Environmental influence on yield and quality of stevia

(*Stevia rebaudiana* Bertoni)

By

SOMANATHAM SUHAS

(2018-11-078)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

(Agronomy)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRONOMY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2022

DECLARATION

I, Somanatham Suhas. (2018-11-07 8) hereby declare that the thesis entitled **"Environmental influence on yield and quality of stevia (*Stevia rebaudiana* Bertoni)"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other university or society.

Somanatham Suhas

(2018-11-078)

Vellanikkara

Date: 05-05-2022

CERTIFICATE

Certified that the thesis entitled **"Environmental influence on yield and quality of stevia (*Stevia rebaudiana* Bertoni)."** is a record of research work done independently by **Mr. Somanatham Suhas** (2018-11-078) under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, associate ship or fellowship to him.

Vellanikkara

Date 05.05.2022

Dr. Sindhu P.V.

Assistant Professor

AICRP on MAP & B

COA, Vellanikkara

(Chairperson Advisory Committee)

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Somanatham Suhas** (2018-11-078), a candidate for the degree of Master of Science in Agriculture, with major field in Agronomy, agree that this thesis entitled **"Environmental influence on yield and quality of stevia (Ste*via rebaudiana* Bertoni)."** may be submitted by **Mr. Somanatham Suhas** (2018-11-078) in partial fulfillment of the requirement for the degree.

05.05.2022

Dr. Sindhu P. V.
Assistant Professor (Agronomy)
AICRP on MAP&B
College of Agriculture, Vellanikkara

5/5/22

Dr. P. Prameela
Professor and Head (Agronomy)
College of Agriculture, Vellanikkara
(Member, Advisory Committee)

05/05/22

Dr. C. Beena
Professor (Biochemistry)
AICRP on MAP&B
College of Agriculture, Vellanikkara
(Member, Advisory Committee)

05/05/22

Dr. Savitha Antony
Assistant Professor
AICRP on Weed control
College of Agriculture, Vellanikkara
(Member, Advisory Committee)

ACKNOWLEDGEMENT

No creation in this world is a solo effort, neither is this thesis. I would like to take this opportunity to acknowledge all, who supported me directly or indirectly bringing in this thesis to a really good shape.

Firstly, I thank the almighty ***God*** *for his love and blessings, without which I wouldn't have been able to complete my studies hitherto and present this piece of work.*

With immense pleasure, I avail this opportunity to express my deep sense of reverence, gratitude and indebtedness to my Major Advisor, ***Dr. P. V. Sindhu,*** *Assistant Professor, AICRP on MAP & B for her sustained and valuable guidance, constructive suggestions, unfailing patience, friendly approach, constant support and encouragement during the course of the research work and preparation of this thesis. She has been a support to me during each step of this venture. I really consider it is my greatest fortune in having her guidance for my research work and will be remembered forever.*

I express my heartfelt gratitude to ***Dr. C. George Thomas,*** *former Professor and Head, Department of Agronomy, College of Agriculture for his affectionate advice, valuable suggestions, constant support and cooperation throughout the course ofstudy.*

No words can truly express my profound sense of gratitude to ***Dr. P. Prameela,*** *Professor and head and member of my Advisory Committee for her expert advice, valuable suggestions, critical evaluation and support rendered during thesis work.*

I feel highly privilege to express the deep sense of gratitude to my advisor committee member, ***Dr. Beena C,*** *Professor (Biochemistry), AICRP on MAP & B for her propitious guidance, keen interest, helpful advices and encouragement during the course of study.*

I sincerely thank ***Dr. Savitha Antony,*** *Assistant Professor, Department of Agronomy, College of Agriculture and member of my advisory committee for her expert advice, unfailing support and relevant suggestions during course of study. I thank him for all the help and cooperation he has extended to me.*

I express my heartiest gratitude to my beloved teachers, **Dr. Meera Menon, Dr. S. Anitha, Dr. K. E. Usha, Dr. Sree Laksmi, Dr. Shyama** for their encouragement, valuable help and advice rendered during the course of study.

I wish to express my sincere gratitude to **Mrs. Sreela and Mrs. Shyamala** for their affectionate advice, valuable help, constant support and cooperation throughout the course of study.

I express my sincere gratitude to **Mr. Sijith, Mr. Midhun**, **Mrs. Sethulekshmi, Ms. Ann and Ms. Athira** (Farm Managers, Dept. of Agronomy), for the sincere help, timely suggestions and mental support during the research works.

I am extremely delightful to acknowledge my profound sense of gratitude to labourers (Agronomy), for their sincere help and cooperation during my field experiments.

I wish to express my gratitude to my respected seniors **Mrs. Shobha Rani, Mr. Saravana Kumar, Ms. Daly George, Ms. Durga, Mrs. Akhila, Mr. Venkateshwara Reddy, Ms. Jeena, Ms. Jeen**, **Ms. Sabika, Ms. Athira, Ms. Emily, Ms. Anassoya, Ms. Vidhu, Mr. Kishor** and dear juniors **Ms. Fasna P, Ms. Aswani S, Ms. Murshida S Ms. Dayana Samson, Ms. Ayesha Jezla P, Ms. Oormila, Ms. Saveri Gopakumar, Ms. Shakkira** of Dept. of Agronomy for their help and support during the course of this study.

I am extremely happy to place on record my sincere appreciation to my beloved friends **Ms. Mounisha, Ms. Maria liz kappen, Ms. Minu, Ms. Parmoi, and Ms. Basila** and all batchmates (PG 2018) for the love, support and affection they rendered towards me.

It gives me immense pleasure to express to my beloved friends **Mr. Sunny babu, Mr. Mahesh Yadav, Mr. Shiva Shankar, Mr. Chakravarthy, Mr. Manoj, Mr. Aditya, Mr. Arjun, Mr. Kasi Prudhvi, Ms. Vaitheeswari, Mr. Karthik, Mr. Niranjan**, and **Mr. Nithin Kumar.**

I thankfully remember the services rendered by all the staff members of Student's Computer Club, Library, Office of COH, and Central Library, KAU.

In explicable in fervent to my parents ***Sri Balagangadhar Tilak,*** *and* ***Smt. Nagaman****i under who's grooming I learnt the concepts of life. They have been a foundation of inspiration throughout my life. To their feet, I bow with my head with great reverence. I feel elated to express my bountiful thanks to my family members for their undiminishing love, benign care and zealous encouragement throughout this endeavor and also my special thanks to my brother* ***Mr. Chaitrahaas*** *and my loving sister* ***Ms. Sai Sriya.***

I thank all my well wishers and others who helped me directly or indirectly not placed here, for their kind cooperation and support rendered to me.

Somanatham Suhas

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

1. INTRODUCTION

Medicinal plants are rich sources of bioactive substances that can be used directly or indirectly to treat a variety of human illnesses. Human civilizations have been studying and exploiting numerous plants and plant products to cure many ailments since time immemorial. They have been used as ethno medicine for the treatment of various diseases by indigenous cultures all over the world (Dogra, 2015).

Stevia (*Stevia rebaudiana* Bertoni) is a perennial herb belonging to the family Asteraceae. It is a natural sweetener commonly known as candy leaf, honey leaf, sweet leaf, sweet herb or sweet herb of Paraguay. The plant generates steviol glycosides (SVglys), which are gaining appeal in the human diet as a low-calorie, high-potency sweetener in light of the rising prevalence of diabetes and obesity The plant is native to the valley of the Rio Monday in highlands of north eastern Paraguay in South America. Paraguayans have used the leaves to sweeten bitter beverages for centuries. Stevia was earlier known as *Eupatorium rebaudianum*, however, in 1905, Dr. M. S Bertoni officially discovered stevia and renamed as *Stevia rebaudiana* (Lemus-Mondaca *et al*., 2012).

The plant was first brought to India in the late 1990s at the University of Agricultural Sciences in Bangalore, where studies on its adaptation began. High demand for natural sweeteners has pushed Indian farmers to grow stevia on a huge scale (Goyal *et al*., 2010). It has been successfully grown in many Indian states, including Rajasthan, Maharashtra, Punjab, and Orissa (Singh *et al*., 2014).

Steviol glycosides are mostly found in the leaves of the stevia plant. Stevia has a high concentration of phenols and flavonoids, as well as antioxidant properties.

The two main glycosides - Stevioside (5-10 per cent of the dry weight of the leaves) and Rebaudioside-A (2-4 per cent of the dry weight of the leaves) are the sweetest compounds which give sweetness to the plant. There are also other related compounds including Rebaudioside-C and Dulcoside-A and C, as well as minor glycosides, including flavonoid glycosides, coumarins, cinnamic acids, phenylpropanoids and some essential oils (Khiraoui *et al.*, 2017).

As the leaf is the most valuable component of the stevia plant, production of increased leaf biomass with higher steviol glycosides is the major criterion for evaluating crop performance. Secondary metabolism is influenced by a variety of environmental conditions that affect development, photosynthesis, and other aspects of primary metabolism (Pant *et al.*, 2015). Environmental factors such as temperature, light and atmospheric CO_2 concentrations can influence the levels of secondary metabolites in plants (Fini *et al.*, 2017).

Light is a physical component that can enhance plant growth and development. Both insufficient and excessive light intensities can harm plants, affecting their growth, development, and yield. Studies on crop growth responses to various light intensities are helpful in determining the best conditions for crop cultivation.

In any crop, the time of sowing is critical for better vegetative growth and eventual yield outputs. Sowing too early or too late might affect the crop's growth, yield, and quality. The planting timing has an impact on phenological development and the efficient conversion of biomass into economic produce.

Stage of harvesting is as important as time of planting in determining yield and quality of a crop. In stevia, changes in the contents of secondary metabolites were reported due to variations in growing conditions, management and also stage of harvesting.

Taking into account, the foregoing experiment "Environmental influence on yield and quality of stevia" (*Stevia rebaudiana* Bertoni) was undertaken with the following specific objectives:

1. To assess the effect of shade and time of planting on yield and quality of stevia (*Stevia rebaudiana* Bertoni).
2. Phytochemical evaluation of stevia leaves at different growth stages

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Stevia, often known as "paraguay's sweet plant," is a perennial herb native to Paraguay and Brazil that is now widely utilised in Asia and South America. Stevioside, the major sweet component in this plant's leaves, has a 300-fold sweeter taste than sucrose (Geuns, 2003). Extracts of the stevia leaf have advantageous anti-hypertensive, anti-hyperglycemic, anti-oxidant, non-carcinogenic, chemoprotective, anti-inflammatory, immune modulatory and anti-viral effects on human health, in addition to its natural, non-caloric sweetening characteristics (Chatsudthipong and Muanprasat, 2009).

In the 1970s, when chemical sweeteners were prohibited and substituted with stevia, Japan began marketing stevioside as a sweetene r(Midmore and Rank, 2002). Stevia has recently piqued the interest of a number of multinational food and beverage corporations as it is a low-calorie sweetener (Panpatil and Polasa, 2008). It is being successfully cultivated in many Indian states like Rajasthan, Maharashtra, Punjab and Orissa (Singh *et al*., 2014).

