased. This expected decrease in the pH value during different time after the application of lime might be associated with the presence of high amount of aluminum released by soil (Shamsuddin and Auxtero, 1991).

Gautam (1996) reported that liming increased the soil pH in acid sulphate soil. In acid sulphate soil after liming at the rate of 5.0 t ha^{-1} pH increased from 4.91 to 7.28 on upper 0-15 cm of soils. Significant higher pH was observed in the treatment with 10 t ha^{-1} of lime than 5 t ha^{-1} was due to the contribution of higher adsorbed calcium.

Aluminum toxicity is responsible for poor yields in acid soils. In Bangladesh, because of intensive land use (> 186%), introduction of high yielding varieties, monocropping practices, inadequate and imbalance use of chemical fertilizers not only accelerates the nutrient mining but also results in nutrient imbalance in the soils. It is evident that the area with low nutrient status of P, K and S is generally higher in upland than in wetland-cultivated area. Most soils of Bangladesh showed a decline in the levels of exchangeable K, Ca, Mg and effective cation exchange capacity (Karim and Anwar 2001). The decline crop yields may be indicative of deterioration in some vital physical parameter, such as soil structure and bearing capacity. Most of the soils under high land and medium high land situations are low in fertility lever where especially N, P, K and S are deficiencies of micro nutrients like Mg, Zn, B and Mo have also been detected in some areas (Miah *et al.*, 1992). Surface acidity (top 5 cm) often occurs in grassland due to high rainfall and the use of nitrogenous fertilizer. This reduces the availability of fertilizer phosphorus. For this reason it is better to have frequent small application of lime than one large application at irregular intervals (Culleton*et al.*, 1993). Yearly application of lime and sulphur could be used to meet the sulphur requirements and minimize surface acidity. The lime requirement to bring the soil to 6.5 is based on the premise that optimum production is required.

2.11.5. Effect of lime on soil pH

Newman *et al.*(2009) reported that the primary reasons for liming acidic soils are to increase crop yield and to enhance fertilizer efficiency. Lime also affects the solubility of other elements; therefore, some plant nutrients are made more available by liming while toxicities caused by excessive concentrations of other plant nutrients are reduced. In addition to neutralizing soil acidity, calcitic limestone supplies the plant nutrient calcium, and dolomitic limestone supplies both calcium and magnesium. While a correct liming program is beneficial for plant growth excessive liming can be detrimental. Deficiencies and imbalances of certain plant nutrients may result from excessive lime application.

The pH is an important indicator to express the acidity or alkalinity of soil. The danger level of acidity varies with crop species, variety and soil. For many upland crops, it is at pH 5.50. The principle is that when the pH of the soil decreases below the optimum ranges for the

growth of specific crop variety, lime is needed (Ocampo*et al.,* 2000). The pH is useful as a measure of the degree of acidity/alkalinity of a soil while the lime requirement is an estimate of the amount of lime required to alter the pH in the soil to a target pH (Culleton*et al.,* 1993).

Foyand Fleming(1978) explained the following reason to liming for strongly acid soils:

a) Concentrations of Al and Mn can reach toxic levels because their solubility increases at pH< 5.50. Al starts to decrease more at pH 5.00 and above than Mn.
b) Organisms responsible for decaying organic matter (OM) and transforming NPS may become low in number.
c) Ca and Mg may be deficient (rarely) if the CEC is low (CEC < 15).
d) Symbiotic N fixation by rhizobia and legumes is greatly reduced. This process functions best at pH 6.00- 6.20
e) Acidic clay soils are less highly aggregated resulting in poor aeration, and
f) Availability of P and Mo reduced.

Culleton*et al.* (1993) also reported that liming affects the availability and uptake by crops of both major and trace elements. It may also affect the toxicity to plants of some of these elements. The pH influences nutrient availability by causing deficiency or toxicity. The idea pH achieves a balance between these extremes. Nutrient uptake is influenced by pH in terms of cationvs anion uptake. Curtin and Smillie (1983) showed that liming dramatically changes the composition of the soil solution in ways which must influence plant composition and yield. With some elements, these effects are mainly associated with changes in availability due to conversion to more soluble forms e.g. manganese become more available as soil acidity increases, even to the extend of becoming toxic to crops under extreme conditions. Others become toxic when acid conditions are associated with poor aeration e.g. iron. Molybdenum becomes more available as alkaline conditions increase. Phosphates are converted into less soluble compounds of iron and aluminium as acidity increases. In alkaline conditions phosphates are again rendered less available with the formation of relatively insoluble dicalcium phosphate etc.

2.11.6. Effect of lime on yield of crops

Adam (1980) observed that the root length and dry weight with the ratio either of Al/Ca = 2:1, 1:1 or 1:2, had no ameliorating effect, but was shown to have a negative effect, compared with the control. This suggested that the alleviation effect and its extent of Ca on Al toxicity for the seedling morphological growth are dependent on characters, the degree of Al stress, and the ratio of Al to Ca.

Richard *et al.* (2007) observed that amendments were analyzed for a number of different tests (ash alkalinity, total basic cation content, proton consumption capacity, and $CaCO_3$ content) which have been proposed as predictors of the liming effect of specific types of organic residues, and values were related to the changes in pH observed.

Meng*et al*. (2004) reported that the soil acidity decreased while exchangeable Ca in plough layer (0–20 cm) increased with lime rate and time. The decreased subsoil (20–60 cm) acidity started to occur four years after liming, and the extent of decreased soil acidity increased with lime rate and time.

Sharma *et al.* (2000) reported that lime application significantly increased the yields of crops and the maximum yield increased 68.25% for barley, 58.23% for mungbean, while 57.3% for wheat, 53.4% for sesame, 52.8% for broad bean, 44.1% for potato, 35.1% for rapeseed, 32.1% for cotton, 28.4% for corn, 18.5% for watermelon, 11.0% for cowpea and 8.8 % for soybean.

Khattak*et al.* (1998) reported that a new technique was developed in which only the upper half of the floral bud of mungbean was opened to expose the stigma. Emasculation and pollination times were 17.00-19.00 and 7.00-9.00 hours, respectively. The success rate of crossing using this technique in Faisalabad, Pakistan, averaged 62.8 and 20.0% in summer and spring, respectively. Pods contained an average 6.8 seeds/pod in summer and 5.0 seeds/pod in spring. This high success rate is mainly due to less disturbance on style and ovary in the bud during emasculation.

Malik *et al.* (2006) reported that a study to find out the residual effect of four summer grain legumes viz. clusterbean, soybean, mungbean and mashbean on yield and yield components of follow up wheat (*Triticumaestivum* L.) was carried out. Wheat parameters such as number of tillers per m of row length, number of grains spike^{-1}, 1000 grain weight (g) and grain yield (kg ha^{-1}) showed improvement.

Yang *et al.* (2005) observed that calcium (Ca) plays a very important role in the response of plants to salt stress. Little information is available about ratios of Al/Ca on the growth of mungbean seedlings under Al stress. Mungbean seedlings were grown in solution with combined concentrations of Al (0, 2, and 5 mM) and Ca (0-10 mM) to evaluate effects of the ratios on alleviation of Al toxicity for the morphological growth under Al stress.

Tomohiro and Bell (1991) reported that adding either $CaCO_3$ or organic matter increased root length in mungbean largely by decreasing the activity of monomeric Al in the soil solution. With organic matter, the major mechanisms of this decrease were presumed to be precipitation of soluble Al and the formation of Al-organic matter complexes. The former effect was predicted from the pH increase accompanying the organic matter addition, the increase being larger with legume leaves which had the higher exchangeable and soluble Ca and Mg contents. The concentration of Al complexed with soluble organic matter also was shown to increase with increasing rate of organic matter addition, the effect again being larger with legume leaves.

Sharma *et al.* (2000) observed that mungbean was compared with summer fallow, followed by rice in the rainy season and wheat in winter. Timely sown summer mungbean yielded 0.4-1.3 t ha^{-1} protein-rich seed and, on average, increased rice yields by 0.5-0.9 t ha^{-1} and the yields of the succeeding wheat by 0.4-0.7 t ha$^{-1.}$ Sharma and Sharma (2005) revealed that partial diversification by including mungbean during summer (May-June) in the rice-wheat system resulted in an increase in productivity and profitability. Tomohiro and Bell (1991) revealed that a soil incubation and short-term root growth experiment was conducted to investigate the effects of organic matter application on Al toxicity alleviation in a highly

weathered acid soil. Ground leaves of a tree legume (*Calliandracalothyrsus*Meissn.), ground barley (*Hordeumvulgare* L.) straw, or $CaCO_3$ were mixed at various rates with A-horizon soil of a red podzolic soil (EpiaquicHaplustult) and incubated at 90% of field capacity for 4 or 10 weeks. After the incubation, a short term (48 h) root growth test was conducted using mung bean (*Vignaradiata* (L.) Wilczek), followed by the analysis of the solution and solid phases of the post-harvest soil.

Haynes and Naramabuye (2006) observed that the major mechanisms responsible for the elevations in pH were suggested to be the substantial $CaCO_3$ content of poultry and pig manures and filter cake, the proton consumption capacity of humic material present in household compost and manures, and decarboxylation of organic acid anions during decomposition of plant residues and manures. Ash alkalinity and basic cation content were the tests most closely correlated with increase in soil pH.

Voigt (1998) reported that liming hastened mungbean root emergence in three of the four soils. Days to 40% emergence were closely related ($P < 0.01$) to soil pH and 40% root emergence on were 0.95, 0.96, 0.94 and 0.96, respectively.

Menzies and Edwards (1994) reported that Short term root growth bioassays using mungbean (*Vignaradiata*) were conducted on 39 surface soils in the unamended state and following the addition of $CaCO_3$ or $CaSO_4.2H_2O$. Root length after 48 h growth was related to solid phase and soil solution Ca and Al attributes.

Taburada (1994) reported that an acidic (pH 4.0) typicTropohemist of Basey, Samar with very high (69 percent) organic matter content was tested for lime and cropping response using mungbean, upland rice and corn. Results revealed the positive effect of liming (5 tons dolomite/ha) in the neutralization of soil acidity even during the first cropping. It is reported that growth and yield of all test crops remained unaffected by lime application until the second cropping period where significant yield increase of mungbean and corn were noted.

Similar effect was observed in the succeeding cropping periods until the prolonged drought which hit the area towards the third cropping year.

Smyth *et al*. (2007) showed that roots of mungbean cultivar plant extending from a limed surface soil compartment grew for 28 days into a subsurface compartment containing acid subsoils from the Cecil (oxidic and kaolinitic), Creedmoor (montmorillonitic) and Norfolk (kaolinitic) series.

Suhartatik (1990) observed that the objective of the experiment was to obtain the residual effect of lime and organic fertilizer on the growth and yield of mungbean. The experiment using 12 treatments of organic fertilizer (manure, *Crotalaria juncea* and *Setaria sp*.), liming and NPK fertilizer in selected combinations and were arranged in a Randomized Complete Block Design with three replications. The first crop was soybean which was followed by mungbean. The results indicated that lime residue had effectively increased mungbean yield and improved soil productivity for two cropping season.

Suhartatik (1991) observed that residue of lime with NPK fertilizer significantly increased: plant height, leaf area, nutrient uptake, number of pod, length of pod, and dry seed weight per plant. The residue of organic fertilizer with NPK did not significantly increase seed weight per plant. Compared to organic fertilizer, the role of lime residue in increasing mungbean yield was greater.

Delfin and Banos (2003) showed that a field trial was conducted to evaluate the effect of liming, application of inorganic nitrogen and phosphorus and inoculation with rhizobia and mycorrhiza on the growth, nitrogen uptake, phosphorus uptake and yield of mungbean.

A field experiment was conducted at Tamanbogo Experimental Farm (Central Lampung) in the 1988/1989 wet season to evaluate the effect of dolomite, calcite, phosphorus, and their combinations on the growth and yield of mungbean. He observed that either liming or P fertilization increased grain yield of mungbean significantly. The levels of grain yields in

mungbean obtained in this experiment varied from 1.5 t mungbean to 1.7 t ha^{-1} compared to the control plot which produced only 1.3 t ha^{-1} by the application of lime in different dozes (Marzuki, 1991).

Samonte (1985) observed that lime-rate field experiments conducted in four soil types indicated that strong to very strong soil acidity reduced the yield of mungbean by 23 to 63% and that of soybean by 22 to 36%. Optimum yields of mungbean were obtained at pH 6.03 to 6.30 while that of soybean occurred at pH 6.1 to 6.4. Lime requirements to obtain high profits for mungbean on Alaminos sandy clay loam, Luisiana sandy clay loam, Guadalupe clay, and Adtuyon sandy clay are 5.6, 7.6, 5.7, and 5.0 tons $CaCO_3$ ha^{-1}, respectively. According to Fertilizer Recommendation Guide (2005), the rate of dolomite application would be 3-5, 2-3 and 1-2 and 1-2 t ha^{-1} for soils having pH 3.5-4.5, 6-5.5 and 5.6-6.5, respectively.

Hashimoto *et al*. (2007) showed that the study was to assess the ameliorative effects of Mg on mungbean root growth in acidic subsoils and to relate the soil solution ionic compositions to mungbean root growth.

Smyth *et al*. (2007) showed that roots of mungbean cultivar plant extending from a limed surface soil compartment grew for 28 days into a subsurface compartment containing acid sub soils from the Cecil (oxidic and kaolinitic), Creedmoor (montmorillonitic) and Norfolk (kaolinitic) series.

Richard *et al.* (2007) observed that amendments were analyzed for a number of different tests (ash alkalinity, total basic cation content, proton consumption capacity, and $CaCO_3$ content) which have been proposed as predictors of the liming effect of specific types of organic residues, and values were related to the changes in pH observed.

Malik *et al.* (2006) reported that a study to find out the residual effect of four summer grain legumes viz. clusterbean, soybean, mungbean and mashbean on yield and yield components of follow up wheat (*Triticumaestivum* L.) was carried out. Wheat parameters such as number

of tillers per m of row length, number of grains spike^{-1}, 1000 grain weight (g) and grain yield (kg ha^{-1}) showed improvement.

Sharma and Sharma (2005) revealed that partial diversification by including mungbean during summer (May-June) in the rice-wheat system resulted in an increase in productivity and profitability. Sharma *et al*. (2000) reported that lime application significantly increased the yields of crops and the maximum yield increased 68.25% for barley, 58.23% for mungbean, while 57.3% for wheat, 53.4% for sesame, 52.8% for broad bean, 44.1% for potato, 35.1% for rapeseed, 32.1% for cotton, 28.4% for corn, 18.5% for watermelon, 11.0% for cowpea and 8.8 % for soybean.

Pandey and Singh (2000) revealed that to elucidate the nature of growth pattern in relation to yield in mungbean, 10 diverse genotypes were planted in summer and kharif at Meerut (Uttar Pradesh) under two environments, with (20 N:40 P:40 K) and without fertilizers.
Khattak*et al.* (1998) reported that a new technique was developed in which only the upper half of the floral bud of mungbean was opened to expose the stigma. Emasculation and pollination times were 17.00-19.00 and 7.00-9.00 hours, respectively. The success rate of crossing using this technique in Faisalabad, Pakistan, averaged 62.8 and 20.0% in summer and spring, respectively. Pods contained on average 6.8 seed pod^{-1} in summer and 5.0 seed pod^{-1} in spring. This high success rate is mainly due to fewer disturbances on style and ovary in the bud during emasculation.

2.12. Effect of gibberelic acid (GA_3) on the morphological characteristics

2.12.1. Plant height

The effect of GA_3 on plant height was studied in various parts of the world by various workers on a variety of crops. It was observed in most cases that GA_3 can remarkably increase plant height of' different crop species, in an experiment with cv. BARI mung 2. Uddin (1999) observed that 150 mg L^{-1} of GA_3 produced the tallest plant. In another study, Haque (2001) reported that 100 mg L^{-1} of GA_3 was more effective in stem elongation in mungbean.

In a field experiment in Maharashtra, soybean seeds were treated with 0-150 mg L^{-1} GA_3 and an increased plant height was obtained with 100 mg L^{-1} (Deotale*et al.,* 1998). Khan and Rao (1969) reported that pre-soaking of seeds and foliar spray of seedlings with 100 and 25 mg L^{-1} of GA_3 respectively, increased plant height in green gram.

Abd-El-Fattah (1997) observed that foliar spray of GA_3 increased plant height in broad bean *(Viciatfaba*L) cv. Aquadolse. In another field experiment conducted on sunflower to study the effect of foliar spray of growth regulators (20 mg L^{-1} each) in different combinations at 20 and *25* days after sowing, it was observed that IBA+ GA_3 increased plant height. Talukder and Paswan (1996) reported that all applied concentrations of GA_3 significantly increased plant height in chrysanthemum with 40 mgL^{-1} being the most effective treatment. Soaking okra seeds in aqueous solution of 50 or 100 mgL^{-1} GA_3 for 24 hours at 25^0C significantly increased plant height compared to control *(Kumer et al. 1996).* Sontakey *et al.* (1991)observed that pre-flowering spray of sesame with 100, 250 or 500 mg L^{-1} GA_3 increased plant height. In french bean *(Pliaseolus vulgaris),* the regulatory effects of GA_3 on stem or shoot elongation or, in other words, growth stimulation was observed by Endo *et al.* (1989), Chakraboriv and Sharma (1982) and Shahan (1976). An increase in concentration of GA_3 increased plant height in faba bean (Omar *et al.,* 1988; Abdul and Said, 1984).

Lee (1990) stated that soaking groundnut seeds in solutions of 50 and 100 mg L^{-1} GA_3 prior to sowing increase the length of' main stem. GA_3 spray on early maturing soybean grown in subtropical area during short days was *found* to increase stem elongation i.e. plant height (Mislevy*et al.,* 1989). Similar increase in plant height due to spray of500, 1000, 1500 or 2000 mg L^{-1} GA_3 was also found in cotton (Kagate*et al.,* 1989).

2.12.2. Seed yield

Suma *et al.* (1987) reported that the highest yield (1166 kg ha^{-1}) was produced by 150 mg L^{-1} of GA_3 in mungbean. Hoque (2001) noted that the highest yield (612 kg ha^{-1}) was produced by 50 mg L^{-1} of GA_3 in case of foliar spraying on mungbean.

Deotale*et al.* (1998) and Maske*et al.* (1998) observed that soybean seeds treated with 100 mgL^{-1} GA_3 had higher seed yield. Soaking of okra seeds in GA_3 at 150 mg L^{-1} gave the highest seed yield (Gulshan and Lal, 1997). Zayed*et al.* (1985) carried out experiments using GA_3 on capsicum plants and found that yield increased with GA_3 at 50 mg L^{-1}. Application of GA_3 on onion plants increased bulb yield (Singh *etal.*, 1983),Hore*et al.*, 1988). The highest onion yield was obtained with GA_3 at 60 and 150 mg L^{-1} (Maurya and Lal, 1987).

Khan *et al.* (1988) sprayed GA_3 with 10 M at 40, 60 or 80 DAS and found that application of GA_3 at 40 or 60 DAS increased seed yield *significantly.* Rahman *et al.* (1989) showed in a pot experiment with grass pea that foliar application of 50 mg L^{-1} of GA_3 increased seed yield. Application of GA_3 at 50 and100 mg L^{-1} on bell pepper had an increased yield (Abdul *et al.*, 1988).

CHAPTER III

MATERIALS AND METHODS

Materials required and methods followed in the experiment during the study period are presented in this chapter under following headings.

3.1. Description of experimental site

3.1.1. Location: Geographically the experimental field is located at 25^0 38' N latitude and 88^o41' E longitude at a height of 34.5 m above the mean sea level. The experiment was conducted during March 2021 to September 2021 at Bangladesh Sugarcane Research Institute (BSRI), The experimental farm of the Regional Sugarcrop Research Station, Thakurgaon, Bangladesh.

3.1.2. Soil and land: The experiment was laid out in farm field soil having good internal drainage. The Agroecological Zone belongs to the AEZ No.1, Old Himalayan Piedmont Plain. The soil is sandy loam, a member of hyper thermic Aeric Haplaquept under the order Inceptisol having only few horizons, developed under acquired moisture regime and variable temperature conditions, Agro ecological Appraisal of Bangladesh, (UNDP and FAO, 1988). According to Fertilizer Recommendation Guide (2018) general characteristics of the soil and chemical characteristics (Table 3.1) of initial composite soil sample (0-15 cm depth), which were collected on February 2021 studying the for initial status and teste of soil.

3.1.3. Climate and weather:

The maximum, minimum and mean air temperature (ºC), relative humidity (%), total rainfall (mm), sunshine (hour's $month^{-1}$) and mean monthly Pan evaporation (mm) during the experimental period are shown in (Table 3.2) The minimum temperature ranged from 17.60 to 26.60^0C, while the maximum temperature ranged from 31.70 to 34.40^0C with the mean temperature range from 24.65 to 29.99^0C. The maximum rainfall occurred in August, 2021 and that was minimum in April, 2021 and there was no rainfall in March, 2021. Higher mean humidity was recorded in the month of August, 2021 followed by July, 2021

3.1. Morphological, physical and chemical characteristics of the soil

A. Morphological description of soil

AEZ	AEZ 1 (Old Himalayan Piedmont Plain)
General soil type	Calcareous Brown Flood Plain
Parent material	Ganges River Alluvium
Soil series	Sara
Soil type	Sandy loam
Land type	High land , medium high land
Drainage	Moderate

B. Physical and chemical properties of the experimental soil

Constituents	**Value**
Physical characteristics	
Sand (2 – 0.05 mm)	60.0%
Silt (0.05 – 0.002 mm)	27.0%
Clay (< 0.002 mm)	13.0%
Textural class	Sandy loam
Chemical characteristics	
pH (Soil : Water = 1 : 2.5)	5.65
Organic carbon (%)	1.10
Total N (%)	0.07
Available P (mg kg^{-1})	37.56
Available S (mg kg^{-1})	18.42
Exchangeable K (meq 100 g^{-1} soil)	0.21
Available Zn (mg kg^{-1})	1.93
Available S(mg kg^{-1})	18.42
Available B(mg kg^{-1})	0.15
Available Mg(mg kg^{-1})	0.48

Fertilizer Recommendation Guide, (2018)

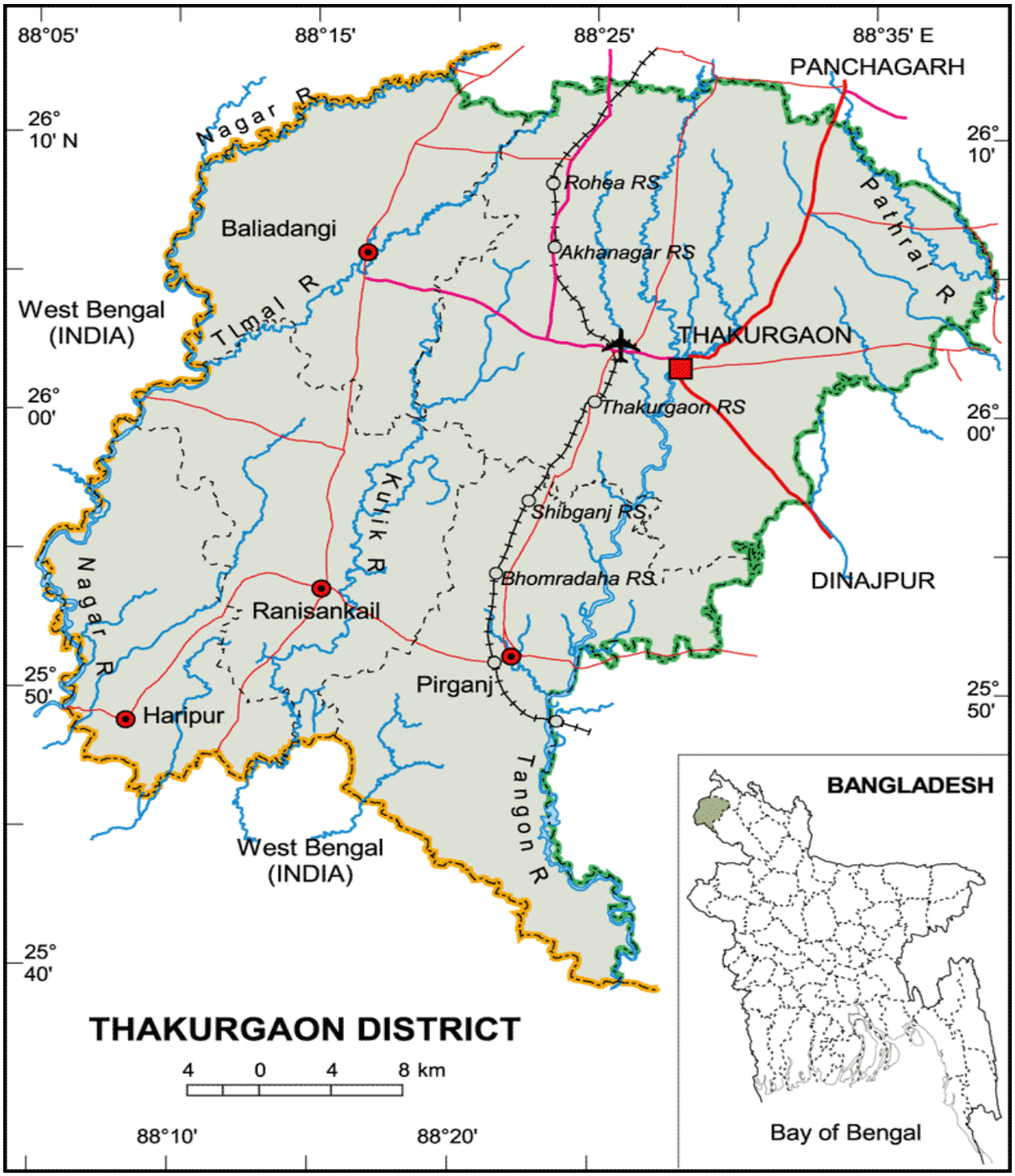

Figure 3.1. Map of Thakurgaon district showing the research conducting area

and September, 2021 and lowest in April 2021. Highest sunshine hour month^{-1} (7.48hrs) was recorded in the month of April, 2021, while the lowest in (3.6hrs) was in the month of August and September, 2021. Highest mean monthly Pan Evaporation (4.73 mm) was recorded in the month of April, 2021 and the lowest in (2.81 mm) was in the month of August, 2021.

Table 3.2. Meteorological data of the experimental period (March to September 2021) at Regional Sugarcrop Research Station, Thakurgaon, BSRI, Bangladesh

Month	Year	** Air temperature (°C)			** Relative Humidity (%)	* Total Rainfall month^{-1} (mm)	Sunshine hours month^{-1}	Mean monthly Pan evaporation (mm)
		Maximum	Minimum	Average				
March	2021	31.7	17.6	24.65	70.4	00	7.42	4.22
April	2021	34.4	20.8	27.6	67.25	19.2	7.48	4.73
May	2021	32.1	23.4	27.75	78.67	337.6	4.87	3.78
June	2021	33.1	25.5	29.8	81.83	246.4	4.91	3.73
July	2021	33.3	26.6	29.95	82.88	181	4.5	3.08
August	2021	33.20	26.56	29.88	86.83	407	3.6	2.81
September	2021	33.96	26.02	29.99	82.23	144	4.07	3.72

* Monthly total, ** Monthly average

Source: Regional Sugarcrop Research Station, Thakurgaon, Bangladesh Sugarcane Research Institute (BSRI).

3.2. Test crop

The test crop was stevia. The stevia seedlings (Plate 1) were collected from Bangladesh Stevia and Food Industries Limited, Dhaka and Bangladesh Sugarcane Research Institute, Regional Sugarcrop Research Station, Thakurgaon, Bangladesh. The seedlings were healthy and vigorous in growth 10-12 cm in height 30 days old.

3.3. Experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD). and There were two factor under the study. factor A different level of lime. L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}. factor B different doses of Gibberellic acid denoted as GA_3 and shown as 'H'. H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm.

The layout of the experiment was prepared for distributing the treatment combinations in each plot of each block. There were 90 plots in total. The unit plot size was 2m×2m. Row to row distance 40 cm, plot to plot distance 50 cm and plant to plant distance 40cm.

Fig.3.2 Stevia seedling

3.4. Land preparation

The land was prepared by using tractor plough and harrow. To achieve good tilth, about 20 cm deep trenches were made Urea, TSP and MOP, were applied @ 152, 50 and 40 kg ha^{-1}, respectively. Full quantity of TSP and one-third of MOP were applied and mixed with soil prior to transplanting of settlings. One-third of urea was applied at 21 days after transplanting (DAT). The second dose of (1/3rd) Urea and 1/3rd MOP were applied as first top dressing at 63 DAT. Similarly final dose of Urea and MOP were applied as top dressing at 105 DAT.

3.5. Field preparation

The land was opened by a tractor drawn disc plough and final preparation was made by ploughing and cross-ploughing with a tractor plough and harrow followed by laddering. The layout of the field was made 7 March 2021 after final land preparation.

Total TSP were applied as basal dose during final soil preparation. The basal dose of urea (1/3 rd) was applied as side dressing at 21 days after transplanting. The rest amount of urea and MOP were applied as top dressing in two equal splits at 63 and 105 DAT.

3.6. Application of lime

Dolomite ($CaCO_3.MgCO_3$) was used as agricultural lime. It was applied during final land preparation and was incorporated with soil with the help of a spade. Five doses of limes were applied. These are respectively control (without lime), 0.5 t ha^{-1}, 1.0 t ha^{-1}, 1.5 t ha^{-1}, and 2.0 t ha^{-1}.and was denoted as L_0, L_1, L_2, L_3, L_4, and L_5, respectively. Each dose was multiplied three times with the experimental field. Each variety was grown without any lime application for comparison with the yields of those plants that were cultivated with lime.

3.7. Application of GA_3

Gibberellic acid spray was taken up at the concentration of control (without GA_3), 150, 200, 250, 300 and 350 ppm denoted as H_1, H_2, H_3, H_4, H_5, and H_6 and sprayed at monthly intervals of 15 days after transplanting up to 90 days. The crop was harvested after 6 months of transplanting.

3.8. Intercultural operations

Intercultural operations like weeding, irrigation drainage etc. was done as and when necessary considering the present situation of the field. On an average weeding was done 15–20 days interval and flooding irrigation was applied at 7–10 days intervals considering rainfall. Insects and pest infestation of stevia plant in this period was trace.

3.8.1. Gap filling

Dead seedlings were replaced by fresh seedlings within 15 days after transplanting.

3.8.2. Harvest

The crop was harvested 147 days after transplanting when it attained maturity. The vegetative part of the plant especially laves were plucked carefully and washed briefly to removed soils and other foreign materials. The fresh leaves were than weighted as plant^{-1}.

3.9. Collection of experimental data

Data were recorded on the following parameters

1. Plant height (cm)
2. Number of leaves plant^{-1}
3. Leaf area plant^{-1} (cm^2)
4. Number of primary branch plant^{-1}
5. Number of secondary branch plant^{-1}
6. Fresh weight plant^{-1} (g)
7. Dry weight plant^{-1} (g)
8. Fresh leaf yield plant^{-1} (g)
9. Dry leaf yield plant^{-1} (g)
10. Nutrients content in stevia leaves (N, P, K, S, Ca, Mg, and Zn%)
11. Initial soil analysis

3.10. Procedure of data collection

3.10.1. Plant height

Plant height was measured from the base of the plant (ground level) to the tip of the upper most leaf and was expressed in cm. It was done just before harvesting.

3.10.2. Number of leaves

Number of leaves plant^{-1} was counted by hand counting and recorded it.

3.10.3. Leaf area

Leaf area of all separated leaves from each plant cm^2 was measured with the help of leaf area meter. It was performed soon after harvesting to avoid curling of the leaves.

3.10.4. Number of branches (Primery and Secondary)

The number of branches plant^{-1} was counted by hand counting and recorded it. It was performed at the time of height measurement.

3.10.5. Fresh weight of plant

Weighting was done just after harvesting the total plant.

3.10.6. Dry weight of plant

Total plant dry weight was obtained after sun and oven drying.

3.10.7. Fresh leaf yield

Weighting was done just after harvesting the total leaves.

3.10.8. Dry leaf yield

Leaf dry weight was obtained after sun and oven drying.

3.11. Analysis of soil sample

3.11.1. Preparation of soil sample

The collected soil a sample were composite and was air-dried, ground and sieved through a 2-mm meshsieve and analyzed for soil texture, soil pH, organic carbon, CEC, total N, available S, P, exchangeable K and Zn.

3.11.2. Particle size analysis

Particle size analysis of the collected soils samples was done by hydrometer method (Black, 1965) and textural classes were identified by plotting the values for % sand, % silt and % clay to the "Marshall's Triangular Coordinate" following the USDA system.

3.11.3. Soil pH

Soil pH was measured using a glass electrode pH meter (WTW pH 522) at a soil-water ratio of 1:2.5 as described by Ghosh *et al.*, 1983. Twenty grams of air dried soil was taken in a plastic container and 50 mL of distilled water was added to it. The suspension was stirred well several times and allowed to stand for about an hour. Then the electrode was immersed into the partly settled soil suspension and pH was measured.

3.11.4. Organic carbon

Organic carbon in soil was determined volumetrically by wet oxidation method of Walkley and Black (1975). The organic matter content was calculated by multiplying the percent organic carbon by 1.73 (Van Bemmelen factor).

3.11.5. Cation exchange capacity

Cation exchange capacity of the soil was determined by sodium saturation method. The sample was saturated with 1 N NaOAc solution followed by replacing Na^+ from the saturated samples by 1 N NaOAc at pH 7.0. The amount of Na^+ in the solution was then determined by flame photometer.

3.11.6. Available phosphorus

Available soil P was extracted with 0.5 M $NaHCO_3$ at a pH 8.5. The P in the extract was determined by $SnCl_2$ method. The intensity of blue color of molybdophosphate blue complex was measured with the help of spectrophotometer (Supertonic® GENESYS ^{TM} 5 336001 CAT) set at 660 nm (Olsen *et al.,* 1954).

3.11.7. Exchangeable potassium content

Exchangeable K was extracted with 1 N NH_4OAc solution. Then K was determined directly with the help of flame emission spectrophotometer (Jenway PFP 7) using specific standard.

3.11.8. Available sulphur

Sulphur was determined by turbidimetric method with the help of a spectrophotometer (Wolf, 1982). $CaCl_2$ solution (0.15%) was used as soil extractant. Twenty gram soil was taken in a 250 ml conical flask and 40 mL $CaCl_2$ solution was added. After 30 minutes shaking, the contents were filtered through filtered through filter paper. About 10 mL extract was taken in tube, 1 mL acid seed solution was added and 0.5 g $BaCl_2$ $2H_2O$ was added and mixed thoroughly. The intensity of colors was read in a spectrophotometer at 420 nm wave length after 20 minutes.

3.12. Plant analyses

The collected plant sample from each plot pot^{-1} was dried in and at 60^0C for about 48 hours and they were ground to pass through a 20-mesh sieve in a grinding mill. The prepared sampled were then put into paper bags and kept in desiccators until use.

3.12.1. Nitrogen determination

The estimation of N was done by Micro- kjeldahl method (Bremner and Mulvaney, 1982), which depends on the fact that organic N, when digested with concentrated sulphuric acid was

converted into ammonium sulphate. Ammonia liberated by making the solution alkaline was distilled into a known volume of standard boric acid, which is then back titrated.