A brief review on the crop stevia, its origin, distribution, morphology, phytochemical constituents, economic importance and influence of growing conditions and agro techniques on growth and yield are presented in this chapter.

2.1 Origin and distribution

Stevia belongs to the Asteraceae family, which has 950 genera. A systematic review of the species of North and Central America was done by Grashoff (1972). Although there are over 200 species, Soejarto *et al*. (1983) has reported *Stevia rebaudiana* to be the sweetest species.

Scientific classification of stevia is as follows

Kingdom	: Plantae
Clade	: Tracheophytes
Clade	: Angiosperms
Clade	: Eudicots
Clade	: Asterids
Order	: Asterales
Family	: Asteraceae
Genus	: *Stevia*
Species	: *rebaudiana*

The natural habitat of the genus Stevia extends from the South Western United States to the Brazilian highlands (Soejarto *et al*., 1983). *Stevia rebaudiana* originated in the highland regions of North Eastern Paraguay on the Brazilian border, between 23° and 24° S latitudes (King and Robinson, 1987).

According to Schmeling (1967), stevia was primarily found in Amambay, especially in the San Pedro, Yhu, and Jejui Guazu zones. It had been used as a sweetener for decades. Europeans first noticed stevia in the 1800s, but it remained largely unknown until it was cultivated and used in England during World War II sugar rationing. Despite of its broad geographic distribution, this genus was only found in the tropical and subtropical areas of the United States, as well as Central and South America. Paraguayans have used this as a sweetener and herbal remedy for generations.

Early sources suggested that the Spanish knew stevia in the 16^{th} century, but it remained unknown to Europeans until M. S. Bertoni brought it back to their notice in 1888.

Stevia was known to the Gurani Indians as *Ka-a-He-e*, and had been used for centuries as a sweetener for bitter drinks such as mate tea. At present the crop is grown in many countries, including Brazil, Korea, Mexico, the United States of America, Indonesia, Tanzania, Canada and India (Brandle and Rosa, 1992).

In 1971, the Japanese imported stevia from Brazil and performed research to assess its ability. Japan is a major producer and marketer of the sweetener, which has been licensed for use in a variety of foods such as cereals, teas, and soft drinks (Ramesh *et al*., 2006).

The plant was originally introduced to India in the late 1990s, and studies on its adaptation began at the University of Agricultural Sciences in Bangalore. High demand for natural sweeteners, as opposed to artificial sweeteners, has pushed Indian farmers to grow stevia on a huge scale (Goyal *et al.,* 2010).

2.2 Morphology

Around 150 species were reported within the genus Stevia. *Stevia dianthoidea*, *S. phlebophylla*, *S. anisostemma*, *S. bertholdii*, *S. crenata*, *S. enigmatica*, *S. eupatoria*, *S. lemmonii*, *S. micrantha*, *S. ovata*, *S. plummerae*, *S. rebaudiana*, *S. salicifolia*, *S. serrate* and*S. viscidaare* were some of the economically important species. Among them, *S. rebaudiana* was the major economic species with the highest sweetness levels (Carakostas *et al*., 2008).

According to Zaidan *et al*. (1980), stevia has sweet tasting leaves. They observed that stevia has an alternate leaf arrangement and herbaceous growth habit.

Madan *et al*. (2010) observed that stevia was a small perennial shrub with height up to 65 cm and leaves which were sessile, lanceolate, opposite and serrated above the middle.

As per Shaffert and Chetobar (1994), stevia was a small shrubby perennial plant, which grows up to 65 cm tall. Leaves were sessile, oppositely arranged, lanceolate to oblanceolate and serrated above the middle. Frederico *et al.* (1996) reported stevia as a diploid with eleven chromosome pairs, which was characteristic for most of the South American members of the genus. Seeds were contained in slender achenes, about 3 mm in length and each achene had persistent pappus bristles (Oddone, 1999).

Stevia plants were bushy shrubs that grew up to a height of 120 cm. The leaves were the major sweet bearing parts of the plant. Leaves were small, oblong, lanceolate and serrated. Seeds had poor viability (Carneiro *et al*,. 1997).

Goettemoeller and Ching (1999) observed that the flowers of stevia had two to six tiny white florets in small corymbs which were arranged in loose panicles. Pollination was by insects. According to them artificial pollination would increase seed setting and the germination.

Dwivedi (1999) reported stevia as a perennial herb, but under some environmental conditions and management circumstances, it acted as an annual or a combination of both types.

Stevia was annual with more or less pubescent stems with extensive, fibrous and filiform root system. The cultivated stevia plant grew vigorously, giving branched bushy shrub like appearance. Dwivedi (1999) reported four distinct developmental stages for stevia: germination, grand growth cycle, flowering, and seed maturity. Flowers were pentamerous, hermaphrodite, and set in corymbs with capitulum. The corolla was white with glandular external hairs.

Leaves were small, sessile, lanceolate to oblanceolate, oblong, serrate above the middle and somewhat folded upwards (Singh and Rao, 2005).

Even though stevia could grow up to 120 cm of height, after four months of growth, the average of stevia height was 34 cm and the lowest was

21 cm (Satpathy and Das, 2010). *Stevia rebaudiana* (Bertoni) was a herbaceous perennial plant having herbaceous, shrub, or sub-shrub nature (Yadav *et al*., 2011). Stevia has the typical reproductive anatomy of male and female gametophytes and was a self- incompatible plant (Angelini *et al*., 2016).

Stevia rebaudiana was a small perennial shrub attaining a height of 80 cm at maturity. Stems were upright and woody with sessile leaves. Leaves were opposite lanceolate to oblanceolate. Leaf surfaces were slightly glandular and had two distinct sizes of the trichome. The inflorescence was a chyme of corymbs with five small white tubular flowers. The fruit was an achene carrying a single seed with a feathery pappus (Pande, 2018).

2.3 Phytochemical constituents

The leaves of stevia plant contain stevioside, rebaudioside-A, polyphenols, flavonoids, vitamins, phytosterols and triterpenes. Compared to young leaves, the old leaves contain more tannic acid, giving bitter taste. Stevia has a bitter smell, attributed to the presence of certain oils and flavonoids along with other constituents (Kinghorn and Douglas, 1987).

The best known compounds present in the leaves of steviawere glycosides, stevioside, several types of rebaudiosides (from A to F), steviolmonoside, rubusoside, dulcoside-A, and steviolbioside, and less common penta-glucosiderebaudioside D and hexa-glucosiderebaudioside M (Starratt *et al*., 2002; Savita *et al*., 2004).

Bondarev *et al*. (2003) observed that plant organs contained different amounts of the steviol glycosides, which was in the order of leaves> flowers> stems> seeds> roots. The highest content of the steviol glycosides was found in upper young actively growing shoot sections, whereas, mature shoot sections exhibited the lowest amount of such compounds. Savita *et al*. (2004) analysed the leaves of stevia on dry weight basis and calculated the energy value as 2.7 kcal/g.

Stevia is a nutrient rich herb that contained significant amounts of other nutrients, like protein, fiber, aminoacids, free sugars, lipids, essential oils, ascorbic acid, beta carotene, riboflavin, thiamine, austroinulin, sterebins A-H, nilacin, rebaudi oxides, gibberellic acid, indole-3-acetonitrile, apigenin, quercetin, isoquercitin, magnesium, iron, phosphorus and trace elements (Hu *et al*., 2010).

Presence of proteins, carbohydrates, lipids, dietary fibers, oils, vitamins, and phenolic compounds in stevia leaves was reported by Goyal *et al.* (2010) and Atteh *et al*. (2011). The dried stevialeaves contained 11.2-16.0 g proteins, 61.9 g carbohydrates, 1.9-3.73 g lipids and 6.8-15.2 g dietary fiber (Abou-Arab*et al*., 2010). As per Kim *et al. (*2011), stevia leaves were an important source of water-soluble vitamins including vitamin C (14.98 mg/100 g), vitamin B_2 (0.43 mg/100 g) and folic acid (52.18 mg/100 g). Furthermore, the plant was rich in macro and microelements such as Zn, Fe, Ca, K, Na, Mg and other minerals that were essential for human health. Rebaudioside-A is a steviol diterpene glycoside and is 180 to 400 times sweeter than cane sugar (Atteh *et al*., 2011; Kaplan and Turgut, 2019).

Taleie *et al*. (2012) reported leaves of stevia as the source of glycosides and a high percentage of phenols, flavonoids and antioxidants.Abdalbasit *et al*. (2014) analyzed stevia leaves for their physiochemical properties, chemical composition and microbiological contamination in addition to rebauidioside-A content. According to them the carbohydrates content was 63.10 per cent, while the moisture, fiber, protein, ash, fat and reducing sugar contents were 10.73, 5.03, 13.68, 12.06, 6.13 and 4.50 per cent respectively. The total soluble sugars were 17.03 per cent and stevia leaves showed good antimicrobial activity.

Leaves of stevia contained around 10 sweetening glycosides of which stevioside (3-10 per cent), rebaudioside-A (13 per cent), and rebaudioside-B, C, D were important (Singh and Verma, 2015).

More than 30 different steviol glycosides in the leaves of *S. rebaudiana*were reported. Among them, stevioside and rebaudioside-A were estimated in the highest levels. Stevioside was first isolated from steviain 1931 and its chemical structure was established in 1952 (Marcinek and Krejpcio, 2015) (Fig.1 and 2).

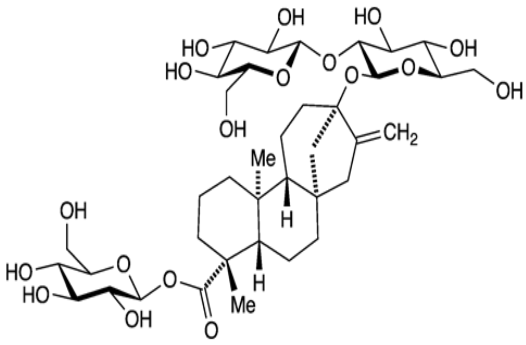


Fig.1. Molecular structure of stevioside

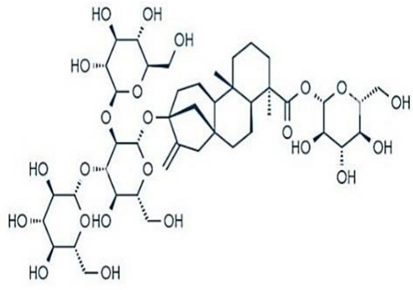


Fig.2. Molecular structure of rebaudioside-A

As per Olsson *et al*. (2016), rebaudioside-D and rebaudioside-M were present in dried *S. rebaudiana*leaves in very low quantities of 0.2 per cent and 0.1 per cent, respectively. Khiraoui *et al.* (2017) reported stevia plants as a good source of carbohydrates (51.50-56.72 per cent), protein (11.75-16.23 per cent) and crude fiber (17.43-19.13 per cent).

Stevia leaves contained a number of phenolic compounds that exhibited strong antioxidant properties, and also oils, which were rich in palmitic, palmitoleic, stearic, oleic, linoleic and linolenic fatty acids (Khiraoui *et al*., 2017).