Reagents

- Mixed indicator: 0.099 g of bromocresol green and 0.065 g of methyl red was dissolved in 100 mL of ethanol (rectified spirit).
- Boric acid (H_3BO_3) indicator solution:20g of boric acid was dissolved to make to volume 1 L with distilled water
- Forty per cent 1N sodium hydroxide (NaOH) solution: 40 g of NaOH was dissolved in distilled water to make the volume 1 L.
- 0.1N Concentrate sulphuric acid (H_2SO_4)
- Catalyst mixture : $CuSO_4.5H_2O$: $K2SO_4$: Se = 1: 5: 0.05

Procedures

The method consists of the following steps :

- Digestion of the sample
- Distillation and
- Titration

Exactly 0.5 g oven dried ground sample was wrapped in a piece of qualitative filter paper and dropped as a package into an 500 mL kjeldahl flask in presence of 5 g potassium sulphate, 1 g copper sulphate and 15 mL concentrated H_2SO_4 and 2 glass beads in the digestion flash tube.The sample mixture was heated at 390^0C for an hour. After the completion of digestion the flask was cooled at room temperature and added about 25 mL of distilled water. Then the flask was swirled to bring any insoluble material into the solution and it was made volume to 100 mL. For performing distillation 10 mL of the digested solution was taken in a distillation unit with 10 mL of 40% NaOH. The distillate was collected in 25 mL 2% boric acid containing mixed indicator to adjust the pH at 5.0 and was titrated against 0.1N sulphuric acid. A blank titration was simultaneously to avoid the N either already present in chemicals or atmospheric nitrogen absorbed during digestion. The percentage was calculated by the following formula with the help of titration value:

$\% N = (T-B) \times 0.014 \times 100 / S$

Where,

T = Sample titration (ml) value of standard H_2SO_4B = Blank titration (ml) value of standard H_2SO_4

N = Strength of H_2SO_4S = Sample weight (g)

3.12.2. Preparation of leaf sample for determination of different nutrient elements

Exactly 1 g of finely ground leaves were taken into a 250 mL conical flask and 10 mL of di-acid mixture (HNO_3:$HCIO_4$ = 2:1) was added to it. Then it was placed on an electric hot plate for heating at 180-200^{0}C until the solid particles disappeared and white fumes were evolved from the flask. Then it was cooled at room temperature, washed with distilled water and filtered into 100 mL volumetric flask through filter paper Whatman No. 1 making the volume up to the mark with distilled water following wet oxidation method as described by Jackson (1973). The solution was used for the analysis of P, K, Ca, Mg, Zn, B, Cu and Na.

3.12.3. Phosphorus content

Phosphorus was determined colorimetrically by stannous chloride method. Stannous chloride ($SnCl_2$. H_2O) was used as a reducing agent to form molybdophosphoric blue complex with sulphomolybdate. Exactly 10 mL aliquot was poured in a 50 mL volumetric flask followed by the addition of 10 mL of sulphomolybdic acid and and 2 mL of stannous chloride solution. The volume was made up to the mark with distilled water and was shaken thoroughly. Finally the intensity of blue color was measured with the help of a spectrophotometer (Supertonic® GENESYS ^{TM} 5 336001 CAT) at 660 nm within 15 minutes after the addition of stannous chloride reagent (Jackson, 1973).

3.12.4. Potassium content

Potassium content of the leaf sample was determined from the previously prepared aliquet directely by flame photometer and the intensity of light emitted by potassium at 768 nm wave lengths was measured by Jackson (1973).

3.12.5. Sulphur content

Sulphur content was determined turbidimetrically as $BaSO_4$ from the prepared leaf sample with the help of a spectrophotometer (Supertonic® GENESYS ^{TM} 5 336001 CAT). Turbidity was developed by using barium chloride ($BaCl_2$. $2H_2O$) and the solution was transferred to a spectrophotometer tube. The reading was taken in spectrophotometer at 420 nm incident light within 2 to 8 minutes as described by Black (1965).

3.12.6. Calcium and Magnesium contents

Five ml leaf sample was transferred into 50 mL volumetric flask using a pipette and 5 mL of $LaCl_3$ solution was added. The volume was made up to the mark with distilled water and was shaken thoroughly. Then the contents of Ca and Mg were measured by Atomic Absorption Spectrometer (AAS).

3.12.7. Zinc content

From the leaf extract, Zn content was directly analyzed by atomic absorption spectrophotometer.

3.12.8. Determination of Boron

Soil extraction

The nitrogen digester was turn on and adjusted it to 150 ^{0}C. When the digester heated to that temperature by the time the samples were ready for digestion. 40 clean and dry digestion tubes were placed in the digestion rack. 7.50 g soil was taken into 38 tubes. The 2 remaining tubes served as blanks. Then 15 mL 0.01 M $CaCl_2$ was added to each tube including the blanks. The rack with the tubes was put beside the digester and placed a glass stopper in each tube. Then the tubes were placed in the digester, which has already been heated to 150 ^{0}C. Reducing the temperature setting to 110 ^{0}C and boiled for exactly 5 minutes from the time when boiling started. Then turned off the digester. Immediately the rack was removed with the tubes from the digester and placed the tubes in a vessel with cold water for 15 minutes. Then it was filtered on a dry filter into a dry plastic bottle.

Determination

Two mL of the filtrate was transferred into another dry plastic bottle. Four mL acetate buffer solution was added and mixed with 4 mL Azomethine-H reagent. After 30 minutes, the absorbance was measured at 420 nm on a spectrophotometer (Model: AA 6300).

3.13. Statistical analysis

Fisher's analysis of variance was used for statistically analysis of collected data and for comparison of differences among treatment means; a least significant difference (LSD) test was used at 5% probability (Steel et al., 1996). Statistics 10 (Tallahassee, FL 32317) was used for the determination of statistical difference.

CHAPTER IV

RESULT AND DISCUSSION

The results of the study on the effect of lime and GA_3 on growth, yeild and nutrient content of stevia and its interaction effects have been presented according to the following headings and sub headings. This chapter contains results and discussion on the basis of data presented in tables and figures. Results were presented on each data of growth, yield and nutrient elements content parameters is stevia leaves.

4.1. Plant height

The plant height of stevia was significantly influenced by different level of lime appilication. The plant height increased up L_3 and then it gradually decreased (Table 4.1 and Appendix I). The highest plant heights (23.50, 35.65, 47.22, 59.87, 70.47, 82.51 and 96.03 cm) was found in L_3 (1.0 t ha^{-1}) treatment at 21 DAT to 147 DAT, respectively. The lowest plant height ere observed in 22.41, 34.55, 45.88, 58.33, 68.79, 81.35 and 93.48 cm at 21 DAT to 147 DAT, in L_1 (control) treatment at 21 DAT 147 DAT, respectively. The results are in agreement with the findings of Hawke (2003) who observed that the mature stevia plant height ranged 65-180 cm when cultivated in field condition. Zaman *et al.,* (2015) found that significantly influenced grown in stevia at different soil type of Bangladesh and reported that stevia plant height varies from 75.33 to 91.33 cm. Noor-E-Ferdous *et al.,* 2021 reported that mature stevia plant height was 112.31 cm when cultivated in field condition.

Plant height was significantly influence by the application of different concentration of GA_3 alone at all growth stage of stevia (Table 4.1 and Appendix I). Applying H_5 (300 ppm GA_3) had the significant effect in increasing height at 21DAT, 42 DAT, 63 DAT, 84 DAT, 105 DAT, 126 DAT and 147 DAT the highest plant height (26.58, 38.10, 51.04, 62.88, 74.99, 89.04 and 97.77 cm), respectively in (H_5) treatment and the lowest was in control (H_1) treatment. It was further noted that plant height increased with the increase in levels of GA_3 upto H_5 though thereafter it decreased at H_6 but the result was statistically similar.

Table 4.1. Effect of lime and GA_3 on plant height of stevia and their interaction

Treatments	Plant height (cm)						
Lime	21DAT	42DAT	63DAT	84DAT	105DAT	126DAT	147DAT
L_1	22.41b	34.55ab	45.88b	58.33ab	68.79c	81.35ab	93.48ab
L_2	22.72b	34.99ab	46.44b	59.08a	69.77ab	81.70ab	94.54ab
L_3	23.50a	35.65a	47.22a	59.87a	70.47a	82.51a	96.03 a
L_4	22.76ab	35.09a	46.33b	59.20a	69.48bc	81.85ab	94.64ab
L_5	22.43b	34.59ab	46.30b	58.59ab	69.49bc	82.80a	93.73ab
LSD (0.05)	0.769	1.770	0.719	1.562	0.933	3.671	3.798
GA_3	-	-	-	-	-	-	-
H_1	18.45e	30.90c	41.14f	53.66e	63.15f	72.05d	90.21c
H_2	20.09d	32.60c	42.90e	56.73d	65.42e	76.11c	92.43bc
H_3	22.81c	34.60b	45.50d	58.81c	68.55d	80.86b	94.35abc
H_4	24.27b	36.62a	48.62c	60.98b	72.17c	84.87b	95.93ab
H_5	26.58a	38.10a	51.04a	62.88a	74.99a	89.04a	97.77a
H_6	24.40b	37.038a	49.41b	61.02b	73.30b	89.33a	96.22ab
LSD (0.05)	0.842	1.939	0.786	1.712	1.022	4.021	4.161
Interaction	-	-	-	-	-	-	-
L_1H_1	18.27g	30.52e	40.94l	52.23k	62.52k	71.28h	88.36b
L_1H_2	19.74fg	32.46c-e	42.78jk	56.37g-j	64.24i-k	75.47gh	91.56ab
L_1H_3	22.29de	34.28a-e	44.24ij	58.54d-h	67.45f-h	80.25d-h	93.76ab
L_1H_4	23.73b-d	36.21a-c	47.76fg	60.23a-f	71.62de	84.55b-f	94.34ab
L_1H_5	26.46a	37.43ab	50.26a-d	62.37a-c	74.45a-c	89.10a-d	97.23ab
L_1H_6	23.98b-d	36.45a-c	49.32c-f	60.25a-e	72.46cd	87.46a-d	95.65ab
L_2H_1	18.30g	30.52e	40.95l	53.34jk	63.55jk	71.28h	90.75ab
L_2H_2	19.87fg	32.51c-e	42.85jk	56.75e-j	65.45h-j	75.77f-h	91.89ab
L_2H_3	22.88c-e	34.67a-e	45.76hi	58.97c-g	68.87f	81.24b-g	93.82ab
L_2H_4	24.35bc	36.35a-c	48.87d-f	61.28a-d	71.88de	84.56b-f	96.43ab
L_2H_5	26.51a	38.34a	50.78a-c	62.82ab	75.21ab	89.48a-c	98.05a
L_2H_6	24.41bc	37.54a	49.46c-f	61.31a-d	73.65a-d	87.85a-d	96.32ab
L_3H_1	19.04g	31.65de	41.95kl	55.14h-k	64.57i-k	73.23gh	91.37ab
L_3H_2	21.18ef	33.12b-e	43.27jk	57.31e-i	66.43g-i	76.86e-h	94.87ab
L_3H_3	23.53b-d	35.27a-d	46.96gh	59.45b-g	69.67ef	81.74b-g	96.46ab
L_3H_4	25.11ab	37.63a	49.98a-e	61.87a-d	72.44cd	85.12b-e	97.65ab
L_3H_5	26.94a	38.50a	51.63a	63.56a	75.85a	89.89ab	98.72a
L_3H_6	25.21ab	37.72a	49.53b-e	61.92a-d	73.86a-d	88.22a-d	97.12ab
L_4H_1	18.35g	31.28de	40.94l	54.37i-k	62.56k	73.16gh	90.84ab
L_4H_2	19.92fg	32.45c-e	43.26jk	56.81e-j	65.64h-j	76.23e-h	92.28ab
L_4H_3	23.03c-e	34.56a-e	45.13i	58.57c-h	68.38fg	80.86c-g	93.97ab
L_4H_4	24.38bc	36.72a-c	48.24e-g	61.29a-d	72.67cd	85.05b-e	96.88ab
L_4H_5	26.52a	38.35a	51.28ab	62.84ab	74.35a-c	88.37a-d	97.53ab
L_4H_6	24.39bc	37.16ab	49.11c-f	61.33a-d	73.26b-d	87.43a-d	96.34ab
L_5H_1	18.28g	30.51e	40.93l	53.24jk	62.56k	71.30h	89.71ab
L_5H_2	19.75fg	32.45c-e	42.34kl	56.41f-j	65.34h-j	76.22e-h	91.57ab
L_5H_3	22.32de	34.17a-e	45.42hi	58.54d-h	68.38fg	80.22d-h	93.76ab
L_5H_4	23.76b-d	36.20a-c	48.24e-g	60.23a-f	72.23cd	85.07b-e	94.34ab
L_5H_5	26.48a	37.87a	51.23ab	62.83ab	75.13ab	88.34a-d	97.32ab
L_5H_6	23.99b-d	36.32a-c	49.65b-e	60.27a-e	73.29b-d	86.57a	95.68ab
LSD (0.05)	1.884	4.335	1.758	3.827	2.286	8.991	9.304

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT.
NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

The intraction effect of lime and different concentration of GA_3 was on plant height stevia also ststisticaly significant at all growth stages (Table 4.1 and Appendix I). The highest plant heights (26.94, 38.50, 51.63, 63.56, 75.85, 89.89 and 98.72 cm) were found in L_3H_5 (lime 1.0 t ha^{-1} × GA_3 300 ppm) treatment. The lowest plant heights (18.27, 30.52, 40.94, 52.23, 62.52, 71.28 and 88.36 cm) were found in L_1H_1 treatment at all growth stages. It was further noticed that is all levels of lime and GA_3 at H_5 level produced highest plant height is whatever might be level of lime. lime continued to exert its effect upto H_5 level (300 ppm).

4.2. Number of leaves plant^{-1}

A significant variation was observed in number of leaves plant^{-1} among the different level of lime application (Table 4.2 and Appendix II). Lime140 kg ha^{-1} L_3 (1.0 t ha^{-1}) had the highest number of leaves plant^{-1}*i.e.* 17.45, 45.20, 210.98, 500.60, 742.56, 933.03 and 1090.80 found in respectively which were statistically different among other level of lime application. Significantly the lowest number of leaves plant^{-1} was observed in L_1 (control) treatment. It was reportrd that (16.12, 42.48, 205.21, 494.80, 735.17, 899.56, 1083.20) number of leaves plant^{-1} at 21 DAT 147 DAT, respectively (Table 4.2 and Appendix II). Noor-E-Ferdous *et al.,* 2021 observed that number of leaf plant^{-1} was recorded in field condition (1215.32) and the lowest number of leaf (659.73) in pot condition.

Number of leaves plant^{-1} was significantly influence by the application of different level of GA_3 application at all growth stage of stevia (Table 4.2 and Appendix II). The concentration of 300 ppm GA_3 produced significantly the highest number of leaves (17.88, 46.36, 230.70, 519.31, 813.45, 1000.40 and 1123.40) was obtained in H_5 ($GA_3$300 ppm) treatment at 21 DAT to 147 DAT, respectively. The lowest number of leaves plant^{-1} was observed in H_1 (control) treatment. Table 4.2 reported that the lowest number of leaves plant^{-1} was observed in (15.69, 40.52, 188.19, 465.09, 660.31, 830.00 and 1033.70) at 21 DAT to 147 DAT, respectively.

The intraction effect of lime and GA_3 showed significantly influenced number of leaves at all growth stages (Table 4.2 and Appendix II). From the Table 4.2, it was found that the highest number of leaves plant^{-1} (18.76, 47.56, 232.74, 520.54, 815.18, 1002.35 and 1142.64) at 21 DAT to 147 DAT, respectively was found in L_3H_5 (lime 1.0 t ha^{-1} 400 g × GA_3 300 ppm). The lowest number of leaves plant^{-1} was observed in L_1H_1 (control) treatment. (15.20, 38.54, 185.77, 462.27, 656.54, 825.76 and 1032.23) at 21 DAT to 147 DAT, respectively.

Table 4.2. Effect of lime and GA_3 on Number of leaves plant^{-1} of stevia and their interaction

Treatments	Number of leaves plant^{-1}						
Lime	21DAT	42DAT	63DAT	84DAT	105DAT	126DAT	147DAT
L_1	16.12ab	42.48b	205.21c	494.80bc	735.17c	899.56d	1083.2a
L_2	16.63ab	43.10b	207.34b	498.31a	738.46b	923.61c	1087.1a
L_3	17.45a	45.20a	210.98a	500.60a	742.56a	933.03a	1090.8a
L_4	17.13a	44.01ab	208.18b	497.87ab	737.64b	927.12b	1067.9b
L_5	16.31ab	43.44b	205.56c	493.93c	737.88b	926.67b	1058.7c
LSD (0.05)	1.342	1.537	1.125	3.289	1.903	2.450	7.758
GA_3	-	-	-	-	-	-	-
H_1	15.69c	40.52c	188.19f	465.09e	660.31f	830.0f	1033.7e
H_2	16.20bc	41.62c	193.44e	489.78d	673.79e	856.7e	1061.9d
H_3	16.80abc	43.59b	204.68d	496.83c	721.22d	912.2d	1069.3d
H_4	17.17ab	44.95ab	212.72c	506.49b	770.39c	962.3c	1094.3b
H_5	17.88a	46.36a	230.70a	519.31a	813.45a	1000.4a	1123.4a
H_6	15.69c	44.85ab	214.97b	505.11b	790.87b	970.5b	1082.6c
LSD (0.05)	1.470	1.684	1.233	3.603	2.085	2.684	8.499
Interaction	-	-	-	-	-	-	-
L_1H_1	15.20b	38.54ijk	185.77q	462.27m	656.54l	825.76l	1032.23k
L_1H_2	15.67ab	40.56ijk	191.45n	488.89kl	671.19j	852.27j	1056.82h-j
L_1H_3	16.32ab	41.98e-k	203.38l	494.32g-k	718.92h	892.20h	1082.39d-f
L_1H_4	16.03ab	42.76d-k	209.46i	501.45e-g	765.21f	912.24g	1092.47b-e
L_1H_5	17.37ab	45.38a-e	228.54b	518.39ab	812.27a	998.38a	1139.56a
L_1H_6	16.12ab	43.66b-j	212.66f-h	503.46d-f	786.87cd	916.51g	1095.48b-d
L_2H_1	15.64ab	39.57k	187.34pq	465.18m	660.28kl	828.20l	1034.29k
L_2H_2	16.06ab	40.76h-k	193.29n	490.43j-l	673.92ij	855.18j	1062.38g-i
L_2H_3	16.78ab	42.87d-k	205.36kl	498.83f-i	721.23gh	912.29g	1084.36de
L_2H_4	17.21ab	44.31a-i	212.33gh	509.54cd	770.77e	962.39e	1099.64b-d
L_2H_5	17.87ab	46.73a-c	230.47ab	520.11a	813.34a	1000.25a	1141.88a
L_2H_6	16.23ab	44.38a-h	215.23d-f	505.75c-f	791.20bc	983.37c	1100.22b-d
L_3H_1	16.23ab	41.46f-k	190.76no	468.58m	662.28k	835.26k	1035.17k
L_3H_2	16.77ab	43.22c-k	197.57m	494.24g-k	676.44i	863.66i	1065.25f-h
L_3H_3	17.14ab	45.36a-e	208.85ij	500.37e-h	725.54g	922.91f	1091.52b-e
L_3H_4	17.87ab	47.17ab	217.76cd	511.63bc	783.73d	982.76cd	1103.46bc
L_3H_5	18.76a	47.56a	232.74a	520.54a	815.18a	1002.35a	1142.64a
L_3H_6	17.92ab	46.42a-d	218.21c	508.24c-e	792.17b	991.22b	1106.51b
L_4H_1	16.03ab	40.67h-k	188.43o-q	465.87m	660.73kl	830.63kl	1034.12k
L_4H_2	16.65ab	42.27e-k	193.45n	491.42i-l	674.17ij	856.92j	1063.46f-i
L_4H_3	17.29ab	43.86a-j	206.56jk	498.29f-j	719.92h	915.83g	1045.64i-k
L_4H_4	17.77ab	45.74a-e	213.7e-g	508.13c-e	766.27ef	976.98d	1089.65b-e
L_4H_5	18.20ab	46.88abc	231.38a	519.28ab	813.19a	1000.54a	1098.24b-d
L_4H_6	16.87ab	44.65a-g	215.53c-e	504.26c-f	791.54b	981.83cd	1076.23e-g
L_5H_1	15.34b	40.32jk	188.67op	463.53m	661.74k	830.29kl	1032.46k
L_5H_2	15.86ab	41.28g-k	191.46n	483.93l	673.24ij	855.28j	1061.74g-i
L_5H_3	16.45ab	43.87a-j	199.27m	492.32h-k	720.51h	917.63fg	1042.35jk
L_5H_4	16.98ab	44.76a-g	210.32hi	501.72d-g	765.95f	976.90d	1086.46c-e
L_5H_5	17.20ab	45.27a-e	230.39ab	518.25ab	813.28a	1000.56a	1094.65b-e
L_5H_6	16.02ab	45.13a-f	213.24e-g	503.83c-f	792.57b	979.35cd	1034.63k
LSD (0.05)	3.288	3.765	2.756	8.057	4.662	6.002	19.003

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT.
NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

4.3. Leaf area plant^{-1} of stevia

Effects of different level of lime application had significantly effect on leaf area plant $^{-1}$ of stevia. The leaf area plant of stevia increased significantly upto L_3 then it reduced to some extents (Table 4.3 and Appendix III). The highest leaf area plant $^{-1}$ (57.15, 729.58, 1104. 40, 2336.90, 3142.00, 3696.90 and 4083.30 cm^2) was observed in found in L_3 (1.0 t ha^{-1}) at 21 DAT to 147 DAT, respectively. The lowest leaf area plant $^{-1}$ (53.60, 703.82, 1086.10, 2256.20, 3019.60, 3648.80 and 4045.70 cm^2) was observed in L_1 (control) treatment in all DATs studied.

Leaf area plant^{-1} of stevia was significantly influence by the application of different levels of GA_3 (Table 4.3 and Appendix III). The leaf area plant^{-1} increased up to (H_5) as level of GA_3 increased the concentration of 300 ppm GA_3. The highest leaf area plant^{-1} (57.38, 809.62, 1220.20, 2501.20, 3321.40, 3817.70 and 4206.50cm^2) was found in H_3 (GA_3 300 ppm) at all study dates, respectively. The lowest leaf area plant^{-1} (52.48, 652.54, 989.50, 2088.30, 2846.90, 3519.80 and 3925.10cm^2) was found in H_1 (control) treatment at 21DAT to 147 DAT, respectively. It was further noted that at highest level of GA_3 (H_6). The leaf area plant^{-1} declined.

A significant variation was found in leaf area plant^{-1} from 21DAT to 147 DAT due to the intraction effect of lime and different concentration of GA_3 (Table 4.3 and Appendix III). at all concentration the highest leaf area plant^{-1} (60.45, 817.10, 1230.73, 2505.79, 3335.15, 3830.71 and 4215.76 cm^2) was found in L_3H_5 treatment (lime 1.0 t ha^{-1} × GA_3 300 ppm). The lowest leaf area plant $^{-1}$ (51.87, 648.86, 982.36, 2086.65, 2844.59, 3516.42 and 3921.40 cm^{-2}) was found in L_1H_1 treatment at all growth stages. Noor-E-Ferdoud et al., (2020) reported that the highest number of leaf was recorded in field cultivation (1215.32) and the lowest number of leaf in pot cultivation (659.73). Zaman *et al.*, (2015) reported that the area of total leaves plant^{-1} was significantly affected by different soil types. Maximum leaf area (2010 cm^2 plant^{-1}) was measured from the plant grown in non-calcareous soil which was statistically identical with the leaf area of the plants grown in acid (1865 cm^2 plant^{-1}) and calcareous (1555 cm^2 plant^{-1}) soils.

Table 4.3. Effect of lime and GA_3on Leaf area plant^{-1} of stevia and their interaction

Treatments	Leaf area plant^{-1} (cm^2)						
Lime	21DAT	42DAT	63DAT	84DAT	105DAT	126DAT	147DAT
L_1	53.60b	703.82b	1086.1b	2256.2c	3019.6d	3648.8c	4045.7b
L_2	54.93b	723.47a	1095.0ab	2282.9b	3077.3b	3653.3bc	4054.5b
L_3	57.15a	729.58a	1104.4a	2336.9a	3142.0a	3696.9a	4083.3a
L_4	55.61ab	725.12a	1097.1ab	2288.6b	3074.2b	3656.8b	4058.5ab
L_5	53.53b	707.47b	1071.6c	2263.8c	3026.4c	3650.4c	4048.2b
LSD (0.05)	2.174	10.193	13.537	11.509	5.078	6.174	25.049
GA_3	-	-	-	-	-	-	-
H_1	52.48d	652.54f	989.5f	2088.3e	2846.9f	3519.8f	3925.1e
H_2	53.10cd	679.20e	1019.1e	2130.6d	2951.1e	3585.5e	3989.1d
H_3	54.99bc	698.20d	1051.3d	2299.3c	3009.8d	3638.2d	4027.2c
H_4	55.75ab	748.67b	1113.5c	2383.6b	3113.0c	3709.4b	4097.3b
H_5	57.38a	809.62a	1220.2a	2501.2a	3321.4a	3817.7a	4206.5a
H_6	56.09ab	719.14c	1151.6b	2311.2c	3165.3b	3696.8c	4103.0b
LSD (0.05)	2.381	11.166	14.829	12.607	5.563	6.763	27.439
Interaction	-	-	-	-	-	-	-
L_1H_1	51.87e	648.86h	982.36i	2086.6l	2844.5k	3516.42i	3921.40k
L_1H_2	52.13de	663.83gh	1006.28f-i	2117.83jk	2887.9j	3572.43h	3976.77h-k
L_1H_3	53.64c-e	684.57fg	1042.92de	2271.01fg	2924.46i	3612.28g	4012.34gh
L_1H_4	54.29b-e	713.56de	1126.72c	2346.71de	3024.25f	3686.29f	4079.30c-f
L_1H_5	55.38a-e	795.57ab	1215.20a	2497.23a	3310.34b	3812.93b	4198.65ab
L_1H_6	54.30b-e	716.54d	1143.18bc	2217.47h	3125.93e	3692.54ef	4085.45c-e
L_2H_1	52.18de	651.43h	991.72hi	2087.46l	2846.32k	3520.12i	3926.66ijk
L_2H_2	53.26c-e	687.82fg	1023.16e-h	2122.65j	2972.49g	3581.32h	3983.47h-j
L_2H_3	54.82b-e	704.63d-f	1052.73de	2277.13f	3017.38f	3615.63g	4022.77f-h
L_2H_4	55.46a-e	765.64c	1131.20bc	2367.36b-e	3126.84e	3691.26ef	4093.44cd
L_2H_5	57.29a-d	812.83a	1218.33a	2501.64a	3323.78a	3814.54b	4207.46a
L_2H_6	56.55a-e	718.45d	1152.71bc	2341.32e	3176.73d	3696.71ef	4093.32cd
L_3H_1	53.82b-e	658.82h	995.15g-i	2091.38kl	2850.18k	3523.15i	3931.22i-k
L_3H_2	54.37b-e	691.41ef	1031.22d-f	2175.82i	3019.34f	3612.18g	4022.32f-h
L_3H_3	56.92a-e	708.34d-f	1061.73d	2391.02b	3127.67e	3728.29d	4056.76d-g
L_3H_4	58.22a-c	778.66bc	1146.32bc	2482.64a	3258.22c	3784.51c	4135.44c
L_3H_5	60.45a	817.10a	1230.73a	2505.79a	3335.15a	3830.71a	4215.76a
L_3H_6	59.10ab	723.16d	1161.55b	2374.92bc	3261.55c	3702.32e	4138.54bc
L_4H_1	52.62de	652.88h	992.83hi	2088.56l	2847.62k	3521.12i	3923.66i-k
L_4H_2	53.54c-e	687.23fg	1026.37e-g	2112.84j-l	2982.53g	3585.43h	3984.23hi
L_4H_3	55.81a-e	706.12d-f	1053.32de	2279.03f	3027.81f	3620.35g	4027.66e-h
L_4H_4	56.54a-e	769.22c	1134.34bc	2372.36b-d	3128.11e	3696.56ef	4096.11cd
L_4H_5	58.24a-c	815.38a	1220.45a	2502.63a	3326.67a	3818.55ab	4209.38a
L_4H_6	56.92a-e	719.91d	1155.53bc	2376.34bc	3132.71e	3698.91ef	4110.23cd
L_5H_1	51.87e	650.71h	985.56i	2087.23l	2845.88k	3518.23i	3922.34jk
L_5H_2	52.13de	665.70gh	1008.22f-i	2123.83j	2893.27j	3576.21h	3978.76h-k
L_5H_3	53.64c-e	687.33fg	1045.66de	2278.41f	2951.43h	3614.56g	4016.54gh
L_5H_4	54.29b-e	716.25de	1028.91d-f	2348.78c-e	3027.35f	3688.22ef	4082.45c-f
L_5H_5	55.38a-e	807.21a	1216.46a	2498.75a	3310.87b	3811.71b	4201.27a
L_5H_6	54.30c-e	717.63d	1144.80cb	2245.89g	3129.46e	3693.67ef	4087.56c-e
LSD (0.05)	5.3246	24.967	33.160	28.191	12.439	15.123	61.356

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT.
NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.
L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

4.4. Number of primary branches plant^{-1} of stevia

Significantly difference was observed on number of primary branch plant^{-1} of stevia among different level of lime application (Table 4.4 and Appendix IV) from 63 to 147 DATS. It was observed that highest number of primary branch plant^{-1} (7.60, 8.71, 9.91, 10.67 and 12.02 found in) was found in L_3 (1.0 t ha^{-1}) at 63 DAT to 147 DAT, except 21 DAT and 42 DAT, respectively. The lowest number of primary branch plant^{-1} (6.68, 7.84, 9.44, 10.11 and 11.05) was observed 63 DAT 147 DAT and at 21 DAT and 42 DAT, The differences were not statistically significant respectively. Noor-E-Ferdous *et al.,* 2021 observed that primary branches plant^{-1} (6.21, 7.41, 8.71, 9.98, 11.63, 12.72 at 42, 63, 84, 105, 126 and 147 DAT.

Effects of different level of GA_3 application had significantly effect on number of primary branch plant^{-1} of stevia. The number of primary branches plant^{-1} of stevia increased significantly upto L_3 then it was reduced to some extents (Table 4.4 and Appendix IV). The concentration of 300 ppm GA_3 produced the highest number of primary branch (4.63, 6.21, 7.42, 8.54, 9.86, 10.21 and 12.17) was observed in H_3 treatment at 21 DAT to 147 DAT, respectively. The lowest number of primary branch plant^{-1} (3.76, 4.97, 6.49, 7.47, 9.14, 9.98 and 10.51) was found in H_1 (control) treatment in all studied.

Number of primary branches plant^{-1} of stevia was significant different in different level lime and GA_3 application at all growth stages (Table 4.4 and Appendix IV). The highest number of primary branch plant^{-1} (4.68, 6.25, 9.53, 11.38, 12.15, 13.46 and 12.76) was found L_3H_5 treatment (lime 1.0 t ha^{-1} × GA_3 300 ppm) and lowest (3.75, 4.96, 5.02, 6.14, 9.12, 9.96 and 10.30) in L_1H_1 treatment at 21 DAT to 147 DAT, respectively.

Table 4.4. Effect of lime and GA_3 on number of primary branch plant^{-1} of stevia and their interaction

Treatments	Number of primary branch plant^{-1}						
Lime	21DAT	42DAT	63DAT	84DAT	105DAT	126DAT	147DAT
L_1	4.20	5.47	6.86ab	7.84ab	9.50a	10.11ab	11.05b
L_2	4.23	5.51	7.19a	8.16a	9.71a	10.36a	11.12b
L_3	4.27	5.63	7.60a	8.71a	9.91a	10.67a	12.02a
L_4	4.24	5.52	7.21a	8.17a	9.50ab	10.49a	11.13b
L_5	4.21	5.48	6.68ab	8.13a	9.44ab	10.12ab	11.06b
LSD (0.05)	NS	NS	0.481	0.637	0.921	0.870	0.798
GA_3	-	-					-
H_1	3.76d	4.97c	6.49ab	7.47b	9.14b	9.98b	10.51d
H_2	3.96cd	5.18bc	6.97ab	7.91b	9.26b	10.11b	10.72cd
H_3	4.18bc	5.42bc	7.28a	8.11ab	9.41b	10.13b	11.05b-d
H_4	4.40ab	5.65ab	7.33a	8.29ab	9.59b	10.16b	11.40a-c
H_5	4.63a	6.21a	7.23a	9.10a	10.66a	11.56a	12.17a
H_6	4.44ab	5.71ab	7.35a	8.33ab	9.62b	10.17b	11.79ab
LSD (0.05)	0.3430	0.572	0.865	1.026	1.009	1.281	0.875
Interaction	-	-	-	-	-	-	-
L_1H_1	3.75b	4.96b	5.02cd	6.14c	9.12b	9.96b	10.30d
L_1H_2	3.93ab	5.15ab	6.92bc	7.87bc	9.23b	10.11b	10.43b-d
L_1H_3	4.13ab	5.36ab	7.25a-c	8.02bc	9.37b	10.13b	10.78b-d
L_1H_4	4.37ab	5.57ab	7.31a-c	8.24bc	9.51b	10.15b	11.13a-d
L_1H_5	4.60a	6.17ab	7.39a-c	8.52b	10.25ab	10.19b	11.88a-d
L_1H_6	4.42ab	5.63ab	7.32a-c	8.29bc	9.54b	10.16b	11.77a-d
L_2H_1	3.76b	4.97b	6.85bc	7.79bc	9.13b	9.98b	10.31cd
L_2H_2	3.96ab	5.18ab	6.94bc	7.92bc	9.25b	10.12b	10.44b-d
L_2H_3	4.19ab	5.40ab	7.28a-c	8.11bc	9.40b	10.14b	10.80b-d
L_2H_4	4.41ab	5.61ab	7.32a-c	8.27bc	9.63b	10.16b	11.14a-d
L_2H_5	4.63a	6.22ab	7.42a-c	8.54b	11.24ab	11.59ab	12.26a-c
L_2H_6	4.44ab	5.72ab	7.34a-c	8.36bc	9.66b	10.17b	11.79a-d
L_3H_1	3.78b	4.99ab	6.91bc	7.85bc	9.17b	10.02b	11.33a-d
L_3H_2	4.01ab	5.21ab	7.10bc	7.96bc	9.31b	10.07b	11.78a-d
L_3H_3	4.26ab	5.54ab	7.31a-c	8.24bc	9.46b	10.13b	12.07a-d
L_3H_4	4.45ab	5.87ab	7.39a-c	8.41bc	9.67b	10.17b	12.34ab
L_3H_5	4.68a	6.25a	9.53a	11.38a	12.15a	13.46a	12.76a
L_3H_6	4.49ab	5.92ab	7.40a-c	8.43bc	9.71b	10.19b	11.84a-d
L_4H_1	3.77b	4.98ab	6.87bc	7.81bc	9.15b	9.97b	10.31cd
L_4H_2	3.98ab	5.19ab	6.97bc	7.93bc	9.27b	10.13b	10.50b-d
L_4H_3	4.20ab	5.43ab	7.29a-c	8.14bc	9.43b	10.15b	10.83a-d
L_4H_4	4.43ab	5.64ab	7.34a-c	8.30bc	9.64b	10.17b	11.27a-d
L_4H_5	4.64a	6.23ab	7.43ab	8.55b	11.35b	12.38ab	12.08a-d
L_4H_6	4.46ab	5.66ab	7.36a-c	8.33bc	9.66b	10.18b	11.80a-d
L_5H_1	3.75b	4.96b	6.84bc	7.80bc	9.13b	9.97b	10.30d
L_5H_2	3.94ab	5.17ab	6.94bc	7.88bc	9.24b	10.12b	10.46b-d
L_5H_3	4.16ab	5.37ab	7.27a-c	8.06bc	9.39b	10.14b	10.80b-d
L_5H_4	4.38ab	5.60ab	7.31a-c	8.25bc	9.53b	10.15b	11.14a-d
L_5H_5	4.61a	6.19ab	4.42d	8.53b	9.84b	10.20b	11.90a-d
L_5H_6	4.43ab	5.63ab	7.33a-c	8.28bc	9.55b	10.16b	11.78a-d
LSD (0.05)	0.767	1.279	2.404	2.295	2.257	2.866	1.956

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.