Recently, several phenylethanoid glycosides, such as steviophethanoside, salidroside, cuchiloside, tyrosol, and icariside-D have been isolated from the leaves of stevia (He *et al*., 2019).

2.4 Medicinal uses and economic importance

Stevia is a natural sweetener commonly known as sweet leaf, sweet-herb, honey leaf or candy leaf. The leaves of stevia contain specific sweet tasting chemicals known as steviol glycosides, which are of high interest in human diet as a low calorie best alternative for cane sugar. Brazilian and Paraguayan natives used stevia leaves as a sweetener and as food additives (Mizutani *et al.*, 2002).

A reduction in both the systolic and diastolic blood pressures by 14 mm Hg and 14.3 mm Hg, respectively, after just seven days due to administration of 0.25 g stevioside three-times daily was reported by Chan *et al*. (2000).

Stevia can be used as a substitute for commonly used sucrose, so that the consumption of simple sugars can be reduced, which is advisable for the prevention of obesity, type 2 diabetes and other life style related diseases (Savita *et al*.,2004). Protective effects of stevia on the digestive system and skin disorders as well as on common complications associated with metabolic syndrome was reported by Singh and Rao (2005).

The World Health Organization (WHO) had declared stevioside as non genotoxic and had set a temporary daily intake limit of 0-2 mg/kg body weight for steviol glycosides (Beneford *et al*., 2006). Anti-diabetic properties of crystals derived from stevia leaves was also reported by Das *et al.* (2009).

Studies showed that stevia was 300 times sweeter than table sugar and also had added health benefits like regulation of blood sugar level and reduction in hypertension (Lemus-Mondaca *et al*., 2012). In view of the rising number of diabetics and obese people in India, this natural sweetener has a significant market potential (Singh *et al*., 2014).

According to Hossain *et al.* (2017), the economic part is leaves. The remaining parts of the plant, including stems, seeds and flowers that were not selected for industrialization, were collected and processed into animal feed or fertilizers.

Stevia contains a range of natural sweeteners which possess therapeutic potential against several diseases such as Diabetes mellitus, candidiasis, hypertension, inflammation, obesity and cancer (Lemus-Mondaca *et al*., 2018). Stevia has various applications including its use as an alternative sugar for food items, as an ingredient for pharmaceuticals, and as asolubilizing agent (Nguyen *et al*., 2015). Cookies containing stevia leaf powder were found to reduce hunger when compared to the control cookies made from 100 per cent wheat flour (Ahmad *et al.,* 2018).

Stevia leaves contain many biologically active substances, which have beneficial effects for human health. The anti-diabetic, anti-hypertensive, anti-tumor, anti-carcinogenic, anti-inflammatory and bactericidal effects of the herb have been studied extensively by many workers (Lopez*et al*., 2016; Ranjbar *et al*., 2020).

The di-terpenoids stevioside and rebaudioside-A proved as great potential sources of cardiovascular therapeutic agent. Regular intake of these compounds reduced the blood cholesterol content by affecting levels of lipid profiles (Atteh *et al.,* 2008).

European Commission Scientific Committee on Foods (SCF) evaluated safety related issues of this natural sweetener in 1985 and 1999. In 2004, Joint FAO/WHO Expert Committee on Food Additives (JECFA) established tentative purity specifications which were later made permanent. JECFA had established Acceptable Daily Intake (ADI) of 4 mg/Kg body weight/day for purified steviol glycosides in 2008 and validated its uses as sweetener in food and beverages (JECFA, 2008).

2.5 Effect of planting time and harvesting time on yield and quality of stevia

Sowing/planting time is an important non-monetary agronomic input, which plays amajor role in improving the yield and quality of plants by influencing the phenological development, biomass production and conversion of biomass into economic yield. Slight change in planting time leads to significant changes in associated weather parameters and consequently the yield and quality of the crop.

Brandle *et al*. (1998) recommended mid-May planting of stevia in the Northern hemisphere in order to get higher biomass. Ramesh *et al*. (2006) reported March-April as the ideal time of planting of stevia in the agro-climatic conditions of Palampur, in North India. They further revealed that delayed planting during June-July resulted in poor leaf harvest because it entered into flowering during the month of September. Stevia planted on 15th April recorded the highest leaf yield and biomass yield. Delay in planting reduced the yield parameters (Mohammad *et al.,* 2007).

Taleie *et al*. (2012) conducted an experiment to assess the effect of planting days on yield and quality of stevia. In the experiment, tissue culture stevia plantlets were transplanted on 15th March, 30th March and 15th April. Maximum plant height, total fresh and dry herbage biomass, highest phenol and flavonoid contents were obtained when plants were transplanted on 15th March. Delay in transplanting decreased the product quantity, because the time between transplanting and harvesting was less.

Khan *et al*. (2012) conducted field study to assess the optimum date of planting for stevia. Planting was done at 15 days interval staring from 1st January to 15thDecember. The highest yield and yield parameters were obtained when stevia was planted on 1st April. According to them, stevia seedlings can be planted from 15th February to 30th April for better yield.

According to Mordechai *et al*. (2013) the optimal time for planting stevia in an open field was in the spring (April and May). Planting in January-March decreased crop biomass.

Sumida (1980) observed that the harvesting of stevia had to be done before flowering took place, and at this time steviol glycoside content of leaves would be maximum. The optimum time for harvest mostly depended on the growing season of stevia along with the weather conditions. Stevia leaves were to be harvested at its peak vegetative growth phase before the onset of flowering to get higher leaf biomass yield.

Bian (1981) reported that the stevioside content of stevia leaves would be reduced due to flowering and hence harvesting of stevia leaves had to be done before flowering took place or at the time of emergence of flower.

Mastana (2012) conducted experiments on propagation and harvesting of *Stevia rebaudiana* and concluded that the harvesting of the plant should be done at a height of 30 cm from the base to get higher biomass yield.

Stevia is best harvested just before flowering, when the amount of steviol glycoside in the leaves is at its highest (Sumida, 1980).

Stevia could be grown either as an annual crop in sub temperate to temperate regions or as a perennial crop in tropical climates. Usually harvesting time varied depending upon the type of climate. Best time of harvest depended on the cultivar and growing season (Donalisio *et al*., 1982).

According to Shuping and Shizhen (1995), leaves harvested four months after transplanting had maximum crop biomass yield. Li *et al*. (2013) studied the effect of different harvesting dates on the quality and yield of stevia. According to them, the maximum quality and quantity in stevia were obtained when harvested at 95 DAT.

As per reports of Farooqi and Sreeramu (2004), stevia would reach the stage of first harvest at three months of planting. Crop should be harvested 5-8 cm above level to facilitate the regeneration of the plant. The subsequent harvest could be done at 90 days intervals. A minimum of four harvests could be obtained in a year.

Rebaudioside-A content was higher when leaves were harvested at 50 per cent flower bud stage (Kumar *et al*., 2012). According to Kumar *et al*. (2012), the best harvesting time for stevia was 60 DAT and at 50 per cent flower bud stage. Rakesh *et al*. (2012) also noted higher leaf biomass from stevia plants when harvested once at 50 per cent flower bud stage compared with those harvested once at60 and 90 days after transplanting (DAT).

Moraes *et al.* (2013) observed leaf biomass production and yield of diterpene glycosides under three different harvest timings. According to them, the leaf production and glycoside production were higher when plants were harvested once a year, than the yield of multiple harvests.

Higher dry leaf yield and better quality of stevia were recorded when the plants were harvested during summer season (Mohammed *et al*., 2019). As per Benhmimou *et al.* (2017), the dry leaf yield and steviol glycoside yield were significantly higher when harvesting was done during August, than that of October harvesting.

Studies conducted by Guerrero *et al*. (2018) proved the optimum harvest time for stevia as around 96 days after planting when the plants were initiating their flowering stage. Samadpourrigani *et al*. (2019) also reported the optimal cutting time for high stevioside content as the pre-flowering stage.

2.6 Effect of growing conditions on yield and quality of stevia

Weather is one of the most important factors that affect the growth, distribution, survival and quality of medicinal plants. Hang *et al.* (2005) reported that better meteorological conditions, such as sufficient sunshine, stable and suitable temperature, moderate rainfall and rational distribution were essential for the growth of medicinal plants in terms of dry matter production and accumulation of active constituents.

In plants, shading facilitated the production of leaves and slowed flowering. Additionally, shading reduced the thickness of the leaf by adding a thinner layer of palisade (Nygren and Killomaki, 1993). According to Rao and Mitra (1988), shading decreased photosynthetically active radiation (PAR) and improved spectral quality, affect plant photosynthesis, dry matter production and crop yield.

The crop yield depends mainly on the genetic characters, however, the phenotypic expression is greatly influenced by climatic and environmental factors (Metivier and Viana, 1979; Ermakov and Kotechetov, 1996). Valio and Rocha (1977) reported stevia as a short day plant. Even though it was a short day plant, initiation of the flowering could happen after the emergence of a minimum of four leaves.

Metiver and Viana (1979) reported stevia as a semi- humid subtropical plant. According to them, long days increased leaf area and leaf dry weight compared to short days. In addition to effect on leaf production, long days resulted in 51 per cent increase in stevioside concentration.

Concentration of stevioside was high in tropical and subtropical regions compared to temperate and sub temperate regions of the world, may be due to cultivation under long days (Kinghorn and Soejarto, 1985).

According to Brandle and Rosa (1992), stevia grew in a temperature range of 6°C to 43°C, with an average of 23°C and required partial shade on long and very hot summer days. According to Chalapathi *et al.* (1997), lower temperature and shorter day length affected the growth of stevia and it recorded lower number of leaves plant. Sensitivity of stevia to low temperature was reported by Columbus (1997).

Allam *et al*. (2001) reported that stevia grown in summer months had produced a good yield as compared to that of stevia grown in the winter months. Many workers reported stevia as a short day plant which responded to light (Valio and Rocha, 1977; Zaidan *et al.*, 1980; Kochetov, 2004).

Monteiro *et al*. (2001) reported that stevia plant remained in vegetative stage if it was subjected to continuous long day conditions, *i.e*., 16 hours photoperiod along with an average temperature of about 25°C.

Barathi (2003) reported that under agro-climatic conditions at Palampur, India, stevia could tolerate up to maximum temperature of 40°C during the day time and minimum temperature of 10°C during the night time.

Singh and Rao (2005) observed increase in the concentration of glycoside in the leaves of stevia when the plants were grown under long days. Long-day conditions increased the internodal length, leaf area, and dry weight and reduced the interval between the appearances of successive leaf pairs. Stevia responded to the length of day and night. Singh and Kaul (2005) reported lower content of steviol glycosides in the leaves grown under winter conditions than during summer conditions under agro-climatic conditions of Palampur in India.

The growth and flowering of stevia were affected by radiation, day length, temperature, soil moisture, and wind (Ramesh *et al*., 2006). They also suggested growing of stevia under long day conditions for enhancing leaf biomass.

Aladakatti *et al.* (2012) indicated that the climatic factors like minimum temperature and photoperiod had greater impact on the glycoside content in the leaves.Ceunen *et al.* (2012) and Tavarini and Angelini (2013) also reported significant influence of weather parameters like radiation, day length, temperature, soil moisture and wind on the growth and flowering of stevia.