L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

4.5. Number of secondary branches plant^{-1} of stevia

The number of secondary branches plant^{-1} of stevia was significantly increased gradually with advancement of the growth stage at 21, 42, 63, 84, 105, 126 and 147 DAT, respectively with increase in different levels of lime applied (Table 4.5 and Appendix V). The highest number of secondary branch plant^{-1} (10.43, 13.07, 15.28, 26.31, 30.02, 32.08 and 34.35) was found in found in L_3 (1.0 t ha^{-1}) from 21 DAT to 147 DAT, respectively, The lowest number of secondary branch plant^{-1} (9.65, 12.05, 14.37, 25.67, 28.99, 30.92 and 33.33) was observed in L_1 (control) treatment and observed in on 21, 42, 63, 84, 105, 126 and 147 days after transplanting, respectively.

Number of secondary branch plant^{-1} was significantly influenced by the application of different levels of GA_3 at all growth stages of stevia (Table 4.5 and Appendix V). produced the highest number of secondary branch (11.19, 13.73, 15.95, 27.37, 30.55, 32.81 and 35.71) in H_5 treatment at all growth stages (21 DAT to 147 DAT) respectively. The lowest number of secondary branched (8.89, 11.46, 13.94, 24.82, 28.30, 30.34 and 31.26) was reported from H_1 (control) with no GA_3 application at all the growth stages studied (21, 42, 63, 84, 105, 126 and 147 days after transplanting, respectively).

The intraction effect of lime and GA_3 influenced significantly the number of secondary branch at all growth stages (Table 4.5 and Appendix V). The highest number of secondary branch plant^{-1} (11.62, 15.23, 18.36, 28.10, 32.25, 35.53 and 36.87) was found in L_3H_5 (lime 1.0 t ha^{-1} × GA_3 300 ppm). The lowest number of secondary branch (8.87, 9.81, 13.07, 23.16, 27.15, 28.52 and 31.25) was observed in L_1H_1 treatment at all growth stages. The number of secondary branches increased proportionately with increase of GA_3 upto H_5 level in combination with all the levels of lime after that at H_6 level the number of secondary branches reduced considerabily.

Table 4.5. Effect of lime and GA_3 on secondary branch plant^{-1} of stevia and their interaction

Treatments	Number of secondary branches plant^{-1}						
Lime	21DAT	42DAT	63DAT	84DAT	105DAT	126DAT	147DAT
L_1	9.65b	12.05c	14.37b	25.67ab	28.99b	30.92b	33.33ab
L_2	9.79ab	12.56b	14.79b	26.07a	29.25ab	31.36ab	33.80ab
L_3	10.43a	13.07a	15.28a	26.31a	30.02a	32.08a	34.35a
L_4	9.83ab	12.59b	14.60b	26.09a	29.26ab	31.40ab	33.80ab
L_5	9.67b	12.41bc	14.54b	26.03a	29.20ab	31.31ab	33.35ab
LSD (0.05)	0.740	0.466	0.493	0.329	0.473	0.983	0.931
GA_3	-		-	-	-	-	-
H_1	8.89c	11.46d	13.94c	24.82c	28.30c	30.34b	31.26c
H_2	9.35bc	12.10c	14.35bc	25.65bc	28.85bc	30.96b	32.33c
H_3	9.66bc	12.44bc	14.51b	25.94b	29.10bc	31.21ab	33.61b
H_4	10.04b	12.75b	14.74b	26.17b	29.61ab	31.56ab	34.64ab
H_5	11.19a	13.73a	15.95a	27.37a	30.55a	32.81a	35.71a
H_6	10.13b	12.74b	14.80b	26.22b	29.66ab	31.61ab	34.80ab
LSD (0.05)	0.811	0.510	0.540	0.919	1.147	1.624	1.198
Interaction	-	-	-	-	-	-	-
L_1H_1	8.87e	9.81af	13.07d	23.16d	27.15c	28.52c	31.25f
L_1H_2	9.13de	11.96e	14.29c	25.56bc	28.67bc	30.89bc	32.22ef
L_1H_3	9.45c-e	12.28de	14.41c	25.87bc	28.94bc	31.11bc	33.18b-f
L_1H_4	9.76b-e	12.53b-e	14.67c	26.10a-c	29.51bc	31.45bc	33.73b-f
L_1H_5	10.83a-d	13.14b-d	15.01c	27.21a-c	30.10ab	32.07a-c	35.06a-c
L_1H_6	9.86a-e	12.61b-e	14.78c	26.16a-c	29.61bc	31.52bc	34.54a-e
L_2H_1	8.90e	11.86e	14.15cd	25.23c	28.41bc	30.79bc	31.26f
L_2H_2	9.16de	12.10de	14.34c	25.61bc	28.76bc	30.93bc	32.36d-f
L_2H_3	9.51c-e	12.36de	14.45c	25.92bc	28.98bc	31.21bc	33.86b-f
L_2H_4	9.93a-e	12.75b-e	14.72c	26.18a-c	29.57bc	31.57bc	34.92a-d
L_2H_5	11.25a-c	13.52bc	16.30b	27.26a-c	30.15ab	32.13a-c	35.75ab
L_2H_6	10.02a-e	12.78b-e	14.79c	26.24a-c	29.68bc	31.58bc	34.66a-e
L_3H_1	8.92 e	11.91e	14.21cd	25.26bc	29.13bc	30.84bc	31.28f
L_3H_2	10.12a-e	12.37de	14.48c	25.89bc	29.38bc	31.15bc	32.43c-f
L_3H_3	10.35a-e	12.87b-e	14.81c	26.08a-c	29.67bc	31.38bc	33.97b-e
L_3H_4	10.84 a-d	13.15b-d	14.91c	26.27a-c	29.85ab	31.76bc	35.83ab
L_3H_5	11.62 a	15.23a	18.36a	28.10a	32.25a	35.53a	36.87a
L_3H_6	10.87a-d	12.92b-e	14.95c	26.30a-c	29.89ab	31.86bc	35.72ab
L_4H_1	8.91e	11.87e	14.16cd	25.24c	28.45bc	30.81bc	31.27f
L_4H_2	9.20de	12.15de	14.36c	25.62bc	28.77bc	30.96bc	32.40c-f
L_4H_3	9.56b-e	12.41c-e	14.46c	25.94bc	28.99bc	31.25bc	33.88b-f
L_4H_4	9.94a-e	12.79b-e	14.76c	26.21a-c	29.60bc	31.58bc	34.96a-d
L_4H_5	11.34ab	13.56b	15.08c	27.31ab	30.18ab	32.24ab	35.77ab
L_4H_6	10.05a-e	12.81b-e	14.81c	26.25a-c	29.61bc	31.60bc	34.56a-e
L_5H_1	8.89e	11.85e	14.13cd	25.22c	28.40bc	30.77bc	31.25f
L_5H_2	9.14de	11.96e	14.30c	25.58bc	28.68bc	30.91bc	32.24ef
L_5H_3	9.47ce	12.30de	14.45c	25.89bc	28.96bc	31.13bc	33.19b-f
L_5H_4	9.77b-e	12.56b-e	14.68c	26.11a-c	29.52bc	31.46bc	33.76b-f
L_5H_5	10.92a-d	13.20b-d	15.03c	27.24a-c	30.11ab	32.10a-c	35.12ab
L_5H_6	9.88a-e	12.62b-e	14.70c	26.17a-c	29.54abc	31.53bc	34.55a-e
LSD (0.05)	1.813	1.141	1.209	2.056	2.565	3.633	2.678

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.

L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

4.6. Nutrient content in stevia leaf

4.6.1. Nitrogen

Nitrogen (N) content of stevia was significantly influenced by the application of different levels of lime (Table 4.6 and Appendix VI). The highest N content (1.80%) in was observed when the plot was treated with lime 1.50 t ha^{-1} (L_4). The lowest N content (1.76%) was recorded in control (L_1) plot which was significantly inferior to all the treatments (Table 4.6 and Appendix VI). The results indicated that N content of stevia was also significantly affected by different levels of GA_3. The content of nitrogen in varied from 1.58 to1.96%. The highest N content (1.96%) was observed when the plot was treated with 300 ppm GA_3 (H_5). The lowest N content (1.58%) was recorded in H_1 treatment. Nitrogen content of stevia was significantly affected by different levels of lime and GA_3 (Table 4.6 and Appendix VI). The highest N content (1.98%) was observed when the plot was treated with lime1.0 t ha^{-1}× 300 ppm GA_3 (L_3H_5). The lowest N content (1.57%) was recorded in the control plot which was significantly inferior to all treatments. Present findings agree with that of Katayama et al., (1976) who also obtained stevia plants consist of 1.4% N. Noor-E-Ferdous *et al.,* 2021 observed that the N content in stevia plant ranged from 1.62 to1.71%. It was noticed (Table 4.6) that application of lime alone increased N content in stevia upto L_4, similarity GA_3 increased N content upto the GA_3 level H_5. Both lime and GA_3 at further increased dose reduced N content.

4.6.2. Phosphorus

Table 4.6 and Appendix VI shows that phosphorus (P) content of stevia was significant influenced by the application of different levels of lime and it was hight with L_3 (0.35% P) and it was lowest (0.133%) is control treatment when no lime was added. The highest P content (0.147%) was observed when the plot was treated with only 300 ppm GA_3 (H_5) (0.147% P) and the lowest P content (0.118%) was recorded in H_1 treatment (Table 4.6 and Appendix VI). Phosphorus content of stevia was significantly influenced by combined effect of levels of lime and GA_3 (Table 4.6 and Appendix VI). The highest P content (0.148%) was observed when the plot was treated with all the plots having no GA_3 with any dose of lime. The lowest P content (0.117%) was recorded in the control plot which

was significantly inferior to all treatments. From the results it appeared that effect of GA_3 was prominent than that of lime. P content increased with augmentation of GA_3 dose.

4.6.3. Potassium

Potassium (K) content of stevia was non significantly influenced by the application of different levels of lime alone (Table 4.6 and Appendix VI). The highest K content (0.147%) in was observed when the plot was treated with lime at 1.0 t ha^{-1} (L_3). The lowest K content (0.144%) was recorded in control (L_1) plot which was significantly inferior to all treatments (Table 4.6 and Appendix VI). The results indicated that K content of stevia was also significantly influenced by different levels of GA_3. The content of potassium in varied from 0.128 to 0.160%. The highest K content (0.160%) was observed when the plot was treated with 300 ppm GA_3 (H_5) alone and the lowest K content (0.128%) was recorded in H_1 treatment. Potassium content of stevia was significantly augmented as levels of both lime and GA_3 were used (Table 4.6). It was deserved that GA_3 gradually increased the K content in stevia whatever was the level of lime and in all levels of lime GA_3 at hight level used reduced K content of stevia.

4.6.4. Sulphur

It appears from the data presented in that in different level of lime application (Table 4.6 and Appendix VI). The content of sulphur is varried from 0.260 to 0.265% in different lime treatments. Sulphur (S) content of stevia was significantly on the other hand influenced by the application of different levels of GA_3. The highest S content (0.288%) was observed when the plot was treated with 300 ppm GA_3 (H_5) alone. The lowest S content (0.230%) was recorded in H_1 treatment (Table 4.6 and Appendix VI). It was further noted that S content increased in L_1H_1, L_2H_2, L_3H_3, gradually with increases in GA_3 level upto H_5 then again it reduced. Sulphur content of stevia was significantly increased with rise in GA_3 levels and did not maintain regular pattern by different levels of lime and GA_3 (Table 4.6 and Appendix VI). The conten of S (0.29%) was observed when the plot was treated with lime 1.0 t ha^{-1} × 300 ppm GA_3 (L_3H_5). The lowest S content (0.23%) was recorded in all control plot.

4.6.5. Calcium

Table 4.6 and Appendix VI, shows that calcium (Ca) content in stevia was significantly effect due to application of different level of lime. It ranged from 1.25 to 1.31%, the highest Ca content (1.31%) in was observed when the plot was treated with lime 1.0 t ha^{-1} (L_3). The lowest Ca content (1.25%) was recorded in control (L_1) plot which was significantly inferior to all treatments and at higher level of lime Ca content again reduced. The calcium content of stevia was significantly infiuenced by different level of GA_3.

The results indicated that Ca content of stevia was also significantly influenced by different levels of GA_3. The content of calcium in varied from 1.11% to1.46% when GA_3 alone was used. The highest Ca content (1.46%) was observed when the plot was treated with 300 ppm GA_3 (H_5). The lowest Ca content (1.11%) was recorded in H_1 treatment. Calcium content of stevia was significantly influenced by different levels of lime and GA_3 together (Table 4.6). The highest Ca content (1.46%) was observed when the plot was treated with lime 1.0 t ha^{-1} × 300 ppm GA_3 (L_3H_5). The lowest Ca content (0.98%) was recorded in L_1H_1. It was further noted that lime and GA_3 had little effect on increasing calcium content of stevia. It become clear when we consider the single effect of lime and GA_3 at different levels.

4.6.6. Magnesium

Magnesium (Mg) content of stevia was significant effect influenced by the application of different levels of lime (Table 4.6and Appendix VI). The results indicated that Mg content of stevia was significantly influenced by different levels of GA_3 and it increased with the rise in level of GA_3. The Magnesium content of in varied from 0.096% to 0.135% due to different level of GA_3. The highest Mg content (0.135% was observed when the plot was treated with 300 ppm GA_3 (H_5) alone. The lowest Mg content (0.096%) was recorded in H_1 treatment.The content of Mg in stevia has also been significantly positively induced due to joint effect of lime and GA_3 (Table 4.6 and Appendix VII). further it was noted that GA_3 raised gradually the content of GA_3 highest level at H_5 and there it again reduced at further level of GA_3. The same trend was absenced when lime and GA_3 were used in combination whatever was the level of lime in with all the levels of lime and GA_3 continued increasing Mg levels.

4.6.7. Zinc

The results on zinc (Zn) content of stevia have been presented in the (Table 4.6 and Appendix VII). Zn did not have significant effect by the application of different levels of lime alone due to the application of lime alone. The content of zinc in varied from 65.91 to 67.48 μg g^{-1} but this variation was not regular pattern. The results indicated that Zn content of stevia was also significantly influenced by different levels of GA_3 (Table 4.6 and Appendix VI) and raised upto GA_3 300 ppm (H_5 level) Significantly the highest Zinc content (72.99 μg g^{-1}) was observed when the plot was treated with 300 ppm GA_3 (H_5). The lowest Zinc content (57.94 μg g^{-1}) was recorded in H_1 that is with no GA_3 application. Zinc content of stevia was significantly influenced by different levels of lime and GA_3 (Table 4.6 and Appendix VI) when considered jointly. The highest Zn content (73.07 μg g^{-1}) was obtained in (L_3H_5) while lime 1.0 t ha^{-1} × 300 ppm GA_3 applications and the lowest (57.94 μg g^{-1}) was recorted in control (L_1H_1). The different levels of lime had not significant effect on Zn content of stevia but GA_3 exerted significantly positive effect of Zn content of stevia and it increased with level of GA_3 up to H_5. GA_3 continued its effect on Zn content when it was used in combination with lime whatever might be its level. The results are in agreement with the findings of Nasrin (2008) who reported that the Zn content in stevia leaf (100.46 μg g^{-1}). Noor-E-Ferdous *et al.*, 2021 observed that Zn content in stevia leaf ranged from 55.52 to 62.87 μg g^{-1}.