Balwinder *et al.* (2014) reported partial shade requirement for stevia during very hot and long summer days. According to them, long spring and summer days favored leaf growth whereas short days triggered flowering. Temperature in the range of 24 to 35°C with appropriate soil moisture was required during first two weeks to obtain plantlets from stem cuttings.

Vegetative growth was reduced when temperature was below 20°C and when day length was less than 12 hours. Increasing day length to 16 hours and increasing light intensity enhanced the vegetative growth and stevioside levels (Pal *et al.,* 2015).

Highest levels of stevioside and rabaudioside-A were observed at initiation of flowering, due to the greater expression of genes involved in the steviol glycosides biosynthetic pathway (Yang *et al*., 2015).

Abdulameer *et al*. (2018) documented the agronomic performance of *Stevia rebaudiana* under long day length (>14 hours) with that of short day length (<14 hours) and also under open and shaded conditions. Stevia plants grown under open sun light started flowering after seven weeks whereas, stevia plants under shade did not flower. Also, stevia genotypes showed a higher performance under long day length condition.

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

A field experiment entitled "Environmental influence on yield and quality of stevia (*Stevia rebaudiana* Bertoni)" was conducted during May-November 2020 at the Agronomy Farm, Department of Agronomy, College of Agriculture, Vellanikkara. The details of the materials used and methods adopted for the experiment are presented in this chapter.

3.1 Geographical specification of the experimental site

Location

The experiment was conducted at the Agronomy Farm, Department of Agronomy, College of Agriculture, Vellanikarra, Thrissur, Kerala. The field is situated at13^{o}32'N latitude and 76^{o}26'E longitude, at an altitude of 40 m above mean sea level.

Soil

The texture of the experimental site is sandy clay loam and is strongly acidic in reaction with a pH of 4.76. The chemical properties of the soil are given Table 1.

Season

The experiment was conducted during the period May-November 2020.

Crop

A local cultivar of stevia (*Stevia rebaudiana* Bertoni) was used for the experiment. Stevia is a genus belonging to the Asteraceae family. It can grow to a height of one meter. The herb is a perennial with a large root system and brittle stems that produce small, elliptic leaves. Sessile leaves are 3-4 cm long, elongate-lanceolate or spatulate in shape. The stem is woody at the bottom and weakly pubescent. The pentamerous flowers are tiny, white, and have a pale purple throat. They are composite and enclosed by an epicalyx involucre.

Table 1. Chemical properties of soil

Particulars	Value		Method used
	Before planting	After harvest	
pH	4.76	7.66	1: 2.5 soil water suspension (Jackson, 1958)
Organic carbon (%)	1.98	2.29	Walkley and Black method (Jackson, 1958)
Available N (kg/ha)	193.41	227.84	Alkaline permanganate method (Subbiah and Asija, 1956)
Available P (kg/ha)	27.64	34.28	Ascorbic acid reduced molybdo phosphoric blue colour method (Bray and Kurtz, 1945; Watanabe and Olsen, 1965)
Available K (kg/ha)	169.76	195.05	Neutral normal ammonium acetate extraction and estimation using flame photometry (Jackson, 1958)

Cropping history of the experimental site

The experimental area had been under cultivation with *iruveli* during the previous year.

Experimental details

Experiment No. 1. Effect of growing condition and planting dates on yield and quality of stevia (*Stevia rebaudiana* Bertoni)

The experiment was laid out in split plot design, with eight treatment combinations and three replications. The plot size was 3 m x 3 m, with plant spacing of 30 cm x 30 cm. The treatment details are given below

Treatments

Main plot: Growing condition

1. Open
2. 50 per cent shade

Sub plot: Planting dates

1. May 15th
2. June 15th
3. July 15th
4. August 15th

Experiment No. 2: Phytochemical evaluation of stevia leaves at different growth stages

The experiment was laid out in Completely Randomized Block Design (CRD), with four treatments and five replications. The treatment details are given below

1. Harvesting leaves at 45 DAP
2. Harvesting leaves at flower bud initiation
3. Harvesting leaves at 50 per cent flowering
4. Harvesting leaves at full bloom stage

The experiment was conducted both under open and shaded conditions.

Layout

The layout of the experiment "Effect of growing condition and planting dates on yield and quality of stevia (*Stevia rebaudiana* Bertoni)" is presented in Fig.3.

Land preparation

The experimental field was initially ploughed with disc plough and then brought to a fine tilth by a cultivator. Weeds and stubbles of the previous crop were removed and plots were laid out as per the layout plan. Beds were prepared as per the treatments. Green coloured shade nets were erected to regulate light intensity to 50 per cent in part of the experimental area.

N ⇧

50 % SHADE				**OPEN**		
R_1	**R_2**	**R_3**		**R_1**	**R_2**	**R_3**
A_2B_1	A_2B_3	A_2B_2		A_1B_1	A_1B_2	A_1B_3
A_2B_3	A_2B_2	A_2B_4		A_1B_3	A_1B_1	A_1B_2
A_2B_4	A_2B_1	A_2B_3		A_1B_2	A_1B_4	A_1B_1
A_2B_2	A_2B_4	A_2B_1		A_1B_4	A_1B_3	A_1B_4

3 m (↕)

3 m (↔)

Fig. 3. Layout of the field experiment

A_1- Open — B_1 - May15th

A_2-50 per cent flowering — B_2- June15th

B_3 - July15th

B_4- August 15th

Planting

Planting materials were procured from AICRP on Medicinal and Aromatic Plants, COA, Vellanikkara. One month old rooted two noded stem cuttings were planted at a spacing of 30 cm x 30 cm. Light irrigation was given immediately after planting.

Irrigation

The beds were irrigated twice a day, in the morning and in the evening with a rose can during crop establishment period and later 3 mm hose irrigation was given once in a day.

Weed management

Hand weeding was done in the plots at 30 and 45 days after planting (DAP).

Plant protection

During the cropping period, no major disease or insect damage was observed in the experimental area.

Harvesting

After three months of planting, on the initiation of flower buds the crop was harvested by cutting at the base of the stem.

Observations recorded

3.3.1 Soil analysis

pH, organic carbon, and important micronutrients were assessed before the experiment as per standard protocols outlined in Table 1. Soil samples were collected, dried, powdered, and passed through a 0.5 mm sieve to evaluate organic carbon content, while samples passed through a 2 mm sieve were used to assess major nutrients such as available N, available P, and available K. The pH of the soil in a 1: 2.5 soil: water suspension was measured with a pH meter.

3.3.2Weather observations (on weekly basis)

1. Maximum and minimum temperatures (ºC)
2. Rainfall (mm)

3. Relative humidity (%)

4. Bright sunshine hours

3.3.3Biometric observations

Five plants per treatment per replication were randomly selected and tagged and the following observations were recorded:

Plant height

Plant height was measured from the ground level to the growing tip of plants at 30 DAP, 60 DAP and at harvest and average was expressed in cm.

Number of branches per plant

Branches arising from main stem of the tagged plants were counted at 30 DAP, 60 DAP and at harvest and the average number of branches per plant was worked out.

Number of leaves per plant

Total leaves of the tagged plants were counted at 30 DAP, 60 DAP and at harvest and the average number of leaves per plant was worked out.

Fresh leaf weight

Randomly selected plants in each treatment and replication were uprooted at 30 DAP, 60 DAP and at harvest and their leaf weight was recorded and average fresh leaf weight per plant was calculated and expressed in grams.

Dry leaf weight

Plants used for fresh leaf weight determination at30 DAP, 60 DAP and at harvest were first shade dried and then dried in hot air oven till they attained constant weight. Sample dry weights were recorded and expressed in grams.

Herbage yield

Randomly selected plants in each treatment and replication were uprooted at 30 DAP, 60 DAP and at harvest and weight of above ground portion (stems with leaves) were recorded and average herbage yield per plant was calculated and expressed in grams.

Total biomass yield at harvest

At three months after planting, the whole plants were uprooted, cleaned and total weights (including stems, leaves and roots) were recorded and expressed in kg/ha.

Incidence of pests and diseases

The crop was regularly monitored for recording occurrence of pests and diseases.

3.3.4 Physiological and biochemical observations

Crop growth rate (CGR)

Crop growth rate indicates at what rate the crop is growing *i.e.*, whether the crop was growing at a faster rate or slower rate than normal. It is expressed as gram of dry matter produced per day. Crop growth rate was calculated using the following formula and expressed as g/day/m^2 (Watson, 1952).

$$\text{CGR (g/day/m}^2\text{)} = \frac{W_1 - W_2}{T_2 - T_1}$$

Where W_1 and W_2 are dry weights of plants at time T_1 and T_2, respectively.

Relative growth rate (RGR)

This parameter indicates rate of growth per unit dry matter. It is expressed as gram of dry matter produced by a gram of existing dry matter in a day. Relative growth rate was calculated by the following formula (Blackman, 1919) and expressed as g/g/day.

$$RGR\ (g/g/day) = \frac{W_2 - W_1}{T_2 - T_1}$$

Where W_1 and W_2 are dry weights of plants at time T_1 and T_2, respectively.

Total steviol glycoside content of leaves at harvest

Total steviol glycoside content in the leaves was estimated at harvest using the method given by Parhi and Mohapatra. (2013). Ten grams of dried leaf powderwas extracted with 250 ml of methyl alcohol. Another wash was carried out with same solvent, filtered and this extract was treated with the aqueous saturated lead acetate solution to precipitate tannins, proteins, coloring matter and other non-glycosidal parts. The precipitate formed was filtered and to the filtrate H_2S gas (HCl + ferrous sulphide) was passed to precipitate excess lead as lead sulphide and removed by filtration. Filtrate was evaporated to dryness on water bath. The residue was dried, collected and weighed to get total glycoside content.

Biochemical analysis of stevia leaves at different growth stages

Nutrient contents of the leaves were estimated by collecting leaves from each replication of each treatment. Leaves were cleaned and dried in an oven till constant weight. Then the samples were powdered and used for analysis. The parameters such as total carbohydrate, total fat, total protein, crude fiber, moisture content, total mineral content, calorific value, total soluble sugars, reducing sugars, non reducing sugars, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and total steviol glycoside content were analyzed at 45 DAP, flower bud initiation stage, 50 per cent flowering stage and at full bloom stage. The methods for estimation of biochemical parameters are given in Table 2.

Statistical analysis

The data collected were subjected to analysis of variance using the statistical package "GRAPES" (Gopinath *et al.*, 2020).

Table 2. Methods adopted for biochemical analysis of stevia leaves

Sl.No.	Parameters	Method
1.	Nitrogen	H2SO4 digestion, distillation and titration (Jackson, 1958)
2.	Phosphorus	9:4 HNO_3– $HClO_4$ diacid digestion and Vanado Molybdate yellow colour method using spectrophotometer (Jackson,1958)
3.	Potassium	9:4 HNO_3 – $HClO_4$ diacid digestion and direct reading using flame photometer (Jackson,1958)
4.	Calcium	Atomic Absorption Spectrophotometry (Piper, 1942)
5.	Magnesium	
6.	Sulphur	Turbidimetric method (Chesnin and Yien, 1951)
7.	Crude fibre	Acid-alkaline digestion method (Asp *et al*., 1983)
8.	Total protein	Folin Lowry's method (Lowry *et al*., 1951)
9.	Total fat	Soxhlet extraction method (Kirk and Sawyer,1991)
10.	Total soluble sugars	Fehling's test (Thomas and Dutcher, 1924)
11.	Reducing sugars	
12.	Non reducing sugars	
13.	Total carbohydrate	Anthrone method (Loewus, 1952)
14.	Moisture content	Gravimetric method (AOAC, 1975)
15	Total energy (calorific value)	(Total Carbohydrate × 4) + (Total protein × 4) + (Total fat × 9)
16	Total steviol glycoside content	Parhi and Mohapatra (2013)

Plate1. General field view of experiment 1

Seedling stage

45 DAP

Flower bud intiation

50 percent flowering

Full bloom stage

Plate2. Stevia at different growth stages

4. RESULTS

4. RESULTS

4.1 Direct effect of treatments

4.1.1 Biometric observations

Plant height at 30 DAP, 60 DAP and at harvest

Growing condition and planting dates significantly influenced plant height of stevia at different growth stages (Table 3). Throughout the study period, taller plants were observed under shaded condition. Among different dates of planting, plant height followed the trend May>August>June>July.

At 30 DAP, plant height under 50per cent shade was 20.08 cm, as compared to 15.24 cm under open growing condition. Among different dates of planting, May planted crop recorded taller plants (19.81cm) and was on par with planting in August (18.69 cm). Shorter plants were observed when planted in June (15.56 cm) and were on par with the July planting (16.59 cm).

At 60 DAP also, the taller plants were recorded under 50per cent shade (28.20 cm) while plant height under open condition was 20.85 cm. Among different dates of planting, May planting resulted in taller plants (31.23 cm) and was on par with the August planting (27.34 cm). Shorter plants were observed in the July planting (19.72 cm) and were on par with the June planting (19.80 cm).

At the time of harvest, plant height under shaded condition was 32.22 cm. Under open condition the plant height was 25.38 cm. Among different dates of planting, May planting continued its superiority with respect to plant height (36.27 cm) and was on par with the August planting (31.15 cm). The shorter plants were observed in the July planting (23.56 cm) which were on par with the June planting (24.22 cm).

Number of branches per plant at 30 DAP, 60 DAP and at harvest

Comparing different growing conditions, the open grown plants produced the highest number of branches (2.06) followed by shade (1.58) at 30 DAP (Table 4). Among different dates of planting, May (2.36) planted crop had significantly higher number of branches which was on par with August planting (1.76).

At 60 DAP, the open condition followed the similar trend of higher number of branches (7.45) than shaded condition (5.53). Also, May (8.60) planted crop had the higher number of branches and was on par with August planting (7.08). Lower number of branches was observed in the July planting (4.93) which was on par with the June planting (5.35).

At the time of harvest, the open (8.25) planted crop recorded higher number of branches than shaded condition (6.47). Regarding the date of planting, May planted crop recorded higher number of branches (9.43) and was on par with August planting (7.96). Lower number of branches was observed in the July planted crop (5.51) which was on par with the June planted crop (6.53).

Number of leaves per plant at 30 DAP, 60 DAP and at harvest

During the first month of growth (at 30 DAP), plants grown in open condition showed significantly higher number of leaves per plant (43.08) as compared to plants grown under shaded condition (29.58) (Table 5). Among different dates of planting, May planted crop showed the higher number leaves per plant (50.84) and was on par with the August planting (39.16). The lower number of leaves was observed in the July planting (26.67) which was on par with the June planting (28.66).

At 60 DAP, the open condition followed the similar trend of higher number of leaves plant (164.83) as compared to plants grown under shaded condition (140.91). Among different dates of planting, May planted crop recorded higher number leaves per plant (210) and was on par with August planting (184.5). The lesser number of leaves were observed in the June planting (107.5) and it was on par with the July planting (109.5).

At the time of harvest, the open planted crop recorded the higher number of leaves per plant (245.67) as compared to plants grown under shaded condition (200.00). Among different dates of planting, May planted crop recorded the highest number of leaves per plant (315.32). The lower number of leaves was observed in the July planting (166) and was on par with the June planting (174.33).

Fresh weight of leaves at 30 DAP, 60 DAP and at harvest

During the first month of growth, plants grown in open condition showed significantly higher fresh weight of leaves per plant (10.62 g) as compared to plants grown under shaded condition (7.56 g) (Table 6). Among different dates of planting, May planted crop recorded the higher fresh weight of leaves per plant (12.54 g) and was on par with the August planting (9.50 g). The lower fresh weight of leaves was observed in the July planting (7.08 g) and was on par with the June planting (7.25 g).

At 60 DAP also, higher fresh weight of leaves per plant was observed under open condition (41.4 g) as compared to plants grown under shaded condition (32.93 g). Among different dates of planting, May planted crop recorded the higher fresh weight of leaves per plant (52 g) and was on par with August planting (44.35 g). The lower fresh weight of leaves was observed in June planting (26.04 g) and was on par with the July planting (26.29 g).

At the time of harvest, the open planted crop recorded a leaf fresh weight of 61.58 g/plant as compared to shaded condition (50.08 g/plant). Among different dates of planting, May planted crop recorded the highest fresh weight of leaves per plant (79.12 g). The lower fresh weight of leaves was observed in the July planting (41.58 g) and was on par with the June planting (43.66 g).

Table 3. Effect of growing condition and planting dates on plant height of stevia at 30 DAP, 60 DAP and at harvest

Treatments	Plant height (cm)		
	30 DAP	60 DAP	Harvest
Growing condition (Main plot)			
Open	15.24	20.85	25.38
50 % shade	20.08	28.20	32.22
CD (0.05)	1.39	2.10	4.27
Planting dates (Subplot)			
May15	19.81	31.23	36.27
June15	15.56	19.80	24.22
July15	16.59	19.72	23.56
August15	18.69	27.34	31.15
CD (0.05)	1.90	3.58	3.02

Table 4. Effect of growing condition and planting dates on number of branches of stevia at 30 DAP, 60 DAP and at harvest

Treatments	No. of branches		
	30 DAP	60 DAP	Harvest
Growing condition (Main plot)			
Open	2.06	7.45	8.25
50 % shade	1.58	5.53	6.47
CD (0.05)	0.28	0.87	1.38
Planting dates (Sub plot)			
May15	2.36	8.60	9.43
June15	1.67	5.35	6.53
July15	1.50	4.93	5.51
August15	1.76	7.08	7.96
CD (0.05)	0.31	0.83	1.33

Table 5. Effect of growing condition and planting dates on number of leaves/plant of stevia at 30 DAP, 60 DAP and at harvest

Treatments	No. of leaves/plant		
	30 DAP	**60 DAP**	**Harvest**
Growing condition (Main plot)			
Open	43.08	164.83	245.67
50 % shade	29.58	140.91	200.00
CD (0.05)	4.92	15.72	15.07
Planting dates (Sub plot)			
May15	50.84	210.0	315.32
June15	28.66	107.5	174.33
July15	26.67	109.5	166.00
August15	39.16	184.5	235.67
CD (0.05)	6.39	19.49	21.32

Table 6. Effect of growing condition and planting dates on fresh weight of leaves (g/plant) of stevia at 30 DAP, 60 DAP and at harvest

Treatments	Fresh weight of leaves (g/plant)		
	30 DAP	**60 DAP**	**Harvest**
Growing condition (Main plot)			
Open	10.62	41.41	61.58
50 % shade	7.56	32.93	50.08
CD (0.05)	1.82	5.17	8.54
Planting dates (Subplot)			
May15	12.54	52.00	79.12
June15	7.25	26.04	43.66
July15	7.08	26.29	41.58
August15	9.50	44.35	58.95
CD (0.05)	1.79	6.04	12.00

Dry weight of leaves at 30 DAP, 60 DAP and at harvest

Significantly higher dry weight of leaves per plant at 30 DAP was under open condition (5.95 g) as compared to shaded condition (4.20 g) (Table 7). Among different dates of planting, May planted crop recorded the higher dry weight of leaves per plant (7.02 g) and was on par with the August planting (5.50 g). The lower dry weight of leaves was observed in the July planting (3.72 g) and was on par with the June planting (4.06 g).

At 60 DAP, the open condition followed the similar trend of higher dry weight of leaves per plant (25.07 g). At this stage the dry weight of leaves under shaded condition was 17.72 g/plant. Among different dates of planting, planting in May resulted in higher dry weight of leaves per plant (30.04 g) and was on par with the August planting (24.52 g). The lower dry weight of leaves was observed in the July planting (15.17 g) and was on par with the June planting (15.85 g).

Dry weight of leaves at the time of harvest were 34.84 g/per plant and 28.04 g/plant, respectively under open and shaded conditions. Leaf dry weight in May planted crop was 44.21 g/plant. The lower dry weight of leaves was observed in the July planting (23.30g) which was on par with the June planting (25.25g)

Total herbage yield at 30 DAP, 60 DAP and at harvest

Data on the influence of growing conditions and dates of planting on herbage yield of stevia at harvest are given in Table 8. Among the two growing conditions, open condition recorded significantly higher herbage yield per plant (29.35 g) as compared to plants grown under shaded condition (23.91 g). Among different dates of planting, May planted crop showed the highest herbage yield (32.71 g). The lower herbage yield was observed in the July planting (23.45 g) and was on par with the June planting (23.48 g).

At 60 DAP, the open condition had significantly higher herbage yield per plant (103.66 g) as compared to plants grown under shaded condition (88.63 g). Among different dates of planting, May planted crop recorded the higher herbage yield per plant (115.08 g) and it is on par with the August planting (101.06 g). Lower herbage yield was observed in the June planting (84.09 g) followed by July planting (84.36 g) which were on par.

At the time of harvest, the herbage yield under open condition was 155.54 g/plant. Under shaded condition it was 131.41 g/plant only. May planted crop recorded higher herbage yield of 188.14 g/plant followed by planting in August (176.13 g). Planting in June or July significantly lowered the fresh biomass yield of stevia (103 g/plant and 106.42 g/plant, respectively).

Fresh biomass yield at 30 DAP, 60 DAP and at harvest

The direct effect of treatments on total fresh biomass production of stevia at 30 DAP is given in Table 9. The open condition resulted in the highest biomass yield (31.86 g) compared to shaded condition (26.11 g). Among different dates of planting, the May planted crop recorded the higher fresh biomass yield of 36.77 g and was on par with planting in August (29.13 g). The lower fresh biomass yield was observed in the June planting (25.02 g) and was on par with the July planting (25.03 g).

At 60 DAP, total fresh biomass yields under open and shaded conditions were 110.67 g/plant and 102.02 g/plant, respectively. Total biomass yield in May planted crop was 129.99 g/plant and was on par with planting in August (113.36 g/plant). The lower total fresh biomass yield was observed in the June planting (90.02 g/plant) followed by planting in July (92 g/plant).