Table 4.6. Effect of lime and GA_3 on N, P, K, S, Ca, Mg and Zn of stevia and their interaction

Treatments	N%	P%	K%	S%	Ca%	Mg%	Zn µg g^{-1}
L_1	1.76ab	0.133c	0.144	0.260	1.25b	0.107ab	66.60
L_2	1.78ab	0.134a-c	0.145	0.262	1.31ab	0.112a	67.48
L_3	1.79a	0.136ab	0.147	0.265	1.36a	0.117a	67.42
L_4	1.80a	0.134bc	0.147	0.263	1.30ab	0.113a	66.21
L_5	1.78ab	0.135a	0.145	0.262	1.31ab	0.109ab	65.91
LSD (0.05)	0.024	0.016	NS	NS	0.066	0.010	NS
GA_3	-	-	-	-	-		-
H_1	1.58f	0.118e	0.128e	0.230e	1.11d	0.096d	57.94e
H_2	1.68e	0.126d	0.136d	0.245d	1.23c	0.102cd	62.17d
H_3	1.76d	0.134c	0.143c	0.258c	1.30b	0.109bc	66.74c
H_4	1.84c	0.140b	0.153b	0.275b	1.36b	0.113b	69.71b
H_5	1.96a	0.147a	0.160a	0.288a	1.46a	0.135a	72.99a
H_6	1.87b	0.142b	0.155b	0.279b	1.37b	0.115b	70.80ab
LSD (0.05)	0.027	0.017	0.013	0.015	0.072	0.010	2.879
Interaction	-	-	-	-	-	-	-
L_1H_1	1.57h	0.11hi	0.12m	0.23h	0.98i	0.095c	57.94i
L_1H_2	1.68fg	0.12fg	0.13j-m	0.24f-h	1.21f-h	0.101c	62.76f-i
L_1H_3	1.73ef	0.13de	0.14h-k	0.25d-f	1.28b-h	0.108c	66.38b-h
L_1H_4	1.81cd	0.14a-c	0.15b-f	0.27a-d	1.32b-f	0.112c	69.26a-e
L_1H_5	1.95a	0.15c-e	0.16a-c	0.28a	1.40bc	0.117bc	72.96a
L_1H_6	1.85bc	0.14h	0.15a-d	0.27ab	1.35b-f	0.114c	70.35a-c
L_2H_1	1.57h	0.12ef	0.12m	0.22h	1.15gh	0.095c	57.94i
L_2H_2	1.69fg	0.13de	0.13k-m	0.24f-h	1.23d-h	0.102c	62.87e-i
L_2H_3	1.77de	0.14ab	0.14h-k	0.25d-f	1.31b-g	0.109c	67.98a-g
L_2H_4	1.84bc	0.14c-e	0.15c-f	0.27a-d	1.37b-f	0.110c	70.74a-c
L_2H_5	1.96a	0.15gh	0.16a-c	0.28a	1.42b	0.138ab	73.01a
L_2H_6	1.86bc	0.14fg	0.15a-e	0.28ab	1.38b-e	0.115bc	72.34ab
L_3H_1	1.59h	0.12b-e	0.13lm	0.23gh	1.15gh	0.095c	57.94i
L_3H_2	1.67g	0.13a	0.13h-k	0.24e-g	1.25c-h	0.104c	63.19d-i
L_3H_3	1.77de	0.13a-d	0.14e-h	0.26b-e	1.32b-f	0.112c	68.25a-f
L_3H_4	1.86bc	0.14h	0.15a-e	0.27ab	1.39b-d	0.117bc	70.96a-c
L_3H_5	1.98a	0.15fg	0.16a	0.29a	1.67a	0.157a	73.07a
L_3H_6	1.89b	0.14de	0.15a-d	0.28ab	1.39b-d	0.118bc	71.16a-c
L_4H_1	1.61h	0.12ab	0.12lm	0.23gh	1.15gh	0.098c	57.96i
L_4H_2	1.69fg	0.13b-e	0.13i-l	0.24f-h	1.22e-h	0.103c	60.38hi
L_4H_3	1.78de	0.13gh	0.14g-j	0.25c-f	1.29b-h	0.108c	66.39b-h
L_4H_4	1.87b	0.14de	0.15a-d	0.27ab	1.37b-f	0.112c	69.32a-d
L_4H_5	1.97a	0.15a-d	0.16ab	0.28a	1.43b	0.146a	72.98a
L_4H_6	1.89b	0.14ab	0.15a-d	0.28ab	1.38b-e	0.114c	70.27a-c
L_5H_1	1.58h	0.12a-d	0.12m	0.23gh	1.14hi	0.097c	57.95i
L_5H_2	1.67g	0.13j	0.13j-m	0.24f-h	1.24c-h	0.104c	61.67g-i
L_5H_3	1.76de	0.14j	0.14f-i	0.25d-f	1.32b-f	0.109c	64.74c-h
L_5H_4	1.85bc	0.14ij	0.15d-g	0.27a-c	1.36b-f	0.113c	68.29a-f
L_5H_5	1.97a	0.15ij	0.16a-c	0.28a	1.42b	0.118bc	72.96a
L_5H_6	1.88b	0.14ij	0.15b-f	0.27ab	1.38b-e	0.115bc	69.88a-c
LSD (0.05)	0.060	0.690	0.717	0.019	0.162	0.023	6.437

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

4.7. Fresh and dry weight of stevia plant

4.7. 1. Fresh wt. plant^{-1} (g)

Effects of different levels of lime application varied were significantly on fresh weight plant^{-1} of stevia (Table 4.7 and Appendix VII). The highest fresh weight plant^{-1} was observed in L_3 (138.57g) treatment and lowest was observed in L_1 (134.30g) treatment (Table 4.7 and Appendix VII) the effect of GA_3 on fresh weight plant^{-1} different siognificantly and it was highest in H_5 (150.19g) treatment and lowest in H_1 (122.58g) treatment (Table 4.7 and Appendix VII). The intraction effect of lime and GA_3 also varied significantly and the highest fresh weight plant^{-1} was observed in L_3H_5 (151.36 g) treatment and it was lowest in L_1H_1 (123.02g) treatment (Table 4.7 and Appendix VII).

4.7.2. Fresh wt. ha^{-1}(kg)

The fresh weight of stevia plant ha^{-1} varied siognificantly but it increased upto in L_3 (8660.60kg) Then it again decreased and at H_6 it was lowest 8270 kg ha^{-1} treatment (Table 4.7 and Appendix VII). The effect of the effect of GA_3 also varied significantly and highest fresh wt. Plant was observed in H_5 (9387.30kg) and lowest was observed in H_1 (7661.30 kg) (Table 4.7 and Appendix VII). The intraction effect of Lime and GA_3 showed significantly different among the doses applied. the highest fresh wt. ha^{-1} was observed in L_3H_5 (9460.31 kg) and it was lowest in N_1H_1 (7689.32 kg) treatment (Table 4.7 and Appendix VII).

4.7.3. Dry weight plant^{-1} (g)

Dry weight plant^{-1} differed significantly due to application of different level of lime and different concentration of GA_3 (Table 4.7 and Appendix VII). The dry weight plant^{-1} was observed highest in L_3 (42.58 g) treatment and it was lowest in L_1 (40.40 g) treatment (Table 4.7 and Appendix VII). due to application of the effect of GA_3 highest dry weight plant^{-1} was observed in H_5 (44.29 g) treatment and it was lowest in H_1 (37.93 g) treatment (Table 4.7 and Appendix VII). The intraction effect of lime and GA_3 also showed significantly different due to different levels. The highest dry wt. ha $^{-1}$ of stevia plant was observed in L_3H_5 (45.53 g treatment and lowest was observed in L_1H_1 (36.97g) treatment (Table 4.7 and Appendix VII).

Table 4.7. Effect of lime and GA_3 on fresh wt. plant^{-1}, fresh wt. ha^{-1}, dry wt. plant^{-1} and dry wt. ha^{-1} of stevia and their interaction

Treatments	Fresh wt. plant^{-1} (g)	Fresh wt. ha^{-1}(kg)	Dry wt. plant^{-1}(g)	Dry wt. ha^{-1}(kg)
L_1	134.30bc	8393.8c	40.40b	2525.2c
L_2	136.12a-c	8507.7b	41.32ab	2582.9b
L_3	138.57a	8660.6a	42.58a	2661.2a
L_4	137.65ab	8603.0ab	41.44ab	2590.3b
L_5	132.33c	8270.6d	40.48b	2529.9c
LSD (0.05)	4.101	97.88	1.860	46.55
GA_3	-	-	-	-
H_1	122.58e	7661.3e	37.93c	2371.0e
H_2	128.99d	8062.1d	39.75bc	2485.0d
H_3	134.81c	8425.6c	41.09a-c	2568.2c
H_4	142.46b	8903.5b	42.08a-c	2630.5b
H_5	150.19a	9387.3a	44.29a	2768.1a
H_6	135.73c	8483.1c	42.31ab	2644.6b
LSD (0.05)	4.493	107.23	4.229	50.996
Interaction	-	-	-	-
L_1H_1	123.02j-l	7689.32l	36.97b	2310.63m
L_1H_2	127.73h-k	7983.37j	38.97ab	2436.11kl
L_1H_3	130.50f-k	8156.29h-j	40.73ab	2545.63h-k
L_1H_4	139.62c-g	8726.13ef	41.16ab	2572.41e-i
L_1H_5	148.27a-c	9267.23ab	43.37a	2710.45b-d
L_1H_6	136.65d-h	8540.59fg	41.22ab	2576.26e-i
L_2H_1	123.78i-l	7736.17l	38.20ab	2387.65lm
L_2H_2	130.02g-k	8126.35h-j	39.48ab	2467.82i-l
L_2H_3	136.87d-h	8554.34fg	40.77ab	2548.33g-k
L_2H_4	142.84a-e	8927.38c-e	42.50ab	2656.27c-h
L_2H_5	150.10ab	9381.56a	44.45a	2778.28ab
L_2H_6	133.13e-i	8320.42gh	42.55ab	2659.20c-h
L_3H_1	128.27h-k	8016.67ij	39.20ab	2450.22j-l
L_3H_2	133.24e-i	8327.87gh	41.59ab	2599.27d-h
L_3H_3	140.25b-f	8765.46d-f	42.26a	2641.23c-h
L_3H_4	145.86a-d	9116.37bc	42.94ab	2683.76b-e
L_3H_5	151.36a	9460.31a	45.53a	2845.65a
L_3H_6	132.43f-j	8276.65h	43.95a	2746.86a-c
L_4H_1	123.82i-l	7738.53kl	38.26ab	2391.23lm
L_4H_2	131.90f-k	8243.87hi	39.73ab	2483.36i-l
L_4H_3	138.80c-g	8675.31f	40.97ab	2560.54f-j
L_4H_4	143.66a-d	8978.95cd	42.65ab	2665.85b-f
L_4H_5	151.04a	9439.76a	44.47a	2779.38ab
L_4H_6	136.66d-h	8541.39fg	42.58ab	2661.23c-g
L_5H_1	114.01l	7125.76m	37.05ab	2315.42m
L_5H_2	122.06kl	7628.91l	39.01a	2438.33kl
L_5H_3	127.62h-k	7976.55jk	40.72ab	2545.27h-k
L_5H_4	140.30b-f	8768.86d-f	41.19ab	2574.36e-i
L_5H_5	150.20ab	9387.52a	43.63a	2726.77bc
L_5H_6	139.78c-g	8736.29ef	41.27ab	2579.43e-i
LSD (0.05)	10.046	239.77	9.455	114.03

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1}, L_5: 2.0 ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

4.7.4. Dry weight ha^{-1} (kg)

The highest dry wt. ha^{-1} of stevia plant was observed in L_3 (2661.20kg ha^{-1}) and lowest in L_1 (2525.20 kg ha^{-1}) treatment (Table 4.7 and Appendix VII) when the effect of GA_3 is considered highest dry wt. ha^{-1} of stevia plant was observed in H_5 (2768.10 kg ha^{-1}) and lowest in H_1 (2371.00 kg ha^{-1}) treatment (Table 4.7and Appendix VII). The intraction effect of Lime and GA_3 also showed significantly different due to the different level of lime and GA_3. The highestdry wt. ha^{-1} was observed in L_3H_5 (2845.65 kg ha^{-1}) and lowest was in L_1H_1 (2310.63 kg ha^{-1}) treatment (Table 4.7 and Appendix VII). It was further noticede that lime increased. The dry wt ha^{-1} of stevia plant upto L_3 then it decreased. The effect of GA_3 also increased dry wt. ha^{-1} of stevia upto H_5 level in combination of lime and GA_3 same result was observed.

4.8. Freshand dry leaf yield of stevia plant

4.8.1. Fresh leaf yield $plant^{-1}$ (g)

Fresh leaf yield $plant^{-1}$ of stevia was significantly influenced by the application of different levels of lime (Table 4.8 and Appendix VII). The highest fresh leaf yield $plant^{-1}$ was observed in L_3 (77.34g) treatment and lowest was observed in L_1 (76.06 g) treatment. The Effect of different levels of GA_3 application varied significantly on fresh leaf yield $plant^{-1}$ of stevia (Table 4.8 and Appendix VII) when considered the effect of GA_3. The highest fresh leaf yield $plant^{-1}$ was observed in H_5 (82.92g) treatment and lowest was observed in H_1 (68.79g) treatment (Table 4.8 and appendix VII). The intraction effect of lime and GA_3 was also varied significantly in fresh leaf yield $plant^{-1}$ of stevia (Table 4.8 and Appendix VII). The highest fresh leaf yield $plant^{-1}$ was observed in L_3H_5 (83.37 g) treatment and lowest was observed in L_1H_1 (67.77g) treatment. Noor-E- Ferdous *et al.*, 2020 reported that the fresh leaf yield was produced in field condittion cultivation 91.37 g plant-1. The results further indicated that the effect of lime increased upto L_3 level then at higher dose it decreased GA_3 also increased the fresh leaf yield ha^{-1} upto H_5 level. same result was followed when lime and GA_3 used combinedly.

4.8.2. Fresh leaf yield ha^{-1} (kg)

The highest fresh leaf yield ha^{-1} was observed in L_3 (4834.30 kg) and lowest was in L_1 (4754.00 kg) treatment. Fresh leaf yield ha^{-1} of stevia was significantly influenced by the application of different levels of GA_3 application (Table 4.8 and Appendix VII). The

highest fresh leaf yield was observed in H_5 (5182.60 kg) and lowest was observed in observed (4299.70 kg) in H_1 treatment. The intraction effect of lime and different concentration of GA_3 varied also significantly in fresh leaf yield ha^{-1} of stevia production (Table 4.8 and Appendix VII). The intraction effect of Lime and GA_3 in most of the combination showed significantly the highest fresh wt. ha^{-1} was observed in L_3H_5 (5210.47 kg) and lowest was observed in L_1H_1 (4235.76 kg) Table 4.7 and Appendix VII. The single effect of lime increased fresh leaf yield of stevia ha^{-1} upto level L_3 the effect of GA_3 upto H_5. same phenomenon was followed when lime and GA_3 used in consideration.

4.8.3. Dry leaf yield plant $^{-1}$ (g)

Dry leaf yield plant^{-1}of stevia was significantly varied by the application of different levels of lime, GA_3 alone and in combination (Table 4.8 and Appendix VII). The highest dry leaf yield plant^{-1} was observed in L_3 (20.78 g) treatment and lowest was observed in L_1 (19.79g) treatment (Table 4.8 and Appendix VII). Application of GA_3 the highest dry leaf yielded plant^{-1} in H_5 (22.27 g) treatment and it was lowest in H_1 (18.43 g) treatment (Table 4.8 and Appendix VII). The intraction effect of Lime and GA_3 produced highest dry leaf yielded in L_3H_5 (22.81g) treatment and lowest in L_1H_1 (18.08g) treatment (Table 4.8 and Appendix VII). Mengesha *et al,*. (2014) reports, the dry weight of the leaves can vary from 15 to 35 g plant^{-1}. The effect of lime increased dry leaf plant^{-1} upto L_3 and that of GA_3 upto H_5. similar phenomenon was followed in combined effect of lime and GA_3.

4.8.4. Dry leaf yield ha^{-1} (kg)

(Table 4.8 and Appendix VII) From the Table 4.8, it was observed that L_3 (1.0 t ha^{-1}) treatment had the highest dry leaf yield ha^{-1} (1298.90 kg) and the lowest was observed in L_1 (1237.30 kg) in conrol treatment when considered the effect of GA_3. The highest dry leaf yield ha$_{-1}$ was observed (1392.0 kg) in H_5 (GA_3 350 ppm) treatment and it was lowest (1152.30 kg) in H_1 treatment (Table 4.8 and Appendix VII). The intraction effect of different level of lime and different concentration of GA_3 also varied significantly on production yield ha^{-1} (Table 4.8 and Appendix VII). The highest dry leaf yield ha^{-1} was observed (1425.64 kg) in L_3H_5 (lime 1.0 tha^{-1} × GA_3 300 ppm) treatment and lowest was (1130.19 kg) L_1H_1. Mengesha *et al.*, 2014 observed that an estimated 6,000 kg ha^{-1} dried leaf yield can be obtained. Here again it was found that dry leaf yield of stevia increased with increase in level of lime upto L_3 and GA_3 (H_5) and in combination also at L_3H_5 in both lime and GA_3 have the capacity to increase leaf production of stevia upto a certain level and at high level both have retarding effect of stevia.

Table 4.8. Effect of lime and GA_3 on fresh leaf yield plant^{-1}, fresh leaf yield ha^{-1}, dry leaf yield plant^{-1} and dry leaf yield ha^{-1} of stevia and their interaction

Treatments	Fresh leaf yield plant^{-1} (g)	Fresh leaf yield ha^{-1} (kg)	Dry leaf yield plant^{-1}(g)	Dry leaf yield ha^{-1} (kg)
L_1	76.06ab	4754.0b	19.79ab	1237.3c
L_2	76.38ab	4774.0ab	20.20a	1263.0b
L_3	77.34a	4834.3a	20.78a	1298.9a
L_4	76.52ab	4782.9ab	20.77a	1298.6a
L_5	76.60ab	4788.0ab	20.04a	1252.5bc
LSD (0.05)	15.79	2.319	77.580	3.530
GA_3	-	-	-	-
H_1	68.79e	4299.7f	18.43c	1152.3e
H_2	72.29de	4518.3e	19.23bc	1202.4d
H_3	75.64cd	4728.0d	20.29a-c	1268.4c
H_4	79.00bc	4937.7c	21.24ab	1328.0b
H_5	82.92a	5182.6a	22.27a	1392.0a
H_6	80.85ab	5053.4b	20.43a-c	1277.4c
LSD (0.05)	17.301	2.541	84.985	3.867
Interaction	-	-	-	-
L_1H_1	67.77f	4235.76q	18.08b	1130.19l
L_1H_2	71.95c-f	4497.12l-o	18.87ab	1179.34i-k
L_1H_3	74.88a-f	4680.34i-l	19.62ab	1226.27h
L_1H_4	78.69a-d	4918.13e-g	20.71ab	1294.32e-g
L_1H_5	82.82a	5176.21a-c	21.73ab	1358.26cd
L_1H_6	80.26a-c	5016.38b-e	19.77ab	1235.54h
L_2H_1	68.78ef	4298.77pq	18.77ab	1173.21jk
L_2H_2	71.30d-f	4456.08m-p	19.27ab	1204.43h-j
L_2H_3	76.60a-e	4787.70f-i	20.42ab	1276.29g
L_2H_4	78.84a-d	4927.43ef	21.19ab	1324.65de
L_2H_5	82.87a	5179.67a-c	22.18a	1386.24bc
L_2H_6	79.91a-d	4994.39c-e	19.41ab	1213.37hi
L_3H_1	69.81ef	4363.25n-q	18.32ab	1145.31kl
L_3H_2	72.59b-f	4536.76k-n	19.28ab	1205.36h-j
L_3H_3	75.64a-f	4727.56h-j	21.07ab	1316.67ef
L_3H_4	79.68a-d	4980.23de	21.99ab	1374.23bc
L_3H_5	83.37a	5210.47a	22.81a	1425.64a
L_3H_6	82.99a	5187.28ab	21.22a	1326.20de
L_4H_1	68.60ef	4287.35pq	18.67ab	1167.17j-l
L_4H_2	72.30b-f	4518.74l-n	19.77ab	1235.56h
L_4H_3	75.41a-f	4712.92i-k	20.74ab	1296.24e-g
L_4H_4	79.27a-d	4954.36ef	21.81ab	1363.28cd
L_4H_5	82.68a	5167.53a-d	22.45ab	1403.17ab
L_4H_6	80.90ab	5056.25a-e	21.22ab	1326.32de
L_5H_1	69.02ef	4313.45o-q	18.33ab	1145.43kl
L_5H_2	73.32b-f	4582.62j-m	18.99ab	1187.28ij
L_5H_3	75.71a-f	4731.67g-j	19.63ab	1226.72h
L_5H_4	78.54a-d	4908.54e-h	20.53ab	1283.38fg
L_5H_5	82.86a	5178.91a-c	22.19ab	1386.74bc
L_5H_6	80.20a-c	5012.76b-e	20.57ab	1285.45fg
LSD (0.05)	38.686	5.682	190.03	8.647

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant.L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1}, L_5: 2.0 ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

Midmore and Rank (2002) reported that dry leaf yield 1,600–2,000 kg ha^{-1}. Noor-E-Ferdous *et al.*, 2020 observed that that dry leaf yield was obtained 1226.17 kg ha^{-1} in field condition.