Table 7. Effect of growing condition and planting dates on dry weight of leaves (g/plant) of stevia at 30 DAP, 60 DAP and at harvest

Treatments	Dry weight of leaves (g/plant)		
	30 DAP	60 DAP	Harvest
Growing condition (Main plot)			
Open	5.95	25.07	34.84
50 % shade	4.20	17.72	28.04
CD (0.05)	0.92	5.72	4.12
Planting dates (Sub plot)			
May15	7.02	30.04	44.21
June15	4.06	15.85	25.25
July15	3.72	15.17	23.30
August15	5.50	24.52	33.01
CD (0.05)	1.08	3.48	6.31

Table 8. Effect of growing condition and planting dates on total herbage yield (g/plant) of stevia at 30 DAP, 60 DAP and at harvest

Treatments	Herbage yield (g/plant)		
	30 DAP	60 DAP	Harvest
Growing condition (Main plot)			
Open	29.35	103.66	155.44
50 % shade	23.91	88.63	131.41
CD (0.05)	3.58	8.97	23.77
Planting dates (Sub plot)			
May15	32.71	115.08	188.14
June15	23.48	84.09	103.00
July15	23.45	84.36	106.42
August15	26.89	101.06	176.13
CD (0.05)	2.36	11.27	15.52

At the time of harvest, the open planted crop recorded the higher total fresh biomass yield per plant (172.23 g) as compared to plants grown under shaded condition (145.85 g). Among different dates of planting, May planted crop recorded the higher fresh biomass yield per plant (231.32 g) and was on par with the August planting (200.96 g). The lower total fresh biomass yield was observed in the June planting (126.29 g) and was on par with the July planting (130.35 g).

Dry biomass yield at 30 DAP, 60 DAP and at harvest

Total dry biomass yield also followed same trend of total fresh biomass yield with higher yields under open growing condition and in May planting (17.37 g/plant and 19.82 g/plant, respectively at 30 DAP) (Table 10). The total dry biomass yield under shaded condition was 15.10 g/ plant. Lower dry biomass yield was observed in the July planting (14.11 g/plant) and was on par with the June planting (14.96 g/plant).

At 60 DAP, total dry biomass yield per plant under open and shaded conditions were 63.47 g/plant and 53.68 g/plant, respectively. May planted crop recorded the higher total fresh biomass yield per plant (72.50 g/plant) followed by planting in August (63.97 g/plant). The lower total dry biomass yield was in June planting (48.16 g/plant) and was on par with the July planting (49.68 g/plant).

At the time of harvest, the open planted crop recorded the higher total dry biomass yield per plant (99.15 g) as compared to plants grown under shaded condition (81.21 g). Among different dates of planting, May planted crop recorded the higher dry biomass yield per plant (113.98 g) which was on par with the August planting (105.20 g). The lower total fresh biomass yield was observed in the June planting (69.17 g) and was on par with the July planting (72.37 g).

Table 9. Effect of growing condition and planting dates on fresh biomass yield of stevia at 30 DAP, 60 DAP and at harvest

Treatments	Fresh biomass yield (g/plant)		
	30 DAP	60 DAP	Harvest
Growing condition (Main plot)			
Open	31.86	110.67	172.23
50 % shade	26.11	102.02	145.85
CD (0.05)	3.69	7.46	17.81
Planting dates (Subplot)			
May15	36.77	129.99	231.32
June15	25.02	90.02	130.35
July15	25.03	92.00	126.29
August15	29.13	113.36	200.96
CD (0.05)	4.56	5.44	19.79

Table 10. Effect of growing condition and planting dates on dry biomass yield/plant of stevia at 30 DAP, 60 DAP and at harvest

Treatments	Dry biomass yield (g/plant)		
	30 DAP	60 DAP	Harvest
Growing condition (Main plot)			
Open	17.37	63.45	99.15
50 % shade	15.10	53.68	81.21
CD (0.05)	2.19	8.56	11.73
Planting dates (Sub plot)			
May15	19.82	72.50	113.98
June15	14.96	48.16	69.17
July15	14.11	49.68	72.37
August15	16.04	63.97	105.20
CD (0.05)	3.09	3.97	10.61

Total Biomass yield (kg/ha)

Table 11 depicts effect of growing condition and planting dates on total biomass yield of stevia (kg/ha). At the time of harvest, the open planted crop recorded the higher total fresh biomass yield (8406 kg/ha) as compared to plants grown under shaded condition (7328 kg/ha). Among different dates of planting, May planted crop recorded the higher biomass yield of 10162 kg/ha) and was on par with the August planting (9540 kg/ha). The lower total fresh biomass yield was observed in the June planting (5779 kg/ha) and it was on par with the July planting (5989 kg/ha).

Table 11. Effect of growing condition and planting dates on biomass yield of stevia (kg/ha)

Treatments	Biomass yield (kg/ha)
Growing condition (Main plot)	
Open	8406
50 % shade	7328
CD (0.05)	1302
Planting dates (Sub plot)	
May15	10162
June15	5779
July15	5989
August15	9540
CD (0.05)	1042

4.1.2 Physiological and biochemical observations

Crop growth rate

Table 12 shows data on crop growth rate (CGR) under various growing conditions during 0-30 DAP, 30-60 DAP and at 60 DAP-harvest stages. Plants grown in the open condition exhibited higher CGR (0.571 $g/m^2/day$) than those planted in the shaded condition (0.415 $g/m^2/day$) at 30 DAP. However, at 30-60 DAP, the data was non-significant. At 60 DAP-harvest, crop growth rate of plants cultivated in open condition (1.095$g/m^2/day$) was higher than that of plants grown in shade (0.784$g/m^2/day$).

Planting dates exhibited significant influence on CGR at 0-30 DAP, 30-60 DAP, and 60 DAP-harvest, with higher growth rates in May planted crop (0.653$g/m^2/day$) at 0-30 DAP, and at 30-60 DAP (1.772$g/m^2/day$). At 60 DAP-harvest, August planted crop recorded the highest growth rate (1.458$g/m^2/day$), followed by May planted crop (1.377$g/m^2/day$).

Relative growth rate

Data on relative growth rate (RGR) of stevia under different growing conditions during 0-30 DAP, 30-60 DAP and at 60 DAP-harvest are depicted in Table 12. The effect of growing conditions and planting dates on RGR at different growth stages was non significant.

Steviol glycoside content

Growing conditions and planting dates exhibited significant effect on total steviol glycoside content in stevia (Table 13). Under open conditions, the steviol glycoside content was 7.57 per cent as compared to shaded condition (5.09 per cent). Among different planting dates, steviol glycoside content was the highest in May planting (9.32 per cent). Lower contents (4.42 and 4.89 per cent) of steviol glycosides were observed in July and June planting. Planting in August had a steviol glycoside content of 6.69 per cent

Table 12. Effect of growing condition and planting dates on crop growth rate (CGR) ($g/m^2/day$) and relative growth (RGR) (g/g/day) of stevia at different growth stages

Treatments	0-30 DAP		30-60 DAP		60 DAP-Harvest	
	CGR	RGR	CGR	RGR	CGR	RGR
Growing condition						
Open	0.571	0.105	1.478	0.045	1.095	0.014
50 % shade	0.415	0.094	1.461	0.051	0.784	0.013
CD (0.05)	0.0737	NS	NS	NS	0.158	NS
Planting dates (Sub plot)						
May15	0.653	0.111	1.772	0.050	1.377	0.007
June15	0.491	0.093	1.228	0.044	0.440	0.016
July15	0.462	0.091	1.294	0.046	0.482	0.018
August15	0.527	0.104	1.584	0.052	1.458	0.013
CD (0.05)	0.103	NS	0.226	NS	0.435	NS

Table 13. Effect of growing conditions and planting dates on total steviol glycosides at harvest

Treatments	Total steviol glycoside content (%)
Growing condition (Main plot)	
Open	7.57
50 % shade	5.09
CD (0.05)	0.46
Planting dates (Sub plot)	
May15	9.32
June15	4.89
July15	4.42
August15	6.69
CD (0.05)	1.16

4.1.3 Interaction effect of growing condition and planting dates

4.1.3a Biometric observations

Plant height at 30, 60 DAP and at harvest

Data on interaction effect of growing condition and planting dates on plant height of stevia at 30 DAP, 60 DAP and at harvest are presented in Table 14. At all the growth stages the interaction was significant and at all stages of observation taller plants were observed in treatment combination May planting under shaded growing condition (22.79 cm, 35.54 cm and 40.30 cm, respectively at 30 DAP, 60 DAP and at harvest). It was followed by May planting in open growing condition (16.84 cm, 26.93 cm and 32.25 cm, respectively at 30 DAP, 60 DAP and at harvest). All the combinations in shade produces greater height than in the open. The shorter plants were noticed when planting was done either in June or July under open condition. The plant heights in treatment combination June planting under open were 13.57 cm, 17.34 cm and 22.38 cm, respectively at 30 DAP, 60 DAP and at harvest. Plant heights of 14.07 cm, 16.08 cm and 18.76 cm were observed when planting was done in July under open condition. All the combination in shade produced greater height than in the open condition.

Number of branches per plant at 30, 60 DAP and at harvest

Data on interaction effect of growing condition and planting dates on number of branches of stevia at 30 DAP, 60 DAP and at harvest are presented in the Table 15. There was no significant interaction between two factors studied with respect to number of branches per plant at 30 DAP. However, interaction was significant at 60 DAP and at harvest. At 60 DAP the highest number of branches were observed when planting was done in May under open growing condition (10.06 nos.). It was followed by August planting under open (7.80 nos.), May planting under open (7.13 nos.), August planting under shade (6.36 nos.) and June planting in open (6.30 nos.). At harvest stage higher number of branches were noticed in May planted crop under open (11.0 nos.), which was on par with August planting under open (8.2 nos.). The lower number of branches at 60 DAP and at harvest were noticed when planting was under done shaded condition both in June or July.

Number of leaves at 30 DAP, 60 DAP and at harvest

Data on interaction effect of shade and planting dates on number of leaves per plant of stevia at 30 DAP, 60 DAP and at harvest are presented in the Table 16. There was significant interaction between main plot and sub-plot. At all stages of observation higher number of leaves per plant was noticed in stevia planted in May under open growing condition (64.00, 232 and 357.66 nos., respectively at 30 DAP, 60 DAP and at harvest). It was on par with the crop planted in August under open condition (45.66, 195.66 and 256 nos., respectively at 30 DAP, 60 DAP and at harvest). But at the time of harvest the may planted plant at shade condition has 273 nos. Lower number of leaves at 30 DAP was recorded in June planting (23.33, 95.33 and 155.66 nos., respectively at 30 DAP, 60 DAP and at harvest) under shade and was on par with the July planting (24.66, 107 and 156 nos., respectively at 30 DAP, 60 DAP and at harvest) under shade.