4.9. Nutrient status of Soil

4.9.1. Initial Soil

pH 5.63, Organic matter (OM) 1.14%, Nitrogen (N) 0.07%, Phosphorus (P) content 70.78 µg g^{-1}, Potassium (K) 0.21 µg g^{-1}, Sulphur (S) 13.25 µg g^{-1}, Zinc (Zn) 4.89 µg g^{-1}, Boron (B) 1.95 µg g^{-1} and Magnesium (Mg) 1.46 µg g^{-1} of stevia was found in initial Soil respectively.

4.9.2. Post harvest Soil

The changes in soil pH, organic carbon, total N, available P, exchangeable K, S, available Zn, B, and Mg status of post harvest soils due to different treatments of lime (L) and GA_3 (H) alone and their treatments combinations are presented in Table 4.9.2.

4.9.2.1. pH

pH content of post harvest soil of stevia was significantly influenced by the application of different levels of lime (Table 4.9 and and Appendix VIII). The highest pH content (6.78) in was observed when the plot was treated with lime 2.0 tha^{-1} (L_5). It was recorded lowest (5.54) in control (L_1) plot which was significantly inferior to all treatments (Table 4.9). The pH level of post harvest soil increased as level of lime increased. The results indicated that pH content of stevia was also significantly affected by different levels of GA_3 but the soil pH did not very remarkably. The highest pH content (6.43) was observed when the plot was treated with 350 ppm GA_3 (H_6) The pH of post harvest soil did not very stastically. The lowest pH content (6.19) was recorded in H_1 treatment. pH content of stevia was significantly affected by different levels of lime and GA_3 (Table 4.9). The highest pH content (6.87) was observed when the plot was treated with lime 2.0 t ha^{-1} × 350 ppm GA_3 (L_5H_6). The lowest pH content (5.12) was recorded in control (L_1H_1) treatment.

4.9.2.2. Organic matter (OM)

It appears from the data presented in (Table 4.9 and Appendix VIII) that organic matter (OM) content of soil after harvest of stevia was significantly influenced by the application of different levels of lime. The content of OM increased with the increase in level of lime. The highest OM content (2.40%) in was observed in L_5 (lime 2.0 t ha^{-1}) treated plot and the lowest OM content (1.00%) was recorded in control (L_1) treatment. The results indicated that OM content of post harvest soil of stevia did not due to application of different levels of GA_3 (Table 4.6 and Appendix VIII). The highest OM content (1.91%) was observed when the plot was treated with 350 ppm GA_3 (H_6). The lowest OM content (1.81%) was recorded in H_1 treatment. The OM content of post harvest soil of stevia was significantly affected by different levels of lime and GA_3 (Table 4.9 and and Appendix VIII). The highest OM content (2.46%) was observed in (L_5H_6) when the plot was treated with lime 2.0 t ha^{-1} × 350 ppm GA_3 (L_5H_6). The lowest OM content (0.83%) was recorded in the control (L_1H_1) plot.

4.9.2.3. Nitrogen

The results indicated that nitrogen **(**N) content of post harvest soil of stevia was significantly influenced by the application of different levels of lime (Table 4.9 and Appendix VIII). The content of N increased with increase in levels of lime. The highest N content (0.92%) in was observed in (L_5) when the plot was treated with lime 2.0 t ha^{-1} (L_5). The lowest N content (0.10%) was recorded in (L_1) control treatement. The results on N content of post harvest soil influenced of stevia was also significantly influenced by different levels of GA_3 (Table 4.9 and Appendix VIII). The highest N content (0.61%) was observed when the plot was treated with 350 ppm GA_3 (H_6). The lowest N content (0.50%) was recorded in H_1 treatment. The combined effect of lime and GA_3 on N% content of post harvest of stevia was significantly influenced (Table 4.9 and Appendix IX). The highest N content (0.98%) was obtained in (L_5H_2) treatment lime 2.0 t ha^{-1} × 150 ppm GA_3 (L_5H_2) and the lowest N content (0.04%) was found in whithout lime and GA_3 application (L_1H_1).

4.9.2.4. Phosphorus

Phosphorus (P) content of stevia was significantly influenced by the application of different levels of lime (Table 4.9 and Appendix VIII) and P increased with the rise in level of lime. The highest P content (102.79µg g^{-1}) in was observed when the plot was

treated with lime2.0 t ha^{-1} (L_5). The lowest P content (55.84µg g^{-1}) was recorded in control (L_1) plot which was significantly inferior to all other treatments. The results indicated that P content of stevia varied significantly different levels of GA_3 (Table 4.9 and Appendix VIII). The content of P in varied from 76.08 to 80.19µg g^{-1}. The highest P content (80.19µg g^{-1}) was observed when the plot was treated with 350 ppm GA_3 (H_6). The lowest P content (76.08µg g^{-1}) was recorded in H_1 treatment. P content of stevia varied significantly different levels of lime and GA_3 but the differences were very neglible (Table 4.9 and Appendix VIII) in different treatments. The highest P content (106.26µg g^{-1}) was observed when the plot was treated with lime 2.0 t ha^{-1} × 350 ppm GA_3 (L_5H_6).while the lowest P content (60.52) obtained in (L_1H_1) control treatment (without lime × without GA_3).

4.9.2.5. Potassium

The content of potassium (K) in stevia leaves varied significantly due to the different level of lime. (Table 4.9 and Appendix VIII). The highest K content (0.68meq/100g) in was observed when the plot was treated with highest level of lime 2.0 t ha^{-1} (L_5) used in the study and lowest K content (0.20meq/100g) was recorded in control (L_1). It was further noticed that level of Potassium (K) content of stevia was significantly influenced by the application of different levels of GA_3 application (Table 4.9 and Appendix VIII). but the difference was very negligible. The highest K content (0.46% meq/100g) was observed when the plot was treated with 350 ppm GA_3 (H_6). The lowest K content (0.42meq/100g) was recorded in H_1 treatment. Interaction effect of different levels of lime and GA_3 application on K content was significantly varied (Table 4.9 and Appendix VIII). The highest K content (0.72meq/100g) was observed in (L_5H_6) and L_5H_5 treatment, respectively. The lowest K content (0.15meq/100g) was recorded in the (L_1H_1) treatment (without lime × with no GA_3) application in the experimental field. The results also showed variation in K content of stevia but this variation was notably due to the application of higher levels of lime.

4.9.2.6. Sulphur

The content of sulphur (S) in stevia leaves varied significantly due to the application of different level of lime. S content increased in stevia leaves as the levels of lime increased (Table 4.9 and Appendix VIII). Sulphur (S) content of stevia was significantly influenced by the application of different levels of GA_3 but except control treatment content of S did

not vary statistically among the levels of GA_3 used (Table 4.9 and Appendix VIII). The highest S content (32.85µg g^{-1}) was observed when the plot was treated with with 350 ppm GA_3 (H_6). The lowest S content (26.95µg g^{-1}) was recorded in H_1 treatment. S content of stevia was significantly affected by different levels of lime and GA_3 (Table 4.9 and Appendix VIII). The highest S content (55.04µg g^{-1}) was observed when the plot was treated with lime 2.0 t ha^{-1} × 350 ppm GA_3 (L_5H_6).The lowest S content (1.44µg g^{-1}) was recorded in (L_1H_1) which was no lime and no GA_3 application in the field. Here also it was found that joint application lime and GA_3 induced slightiy high content S in stevia leaves but it seems alternative that this was due to higher levels of lime not GA_3.

4.9.2.7. Zinc

It appears from the data presented in Table 4.9 and Appendix VIII that Zinc (Zn) content of stevia significantly differenced due to application of different levels of lime. The highest Zn content (8.21µg g^{-1}) was observed when the plot was treated with lime 2.0 t ha^{-1} L_5 and it was lowest (2.67µg g^{-1}) in control (L_1) plot. Further it was revealed that content of Zn increased in level of lime increased (Table 4.9 and Appendix VIII). The results indicated that Zn content of stevia though different significantly but the difference was very negligible. It ranged from 5.19 to 5.65 µg g^{-1}. The content of Zn in varied from 5.19 to 5.65µg g^{-1}. The highest Zn content (5.65µg g^{-1}) was observed when the plot was treated with 350 ppm GA_3 (H_6) and it was lowest Zn (5.19µg g^{-1}) in H_1 treatment. If we have a look it will be clear than Zn content of stevia was significantly influenced by different levels of lime and GA_3 (Table 4.9 and Appendix VIII). The highest Zn content (8.93µg g^{-1}) was observed when the plot was treated with lime 2.0 t ha^{-1} × 300 ppm GA_3 (L_5H_5). The lowest Zn content (2.29µg g^{-1}) was recorded in the control plot which was significantly inferior to all treatments. If may further be seen that when level of lime increased alone with GA_3, Zn content increased due to higher level of lime effect of GA_3 was very little.

4.9.2.8. Boron

The significant differences effect of boron (B) content of stevia production due to the application of various lime levels (Table 4.9 and Appendix VIII). The highest B content

(3.81μg g^{-1}) in was observed when the plot was treated with lime 2.0 t ha^{-1} (L_5) and it was lowest (1.27μg g^{-1}) in control (L_1) plot. The results indicated that B content of stevia also significantly varied due to different levels of GA_3 but the difference although significant but very neglibible and inconsistant with the levels of lime. The content of B in varied from 2.41 to 2.64 μg g^{-1}. The highest B content (2.64μg g^{-1}) was observed when the plot was treated with 50 ppm GA_3 (H_1). The lowest B content (2.41 μg g^{-1}) was recorded in H_1 treatment. When B content of stevia was considered jointly with by different levels of lime and GA_3. The differences in B content was although varied significantly it was mainly dependent on the concentration of lime (Table 4.9 and Appendix VIII). The highest B content (3.98μg g^{-1}) was observed when the plot was treated with lime 2.0 t ha^{-1} × 250 ppm (L_5H_4) and the lowest B content (1.12μg g^{-1}) was recorded in the control treatments.

Table 4. 9. Effect of lime and GA_3 on nutrient status of post harvest soils changes of stevia production in soil properties and their interaction

Treatmets	Nutrients								
	pH	OM (%)	N%	P µg g^{-1}	K meq/100g	S µg g^{-1}	Zn µg g^{-1}	B µg g^{-1}	Mg meq/100g
Initial soil	5.63	1.14	0.07	70.78	0.21	13.25	4.89	1.95	1.46
Post harvest soil									
Lime	-	-	-	-	-	-	-	-	-
L_1	5.54d	1.00d	0.10e	55.84e	0.20e	7.45e	2.67e	1.27e	0.61e
L_2	6.18c	1.47c	0.37d	66.10d	0.33d	19.61d	4.13d	1.93d	1.03d
L_3	6.44bc	1.99b	0.56c	78.69c	0.46c	32.54c	5.24c	2.46c	1.53c
L_4	6.61ab	2.27ab	0.77b	92.26b	0.57b	39.43b	6.56b	3.17b	2.25b
L_5	6.78a	2.40a	0.92a	102.79a	0.68a	53.13a	8.21a	3.81a	2.90a
LSD (0.05)	0.320	0.381	0.017	3.818	0.021	1.156	0.252	0.081	0.050
GA_3	-	-	-	-	-	-	-	-	-
H_1	6.19b	1.81a	0.50c	76.08b	0.42b	26.95c	5.19bc	2.64a	1.66b
H_2	6.33a	1.81a	0.54b	81.87a	0.46a	30.16b	5.14c	2.41d	1.51d
H_3	6.30a	1.81a	0.51c	77.31b	0.45a	30.54b	5.44ab	2.48cd	1.59c
H_4	6.22a	1.79b	0.50c	79.33ab	0.46a	30.23b	5.31bc	2.57ab	1.69b
H_5	6.39a	1.83a	0.60a	80.01ab	0.46a	31.87a	5.45ab	2.52bc	1.86a
H_6	6.43a	1.91a	0.61a	80.19ab	0.45a	32.85a	5.65a	2.57ab	1.68b
LSD (0.05)	0.351	0.418	0.019	4.182	0.023	1.266	0.276	0.088	0.055
Interaction	-	-	-	-	-	-	-	-	-
L_1H_1	5.12g	0.83i	0.04r	60.52l-o	0.15q	1.44p	2.29rs	1.12u	0.45v
L_1H_2	5.76d-g	1.10g-i	0.15p	57.46n-p	0.24op	7.15o	2.13s	1.25tu	0.50uv
L_1H_3	5.43e-g	0.89hi	0.08qr	52.57op	0.18q	3.46p	2.98pq	1.19tu	0.84rs
L_1H_4	5.36fg	0.86i	0.05r	55.68n-p	0.20pq	12.78lm	2.87p-r	1.17tu	0.61tu
L_1H_5	5.77d-g	1.17f-i	0.12pq	58.63m-p	0.16q	10.43mn	2.76qr	1.36t	0.73st
L_1H_6	5.84c-g	1.19f-i	0.20o	50.17p	0.27o	9.47no	3.02pq	1.58s	0.58u
L_2H_1	6.15a-e	1.48b-i	0.31n	63.12j-n	0.25op	13.54l	3.4op	2.16no	1.07op
L_2H_2	6.05b-f	1.38d-i	0.27n	69.56i-l	0.29no	17.85k	3.76no	1.80qr	0.94qr
L_2H_3	6.20a-e	1.42c-i	0.22o	64.32j-n	0.38k-m	25.33i	4.68lm	1.93pq	0.99pq
L_2H_4	6.06b-f	1.32e-i	0.37m	67.31i-m	0.35lm	20.36jk	4.16mn	1.98o-q	0.89qr
L_2H_5	6.29a-d	1.56a-i	0.45l	61.54k-o	0.33mn	18.13k	3.89no	2.04op	1.20mn
L_2H_6	6.35a-d	1.67a-i	0.60j	70.75i-k	0.40j-l	22.49j	4.85kl	1.71rs	1.14no
L_3H_1	6.37a-d	2.13a-e	0.47l	71.68hi	0.50gh	28.64h	5.36i-k	2.67k	1.84i
L_3H_2	6.46a-d	1.88a-g	0.55k	80.21f-h	0.44ij	34.12g	4.98kl	2.31l-n	1.35l
L_3H_3	6.43a-d	2.15a-e	0.57jk	76.39g-i	0.45h-j	31.24h	5.28j-l	2.39lm	1.49k
L_3H_4	6.38a-d	2.06a-f	0.45l	74.35hi	0.49g-i	29.57h	5.15kl	2.47l	1.55jk
L_3H_5	6.48a-d	1.82a-h	0.68hi	85.36e-g	0.51fg	35.08fg	4.90kl	2.73jk	1.67j
L_3H_6	6.53a-d	1.93a-g	0.65i	84.17e-g	0.41jk	36.59fg	5.77h-j	2.24mn	1.28lm
L_4H_1	6.56a-c	2.20a-e	0.85de	86.24ef	0.53fg	40.98d	7.32dfe	3.35ef	2.18g
L_4H_2	6.67ab	2.33a-c	0.79f	97.13a-d	0.61cd	37.41ef	6.49fg	2.96hi	2.01h
L_4H_3	6.61a-c	2.26a-d	0.74g	91.16de	0.56d-f	39.50de	6.25f-h	3.10gh	1.95hi
L_4H_4	6.62a-c	2.30a-d	0.73g	96.27b-d	0.59c-e	37.15ef	6.58fg	3.27fg	2.37f
L_4H_5	6.65ab	2.24a-e	0.81ef	93.14c-e	0.60cd	40.90d	6.80ef	2.87i	2.48ef
L_4H_6	6.60a-c	2.34a-c	0.72gh	89.63de	0.54e-g	40.67d	5.97g-i	3.50de	2.54e
L_5H_1	6.78ab	2.44a	0.86cd	98.87a-d	0.67ab	50.16c	7.53cd	3.90ab	2.80cd
L_5H_2	6.74ab	2.38ab	0.98a	105.01ab	0.72a	54.27a	8.36ab	3.73bc	2.77cd
L_5H_3	6.85a	2.35a-c	0.94ab	102.13a-c	0.69a	53.18ab	8.03bc	3.79a-c	2.68d
L_5H_4	6.71ab	2.41ab	0.90bc	103.08ab	0.70a	51.29bc	7.81b-d	3.98a	3.05b
L_5H_5	6.77ab	2.40ab	0.97a	101.41a-c	0.71a	54.84a	8.93a	3.62cd	3.24a
L_5H_6	6.87a	2.46a	0.89cd	106.26a	0.63bc	55.04a	8.65a	3.85ab	2.86c
LSD(0.05)	0.785	0.935	0.042	9.352	0.052	2.832	0.618	0.198	0.123

Mean values in a column having the same letter (s) do not differ significantly at 5% level of DMRT. NS = Non significant, ** indicates 1 % level of significant. * indicates 5 % level of significant. L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1}, L_5: 2.0 ha^{-1}, H_1: control, H_2: 150 ppm, H_3: 200 ppm, H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm

4.9.2.9. Magnesium

Application of various lime levels has a substantial impact on The magnesium (Mg) content of stevia varied like other nutrient elements significantly but itincreased with the increase in level of lime. (Table 4.9 and Appendix VIII). The highest Mg content (2.29meq/100g) in was observed when the plot was treated with lime 2.0 t ha^{-1} (L_5) and lowest Mg content (0.61meq/100g) was recorded in control (L_1) plot. The results indicated that Mg content of stevia was also significantly affected by different levels of GA_3 (Table 4.9 and Appendix VIII). but the differences was proportionally increased with increase in GA_3 level. The content of Mg in varied from 1.51 to 1.86meq/100g. The highest Mg content (1.86meq/100g) was observed when the plot was treated with 300 ppm GA_3 (H_5) and the lowest Mg content (1.51meq/100g) was recorded in H_2 treatment. Mg content of stevia varied significantly due to different levels of lime and GA_3 (Table 4.9 and Appendix VIII) When used jointly. The highest Mg content (3.24meq/100g) was recorded in L_5H_5 (lime 2.0 t ha^{-1} × GA_3 300 ppm) treatment and lowest Mg content (0.45meq/100g) was observed in L_1H_1 (with no lime and no GA_3 application) treatment.

The one opinion that the declining photoperiods, different soil classes, with different pH levels, can have interfered with stevia productivity. The environmental and soil conditions generate evolutionary compatibility of the crop with the type of soil and the availability of nutrients and water (Zaman *et al*., 2015). These authors maintained that Soils with a high pH, calcareous soils and soils corrected with high doses of limestone hinder the development of stevia cultures, harming the accumulation of biomass. Thus, the levels of biomass, may have suffered negative influences both by the declining photoperiod, as well as by the inadequate pH range of the soil in the experimental area.

CHAPTER V

SUMMARY AND CONCLUSION

A field experiment was conducted at Regional Sugarcrop Research Station, Bangladesh Sugarcane Research Institute (BSRI), Thakurgaon, Bangladesh during the period from March to September, 2021 to investigate the effect of Lime and Gibberellic Acid on growth, yeild and nutrient content of stevia. The trail was laid out in Randomized Complete Block Design (RCBD) with three replications. Factor A, lime was used, L_1: Control, L_2: 0.5 t ha^{-1}, L_3: 1.0 t ha^{-1}, L_4: 1.5 t ha^{-1} and L_5: 2.0 t ha^{-1} and Factor B, the selected Gibberellic acids (GA_3) were H_1: control, H_2: 150 ppm, H_3: 200 ppm H_4: 250 ppm, H_5: 300 ppm and H_6: 350 ppm. Plant height increased gradually with advancement of the growth stage (21 DAT to 147 DAT) of the stevia plants. Significant variations (P<0.05) were observed on plant height in different level of lime application. The highest plant height was observed in L_3 (lime 1.0 t ha^{-1}) treatment and the lowest plant height was observed in L_1 (control) treatment at 21 DAT to 147 DAT, respectively. significant variation was observed in height due to different doses of GA_3 at 21 DAT to 147 DAT, respectively and the lowest plant height was observed in control (GA_3) treatment. The highest plant heights was found in L_3H_5 (lime 1.0 t ha^{-1} × GA_3 300 ppm) treatment and it was lowest in L_1H_1 (with no lime × with no GA_3) at all growth stages. Lime at 1.0 t ha^{-1} (L_3) had the highest number of leaves $plant^{-1}$ *i.e.* 17.45, 45.20, 210.98, 500.60, 742.56, 933.03 and 1090.80, respectively which were statistically different from other level of lime application. The lowest number of leaves $plant^{-1}$ was observed in L_1 (control) treatment. It was reportrd that (16.12, 42.48, 205.21, 494.80, 735.17, 899.56 and 1083.20) number of leaves $plant^{-1}$ at 21 DAT 147 DAT, respectively. The number of leaves varied significantly due to variation in doses of GA_3. The highest number of leaves was obtained in H_5 (GA_3 300 ppm) treatment and it was lowest leaves in H_1 (control) treatment. The intraction effect of lime and GA_3 showed significantly variation in the highest number of leaves of stevia at all the stage of growth. It was found that the highest number of leaves $plant^{-1}$ were at 17.88, 46.36, 230.70, 519.31, 813.45, 1000.40 and 1123.40 at 21 DAT to 147 DATs, respectively in L_3H_5 treatment (lime 1.0 t ha^{-1} × GA_3 300 ppm). Significantly the lowest number of leaves $plant^{-1}$ was observed in L_1H_1 (control) treatment. It was found number of leaves $plant^{-1}$ (15.69, 40.52, 188.19, 465.09, 660.31, 830.00 and 1033.70) at 21 DAT to 147 DATs, respectively.The highest leaf area $plant^{-1}$ was observed in L_3 (1.0 t ha^{-1}) and the lowest

leaf area plant^{-1} was observed in L_1 (control) treatment at 21 DAT to 147 DAT, respectively. The concentration of 300 ppm GA_3 alone produced the highest leaf area plant^{-1} in H_5 (GA_3 300 ppm) at 21 DAT to 147 DAT, respectively. The leave are plant^{-1} varied according to rise in levels of GA_3 and reached highest level at H_5 and at further increase of GA_3 it again reduced. The lowest leaf area plant^{-1} was found in H_1 (control) treatment at 21DAT to 147 DATs, respectively. A significant variation was found leaf area plant^{-1} at 21 DAT to 147 DAT by the intraction effect of lime and different level of GA_3. The highest leaf area plant^{-1} was found at L_3H_5 treatment (lime 1.0 t ha^{-1} × GA_3 300 ppm). The lowest leaf area plant $^{-1}$ was found in L_1H_1 treatment at all growth stages. Number of primary branch of stevia was significantly different in different levels of lime and GA_3 application at all growth stages. The highest number of primary branch (4.68, 6.25, 9.53, 11.38, 12.15, 13.46 and 12.76) was found L_3H_5 treatment (lime 1.0 t ha^{-1} × GA_3 300 ppm). The lowest number of primary branch (3.75, 4.96, 5.02, 6.14, 9.12, 9.96 and 10.30) was found in L_1H_1 treatment at 21 DAT to 147 DAT, respectively. The intraction effect of lime and GA_3 in most of the combination showed significantly different on the highest number of secondary branch at all growth stages. The highest number of secondary branch was found in L_3H_5 (lime 1.0 t ha^{-1} × GA_3 300 ppm). The lowest number of secondary branch was observed in L_1H_1 treatment at all growth stages. Among the nutrients content of stevia was significantly different due to different levels of lime and GA_3. The highest N content (1.98%) was observed when the plot was treated with lime 1.0 t ha^{-1} × GA_3 300 ppm (L_3H_5). The lowest N content (1.57%) was recorded in the control plot which was significantly inferior to all treatments. The content of K, Mg, Zn in stevia leaves were significantly different the application of lime and GA_3. The effect of different level of lime application were significantly different in fresh weight plant^{-1} of stevia. The highest fresh weight plant^{-1} was observed in L_3 (138.57g) treatment and lowest was observed in L_1 (134.30 g) treatment. The fresh weight plant^{-1} varied significantly due different levels of GA_3 highest fresh weight plant^{-1} was observed in H_5 (150.19g) treatment and lowest was observed in H_1 (122.58g) treatment. The intraction effect of lime and GA_3 in also varied significantly due different combination of lime and GA_3. The highest fresh weight plant^{-1} was observed in L_3H_5 (151.36 g) treatment and it was lowest in L_1H_1 (123.02 g) treatment.The intraction effect of lime and GA_3 on fresh weight ha^{-1} varied significantly. The highest fresh weight ha^{-1} was observed in L_3H_5 ((9460.31 kg) and lowest was observed in L_1H_1 (7689.32 kg) treatment. The highest dry weight ha^{-1} (2845.65 kg) was observed in L_3H_5 (lime 1.0 t ha^{-1}

× GA_3 300 ppm) treatment and lowest dry weight ha^{-1} (2310.63 kg) was observed in L_1H_1 (with no lime application × with no GA_3 application) treatment.

The highest fresh leaf yield ha^{-1} was observed in L_3 (4834.30 kg) treatment and it was lowest in L_1 (4754.00 kg) treatment. The effect of different level of GA_3 on fresh leaf ha^{-1} varied significantly. The highest fresh leaf yield ha^{-1} was observed in H_5 (5182.60 kg) treatment and lowest was observed in H_1 (4299.70 kg) treatment. In combination lime and GA_3 fresh leaf yield ha^{-1} varied significantly. The highest fresh leaf yield ha^{-1} was observed in L_3H_5 (5210.47 kg) treatment and lowest was observed in L_1H_1 (4235.76 kg) treatment. Similarly highest dry leaf yield ha^{-1} was observed in L_3 (1298.90 kg) treatment and lowest was observed in L_1 (1237.30 kg) treatment and highest dry leaf yield $ha^{-1}$was observed in H_5 (1392.0 kg) treatment and lowest was observed in H_1 (1152.30 kg) treatment. The intraction effect of lime and GA_3 in most of the combination showed to very significantly, The highest dry leaf yield ha^{-1} was observed in L_3H_5 (1425.64 kg) treatment and lowest was observed in L_1H_1 (1130.19 kg) treatment.

Stevia grows well in fertile sandy loam or loamy soil having rich in organic matter, prefers lighter acidic to neutral (pH 6-7) soil for better growth and requires a consistent supply of moisture, but not waterlogged conditions. It is harvested just prior to flowering to get maximum steviol glycoside content in the leaves. Diterpene glycosides, stevioside and rebaudiosidediterpene glycosides are responsible for its high sweetening potential of leaves. Food scientists at different organizations are constantly developing new applications for both individual steviol glycosides and tailored steviol glycoside mixes to achieve the required sensory attributes. However, stevia can be taken as a carbohydrate diet source without calories. Farmers of Bangladesh could easily cultivate the plant in their relatively high land. It can help use import of artificial sugar, side by side help create job opportunities for large number of unemployed youths in our country. The application of lime and gibberellic acid (GA_3) had positive impact on leaf yield components resulted in higher yield of study. From the result it can be is apperent that for cultivation of stevia application of lime 1.0 t ha^{-1} and GA_3 300 ppm seems might be suitable for cultivation in Northern part of Bangladesh.

CHAPTER IV

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Appendix I: Analysis of variance (mean square) on plant height (cm) of stevia under Lime with GA_3

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Plant height (cm) at different days after transplanting (DAT)						
		21	42	63	84	105	126	147
Replication	2	130.876	121.485	256.552	235.760	152.596	19.319	245.388
Factor A	4	3.518*	3.557*	4.276*	6.430*	6.611*	6.452*	17.968*
Factor B	5	135.798*	117.475*	228.575*	170.188*	328.813*	739.262*	115.273*
AB	20	0.116*	0.197*	0.581*	0.396*	0.437*	7.744*	1.086*
Error	58	1.328	7.037	1.157	5.484	1.956	30.266	32.407

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix II: Analysis of variance (mean square) on number of leaves plant^{-1} of stevia under Lime with GA_3

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Number of leaves plant^{-1} at different DAS						
		21	42	63	84	105	126	147
Replication	2	455.676	548.969	672.04	319.61	581.8	407.7	40.7
Factor A	4	5.587*	19.048*	97.26*	133.47*	128.6*	3041.0*	3355.8*
Factor B	5	8.689*	73.322*	3599.23*	5173.08*	59926.9*	68825.8*	13946.4*
AB	20	0.191*	1.023*	4.90*	8.18*	23.5*	555.4*	565.6*
Error	58	4.046	5.305	2.84	24.30	8.1	13.5	135.2

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix III: Analysis of variance (mean square) on leaf area plant^{-1} of stevia under Lime with GA_3

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Leaf area plant^{-1} (cm^{-2}) at different (DAT)						
		21	42	63	84	105	126	147
Replication	2	374.463	683.4	568	21	1466	1373	2138
Factor A	4	40.916*	2369.6*	2855*	17967*	43561*	7304*	4068*
Factor B	5	52.164*	46554.6*	113780*	359698*	424828*	162979*	146989*
AB	20	1.160*	350.7*	985*	3648*	4621*	1330*	216*
Error	58	10.614	233.4	412	298	58	86	1409

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix IV: Analysis of variance (mean square) on number of primary branches plant^{-1} of stevia under Lime with GA_3

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Number of primary branches plant^{-1} of stevia at different DAS						
		21	42	63	84	105	126	147
Replication	2	1.66145	5.91408	2.39701	15.0238	27.1320	9.91875	0.54675
Factor A	4	0.01703NS	0.06859NS	2.26056*	1.7669*	0.6855*	1.04354*	3.12626*
Factor B	5	1.59683*	2.88145*	1.63820*	4.3694*	4.5087*	5.34278*	6.11654*
AB	20	0.00045*	0.00817*	1.95578*	0.9641*	0.4764*	0.99361*	0.14901*
Error	58	0.22021	0.61274	2.16438	1.9719	1.9077	3.07589	1.43183

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix V: Analysis of variance (mean square) on number of secondary branches plant^{-1} of stevia under Lime with GA_3

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values						
		Number of secondary branches plant^{-1} of stevia at different DAS						
		21	42	63	84	105	126	147
Replication	2	32.8235	75.2083	199.434	44.6032	398.289	49.6139	69.9824
Factor A	4	1.9425*	2.4342*	2.206*	0.9572*	2.800*	3.1560*	3.1434*
Factor B	5	9.3157*	8.6048*	6.941*	10.8340*	9.057*	10.2077*	41.9584*
AB	20	0.1008*	0.5668*	0.996*	0.4332*	0.421*	1.4146*	0.4269*
Error	58	1.2299	0.4878	0.547	1.5836	2.464	4.9410	2.6856

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance

Appendix VI: Analysis of variance (mean square) on N (%), P (%), K (%), S (%), Ca (%), Mg (%) and Zn (µg g^{-1}) contents of stevia leaf under Lime with GA_3

Source of Variance	**Degree of freedom (d.f.)**	**Mean sum of square values**						
		Stevia leaf						
		N (%)	**P (%)**	**K (%)**	**S (%)**	**Ca (%)**	**Mg (%)**	**Zn (µg g^{-1})**
Replication	2	1.61936	3.081E-03	0.01125	0.06505	0.00133	0.02358	6273.62
Factor A	4	0.00318*	3.385E-05*	2.900E-05NS	0.00006NS	0.02484*	0.00024*	8.97NS
Factor B	5	0.28907*	1.719E-03*	2.254E-03*	0.00753*	0.23061*	0.00270*	487.94*
AB	20	0.00041*	5.470E-06*	3.680E-06*	0.00001*	0.00702*	0.00014*	1.54*
Error	58	0.00134	1.212E-05	2.845E-05	0.00013	0.00991	0.00020	15.51

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix VII: Analysis of variance (mean square) on fresh wt. plant^{-1}(g), fresh wt. ha^{-1} (kg), dry wt. plant^{-1}(g) and dry wt. ha^{-1} (kg), leaf yield plant^{-1} (g), fresh leaf yield ha^{-1} (kg), dry leaf yield plant $^{-1}$ (g) and dry leaf yield ha^{-1} (kg) of stevia of under Lime with GA_3

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values							
		Stevia plant weight				Stevia leaf yield			
		Fresh wt. plant^{-1} (g)	Fresh wt. ha^{-1} (kg)	Dry wt. plant^{-1}(g)	Dry wt. ha^{-1} (kg)	Fresh leaf yield plant^{-1} (g)	Fresh leaf yield ha^{-1} (kg)	Dry leaf yield plant $^{-1}$ (g)	Dry leaf yield ha^{-1} (kg)
Replication	2	1194.61	2677798	64.0356	1163277	3349.21	1704612	200.570	2218
Factor A	4	114.69*	447699*	14.0386*	54830*	4.04*	15792*	3.541*	13848*
Factor B	5	1420.87*	5550439*	72.9482*	284761*	432.50*	1689911*	28.243*	110210*
AB	20	33.11*	129355*	0.2979*	1163*	1.19*	4682*	0.426*	1650*
Error	58	37.78	21521	33.4697	4868	27.99	13519	12.087	560

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.

Appendix VIII: Analysis of variance (mean square) on nutrient status of post harvest soil (pH, OM, N (%), P (%), K (%), S (%), Zn (µg g^{-1}), B% and Mg (%) of stevia field under Lime with GA_3

Source of Variance	Degree of freedom (d.f.)	Mean sum of square values								
		Post harvest soil of stevia field								
		pH	OM	N (%)	P (%)	K (%)	S (%)	Zn (µg g^{-1})	B%	Mg (%)
Replication	2	10.0804	4.37008	0.13601	8.45	0.05808	8.45	0.0252	0.4037	0.3000
Factor A	4	4.2219*	6.15167*	1.88231*	6501.90*	0.66235*	6501.90*	82.5935*	17.9151*	15.2087*
Factor B	5	0.1311*	0.03030*	0.03890*	66.17*	0.00416*	66.17*	0.5326*	0.0990*	0.2071*
AB	20	0.0469*	0.04252*	0.01759*	45.39*	0.00575*	45.39*	0.6342*	0.1012*	0.0845*
Error	58	0.2311	0.32730	0.00069	32.74	0.00104	32.74	0.1431	0.0148	0.0057

NS = Not significant, ** indicates 1 % level of significance and * indicates 5 % level of significance.