Fresh weight of leaves at 30 DAP, 60 DAP and at harvest

The interaction was significant for fresh weight of leaves at 30 DAP, 60 DAP and at harvest (Table 17). At 30 DAP, 60 DAP and at harvest higher fresh weight of leaves per plant was noticed in the treatment combination of planting in May under open condition (15.58, 56.42 and 89.91 g/plant, respectively). Lower fresh weight of leaves was recorded in June planting (5.58, 21.83 and 39.08 g/plant, respectively at 30 DAP, 60 DAP and at harvest) under 50 per cent shade and was on par with the July planting (6.99, 23.16 and 39.16 g/plant, respectively) under 50 per cent shade.

Table 14. Interaction effect of growing condition and planting dates on plant height of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	Plant height (cm)		
	30 DAP	60 DAP	Harvest
Open × May	16.84	26.93	32.25
Open × June	13.57	17.34	22.38
Open × July	14.07	16.08	18.76
Open × August	16.49	23.06	28.14
Shade × May	22.79	35.54	40.30
Shade × June	17.55	22.26	26.06
Shade × July	19.11	23.36	28.36
Shade × August	20.89	31.63	34.17
CD (0.05)	2.12	4.08	6.36

Table 15. Interaction effect of growing condition and planting dates on number of branches of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	No. of branches		
	30 DAP	60 DAP	Harvest
Open × May	2.86	10.06	11.00
Open × June	1.86	6.30	7.76
Open × July	1.53	5.63	6.03
Open × August	2.00	7.80	8.20
Shade × May	1.86	7.13	7.86
Shade × June	1.46	4.40	5.30
Shade × July	1.46	4.23	5.00
Shade × August	1.53	6.36	7.73
CD (0.05)	NS	2.13	2.94

Table 16. Interaction effect of growing condition and planting dates on number of leaves of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	No. of leaves/plant		
	30 DAP	60 DAP	Harvest
Open × May	64.00	232.00	357.66
Open × June	34.00	119.66	193.00
Open × July	28.66	112.00	176.00
Open × August	45.66	195.66	256.00
Shade × May	37.66	188.00	273.00
Shade × June	23.33	95.33	155.66
Shade × July	24.66	107.00	156.00
Shade × August	32.67	173.33	215.33
CD (0.05)	18.68	38.36	86.44

Table 17. Interaction effect of growing condition and planting dates on fresh weight of leaves of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	Fresh weight of leaves (g/plant)		
	30 DAP	60 DAP	Harvest
Open × May	15.58	56.42	89.91
Open × June	8.92	30.25	48.25
Open × July	7.17	29.42	44.00
Open × August	10.83	49.54	64.16
Shade × May	9.50	47.58	68.33
Shade × June	5.58	21.83	39.08
Shade × July	6.99	23.16	39.16
Shade × August	8.16	39.17	53.75
CD (0.05)	4.92	8.05	22.43

Dry weight of leaves at 30 DAP, 60 DAP and at harvest

The interaction was significant for dry weight of leaves at 30 DAP, 60 DAP and at harvest (Table 18). At 30 DAP, 60 DAP and at harvest higher fresh weight of leaves per plant was noticed in the treatment combination of planting in May under open condition (8.73, 31.59 and 50.16 g/plant, respectively) Lower fresh weight of leaves was recorded in June planting (3.12 ,12.88 and 21.88 g/plant, respectively at 30 DAP, 60 DAP and at harvest) under 50 per cent shade and was on par with the July planting (3.44, 12.97 and 21.93 g/plant, respectively) under 50 per cent shade

Total herbage yield of stevia at 30 DAP, 60 DAP and at harvest

Table 19 depicts data on interaction effect of growing condition and planting dates on fresh herbage yield per plant of stevia at 30 DAP, 60 DAP and at harvest. Higher herbage yields of 36.68, 127.35 and 202.68 g/plant, respectively at 30 DAP, 60 DAP and at harvest was observed when stevia was planted under open condition in the month of May. It was on par with planting in August under open growing condition (29.60, 109.35 and 189.10 g/plant, respectively). Lower fresh herbage yields were reordered when planting was done either in June or July under shaded condition (20.91, 78.37 and 91.40 g/plant, respectively at 30 DAP, 60 DAP and at harvest in June planting under shade, and 21.83, 80.58 and 97.48 g/plant, respectively at 30 DAP, 60 DAP and at harvest in July planting under shade.

Table 18. Interaction effect of growing condition and planting dates on dry weight of leaves /plant of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	Dry weight of leaves (g/plant)		
	30 DAP	60 DAP	Harvest
Open × May	8.73	31.59	50.16
Open × June	4.99	16.75	28.62
Open × July	4.00	15.72	24.67
Open × August	6.07	27.44	35.93
Shade × May	5.31	28.03	38.26
Shade × June	3.12	12.88	21.88
Shade × July	3.44	12.97	21.93
Shade × August	4.92	24.96	30.10
CD (0.05)	1.53	5.85	8.92

Table 19. Interaction effect of growing condition and planting dates on total herbage yield of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	Total herbage yield (g/plant)		
	30 DAP	60 DAP	Harvest
Open × May	36.68	127.35	202.68
Open × June	26.06	89.82	114.60
Open × July	25.07	88.13	115.37
Open × August	29.60	109.35	189.10
Shade × May	28.73	102.80	173.59
Shade × June	20.91	78.37	91.40
Shade × July	21.83	80.58	97.48
Shade × August	24.18	92.78	163.17
CD (0.05)	7.25	18.10	13.68

Fresh biomass yield at 30 DAP, 60 DAP and at harvest

Data on interaction effect of growing condition and planting dates on total fresh biomass yield per plant of stevia at 30 DAP, 60 DAP and at harvest are presented in Table 20. As with other biometric observations, higher fresh biomass yield per plant was also higher in the treatment combination May planting under open condition (40.41, 138.87 and 231.32 g/plant, respectively). At 30 DAP and 60 DAP, it was on par with the May planting under shade condition (33.13 and 121.12 g/plant, respectively) and at harvest it was on par with the August planting under open condition (200.96 g/plant). At all stages of observation lower fresh biomass yield per plant was recorded in June planting (21.25, 85.83 and 112.53 g/plant, respectively at 30 DAP, 60 DAP and at harvest) under 50 per cent shade and was on par with the July planting (23.20, 90.40 and 119.01 g/plant, respectively at 30 DAP, 60 DAP and at harvest) under 50 per cent shade.

Total dry biomass yield at 30 DAP, 60 DAP and at harvest

Data on interaction effect of growing condition and planting dates on total dry biomass yield per plant of stevia at 30 DAP, 60 DAP and at harvest are presented in Table 21. As like other biometric observations, higher dry biomass yield per plant was observed in the treatment combination of May planting under open condition (21.09, 77.21 and 125.36 g/plant, respectively). At 30 DAP and 60 DAP, it was on par with the May planting under shaded condition (18.55 and 68.77 g/plant, respectively) and at harvest it was on par with the August planting under open condition (115.91 g/plant). At all stages of observation lower fresh biomass yield per plant was recorded in June planting (13.80, 50.97 and 62.22 g/plant, respectively at 30 DAP, 60 DAP and at harvest) under 50 per cent shade and was on par with the July planting (13.17, 53.07 and 65.52 g/plant, respectively at 30 DAP, 60 DAP and at harvest) under 50 per cent shade.

Table 20. Interaction effect of growing condition and planting dates on fresh biomass yield/plant of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	Fresh biomass yield (g/plant)		
	30 DAP	60 DAP	Harvest
Open × May	40.41	138.87	231.32
Open × June	28.79	94.21	130.35
Open × July	26.87	93.60	126.29
Open × August	31.38	116.00	200.96
Shade × May	33.13	121.12	183.92
Shade × June	21.25	85.83	112.53
Shade × July	23.20	90.40	119.01
Shade × August	26.87	110.72	167.92
CD (0.05)	9.83	18.20	30.98

Table 21. Interaction effect of growing condition and planting dates on total dry biomass yield/plant of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	Total dry biomass yield (g/plant)		
	30 DAP	60 DAP	Harvest
Open × May	21.09	77.21	125.36
Open × June	16.11	52.62	67.79
Open × July	15.03	52.77	69.22
Open × August	17.23	64.29	115.91
Shade × May	18.55	68.77	103.27
Shade × June	13.80	50.97	62.22
Shade × July	13.17	53.07	65.52
Shade × August	14.86	62.86	98.73
CD (0.05)	4.38	8.69	16.58

Biomass yield (kg/ha)

Data on interaction effect of shade and planting dates on total fresh biomass yield (kg/ha) of stevia at harvest are presented in Table 22. The interaction was significant and there was interaction between main plot and sub-plot. At harvest, higher fresh biomass yield per plant was noticed in the treatment combination of May planting under open condition (11143kg/ha) and it is on par with the August planting under open condition (10303kg/ha). Lower fresh biomass yield per plant was recorded in June planting (5531kg/ha) grown under 50 per cent shade and it was on par with the July planting (5824kg/ha) grown under 50 per cent shade.

Table 22. Interaction effect of growing condition and planting dates on biomass yield (kg/ha) of stevia at 30 DAP, 60 DAP and at harvest

Growing condition × planting dates	Biomass yield (Fresh) (kg/ha)
Open × May	11143
Open × June	6026
Open × July	6153
Open × August	10303
Shade × May	9180
Shade × June	5531
Shade × July	5824
Shade × August	8777
CD (0.05)	1473.97

4.1.3bPhysiological and biochemical observations

Crop growth rate

Data on the interaction effect of growing condition and planting dates on crop growth rate are presented in Table 23. At 0-30 DAP, 30-60 DAP and at 60-harvest, the interaction effect on crop growth rate was non-significant.

Relative growth rate

Table 24 shows data on the interaction effect of growing condition and planting date on and relative growth rate of stevia at different growth stages. The interaction was non significant at all growth stages were observed.

Total steviol glycoside content

Table 25 depicts data on the interaction effect of growing condition and planting dates on total steviol glycoside content of stevia at harvest stage. Planting in May under open condition resulted in the highest steviol glycoside content of 9.05 per cent. It was followed by August planting in open condition (8.19 per cent) and May planting in shaded condition (7.60 per cent) which was on par. July planting in shaded conditions had lower steviol glycoside content (3.64 per cent) and was on par to June planting in shaded condition (3.93 per cent).

Table 23. Interaction effect of growing condition and planting dates on crop growth rate of stevia at different growth stages

Treatments	CGR ($g/m^2/day$)					
	0-30 DAP		30-60 DAP		60 DAP-Harvest	
	Open	50 % shade	Open	50 % shade	Open	50 % shade
May	0.69	0.61	1.87	1.67	1.60	1.15
June	0.52	0.45	1.21	1.23	0.50	0.37
July	0.49	0.43	1.25	1.33	0.54	0.41
August	0.56	0.48	1.56	1.60	1.72	1.19
CD(0.05)	NS		NS		NS	

Table 24. Interaction effect of growing condition and planting dates on relative growth rate of stevia at different growth stages

Treatments	RGR(g/g/day)					
	0-30DAP		30-60DAP		60 DAP -Harvest	
	Open	50% shade	Open	50% shade	Open	50% shade
May	0.108	0.103	0.043	0.056	0.015	0.011
June	0.103	0.085	0.041	0.048	0.018	0.018
July	0.090	0.088	0.047	0.045	0.015	0.018
August	0.100	0.101	0.050	0.055	0.009	0.006
CD(0.05)	NS		NS		NS	

Table 25. Interaction effect of growing condition and planting dates on total steviol glycoside content at harvest

Treatment combination	Total steviol glycoside content at harvest (%)
Open × May	9.05
Open × June	5.86
Open × July	5.20
Open × August	8.19
Shade × May	7.60
Shade × June	3.93
Shade × July	3.64
Shade × August	5.20
CD (0.05)	1.64

4.1.4 Weather observations

Tables 26 and 27 shows the various weather parameters experienced in the field from planting to harvest of stevia. Weather characteristics such as maximum and minimum temperature, relative humidity, rainfall, and bright sunshine hours were recorded and presented both as weekly (Table 26) and monthly weather data (Table 27).

Maximum and minimum temperature

The average maximum temperature ranged from 28^{o}C to 34.4^{o}C in open growing condition, and 26.8^{o}C to 33.2^{o}C in shaded conditions. In the open condition, the highest maximum temperature was recorded on the second week of May, which was 34.4^{o}C and the lowest maximum temperature was recorded during 13th week (August) which was 28.0^{o}C. Under shaded condition it followed the similar pattern as open condition. The highest maximum temperature was recorded on the second week of May, which was 33.2^{o}C and the lowest maximum temperature was recorded on 13th week (August) which was 26.8^{o}C.

The average minimum temperature ranged from 21.1^{o}C to 26.2^{o}C in open situation, and 20.9^{o}C to 26.0^{o}C in shaded condition. In the open condition the highest minimum temperature was recorded on the second week of May, which was 26.2^{o}C and the lowest minimum temperature in 24th week (October) which was 21.1^{o}C. Under shaded condition it followed the similar pattern as open condition. The highest minimum temperature was in the 2nd week of May (26^{o}C) and the lowest minimum temperature in 24th week (October) which is 20.9^{o}C.

Relative Humidity

In the morning, the relative humidity (RH) ranged from 74 per cent to 98 per cent in open condition, and 76 per cent to 99 per cent under the shade. The maximum morning relative humidity of 99 percent occurred in the

12thweek in the shaded condition, while the lowest occurred in the 20th week in the open condition.

In both open and shaded conditions, the 26th week had the lowest afternoon relative humidity (53 percent and 55 percent, respectively under open and shade). The afternoon RH ranged from 53 per cent to 95 per cent.

Rainfall

The year 2020 was characterized by heavy showers. In the 13th week, the highest rainfall of 378.9 mm was received. There was no rain during 25th week. The total amount of rain that fell during the cropping season was 2676.7 mm.

Bright sunshine hours

The 16th week had the highest sunlight hours (55), while the 13th week had the least (0.1).

Table 26. Weekly weather data during the cropping period

Weeks	Maximum temperature (°C)		Minimum temperature (°C)		Forenoon Relative humidity (%)		Afternoon Relative humidity (%)		Rainfall (mm)	Bright sunshine hours
	Open	Shade	Open	Shade	Open	Shade	Open	Shade		
May 2020										
1	33.9	32.7	24.2	24.0	94	96	70	73	49.2	32.4
2	34.4	33.2	26.2	26.0	91	93	65	68	7.5	45.0
3	33.0	31.8	24.9	24.7	90	92	70	73	23.6	30.9
June										
4	31.2	30.0	24.3	24.1	94	96	79	82	74.7	14.6
5	31.4	30.2	23.2	23.0	96	98	76	79	125.9	14.3
6	30.7	29.5	23.5	23.3	96	98	75	78	92.1	12.1
7	31.1	29.9	23.4	23.2	92	94	71	74	119.9	25.4
July										
8	29.9	28.7	23.3	23.1	97	98	87	90	163.5	3.8
9	31.0	29.8	23.2	23.0	95	97	71	74	76.0	23.0
10	30.2	29	23.2	23.0	95	97	81	84	84.9	17.1
11	31.5	30.3	23.3	23.1	95	97	73	76	72.3	35.9
12	28.7	27.5	23.5	23.3	98	99	83	86	332.0	5.6
August										
13	28.0	26.8	22.7	22.5	96	98	92	95	378.9	0.1
14	30.5	29.3	23.3	23.1	96	98	75	78	48.1	12.6
15	31.4	30.2	23.5	23.3	96	98	71	74	73.0	38.2
16	32.3	31.1	23.2	23.0	94	96	63	66	12.8	55.0

September										
17	31.3	30.1	22.7	22.5	83	85	88	91	138.1	22.4
18	28.1	26.9	22.2	22.0	86	88	92	95	210.2	3.6
19	29.1	27.9	21.9	21.7	80	82	89	92	206.1	8.1
20	30.7	29.5	22.7	22.5	74	76	85	88	22.0	23.8
October										
21	31.2	30.0	21.7	21.5	94	96	65	68	17.8	53.3
22	30.3	29.1	21.6	21.4	97	99	78	81	187.0	20.0
23	30.1	28.9	21.5	21.3	96	98	74	77	96.3	33.8
24	31.4	30.2	21.1	20.9	94	96	64	67	9.2	40.4
25	33.0	31.8	22.5	22.3	94	96	62	65	0.0	38.0
November										
26	33.7	32.5	22.0	21.8	86	88	53	55	46.8	54.2
27	32.9	31.7	22.6	22.4	79	81	62	65	8.8	37.5

Table 27. Monthly weather data during the cropping period

Months	Maximum Temperature (°C)		Minimum Temperature (°C)		Forenoon relative humidity (%)		Afternoon relative humidity (%)		Rainfall (mm)	Bright Sunshine hours
	Open	Shade	Open	Shade	Open	Shade	Open	Shade		
May	33.76	32.56	25.10	24.90	91.66	93.66	68.33	71.33	80.3	108.3
June	31.10	29.90	23.60	23.40	94.50	96.50	75.25	78.25	412.6	66.4
July	30.26	29.06	23.30	23.10	96.00	98.00	79.00	82.00	728.7	85.4
August	30.55	29.35	23.17	22.97	95.50	97.50	75.25	78.25	512.8	105.9
September	29.80	28.60	22.37	22.17	80.75	82.75	88.50	91.50	576.4	57.9
October	31.20	30.00	21.68	21.48	95.00	97.00	68.60	71.60	310.3	185.5
November	33.30	32.10	22.30	22.10	82.50	84.50	57.50	60.00	55.6	91.7

Crop weather correlations

A simple linear correlation between major morphological, yield, and quality parameters and mean monthly meteorological weather parameters such surface air temperature, relative humidity (forenoon and afternoon), rainfall, and bright sunshine hours was done and the coefficients are presented in Table 28.

Plant height

A strong positive correlation was observed between plant height and maximum and minimum temperatures (0.897 and 0.522). Forenoon relative humidity exhibited no effect on the plant height whereas; afternoon relative humidity had negative correlation with the plant height. Rainfall exhibited negative correlation with the plant height (-0.792). No significant correlation was observed between bright sunshine and plant height.

Number of leaves per plant

Positive correlation was observed between number of leaves per plant and maximum temperature (0.843). Minimum temperature also positively correlated with the number of leaves (0.629). Forenoon relative humidity had no effect on number of leaves per plant. However, afternoon relative humidity exhibited negative correlation with the number of leaves (-0.70). Rainfall also exhibited negative correlation with the number of leaves (-0.708).

Fresh leaf weight

There was a positive correlation between fresh leaf weight and maximum temperature (0.842). Minimum temperature also positively influenced the fresh leaf weight (0.631). While forenoon relative humidity had no effect on the fresh leaf weight, the afternoon relative humidity exhibited significant negative influence on the fresh leaf weight (-0.698).

Rainfall also exhibited negative correlation with the fresh leaf weight (-0.705). Bright sunshine hours had no effect on fresh leaf weight.

Biomass yield

The correlation between maximum temperature and biomass yield was significant with a correlation coefficient of 0.895. Minimum temperature and forenoon relative humidity showed no correlation with biomass yield. Afternoon relative humidity had negative correlation with the biomass yield (-0.848). Correlation between rain fall and biomass yield was strongly negative (-0.852).

Steviol glycoside content

A positive correlation was observed between total steviol glycoside content and maximum temperature (0.874). Minimum temperature and forenoon relative humidity showed no correlation with the total steviol glycosides. Afternoon relative humidity had negative correlation with the steviol glycoside content (-0.789). Rainfall also exhibited negative correlation with the steviol glycoside content (-0.790).

4.1.5 Incidence of pests and diseases

During the cropping period, no major disease or insect damage was observed in the experimental area.

Table 28. Crop weather correlations

	Maximum temperature	Minimum temperature	Forenoon relative humidity	Afternoon relative humidity	Rainfall	Bright sunshine hours
Plant height	0.879**	0.522*	0.272	-0.786**	-0.792**	0.463
No. of leaves	0.843**	0.629**	0.401	-0.700**	-0.708**	0.350
Fresh leaf weight	0.842**	0.631**	0.405	-0.698**	-0.705**	0.347
Total fresh biomass yield	0.895**	0.424	0.162	-0.848**	-0.852**	0.553
Total steviol glycoside content	0.874**	0.492	0.190	-0.789**	-0.790**	0.461

**** Significant at 5% level**

4.2 Phytochemical evaluation of stevia leaves at different growth stages

4.2.1Phytochemical parameters of stevia leaves at different growth stages

Phytochemical parameters such as total carbohydrates, total fat, crude fibre, total protein, total soluble sugars, reducing sugars, non reducing sugars, total mineral content, content of N, P, K, Ca, Mg, S, total steviol glycoside content, moisture content and total energy of stevia leaves at different growth stages are presented in Table 29.

Total carbohydrates (%)

The total carbohydrates content in stevia leaves ranged from 0.62 per cent to 0.73 per cent with maximum at flower bud initiation stage. The lower content of 0.62 per cent was noticed at 45 DAP and the higher content was at flower bud initiation stage (0.73 per cent). After flower bud initiation stage slight reduction in carbohydrate content was observed in stevia leaves (0.69 per cent at 50 per cent flowering stage and 0.70 per cent at full bloom stage).

Total fat (%)

Total fat content ranged from 0.44 per cent to 0.56 per cent with maximum content at flower bud initiation stage (0.56 per cent). After flower bud initiation stage a gradual reduction in fat content was observed.

Crude fibre (%)

The highest content of crude fibre was observed at full bloom stage (7.79 per cent) followed by 50 per cent flowering stage (7.26 per cent). The lower fibre content was at 45 DAP (5.56 per cent).

Moisture content (%)

The moisture content ranged from 11.30 per cent to 15.64 per cent with maximum content at 45 DAP. Moisture content reduced with increase in crop maturity.

Total protein (%)

Total protein content in leaves increased from 45 DAP to flower bud initiation stage and there after it decreased. The lowest content of 5.24 per cent protein was observed at 45 Dap and the highest content of 6.04 per cent at flower bud initiation stage.

Mineral content (%)

The total mineral content increased from 13.29 per cent at 45 DAP to 16.82 per cent at full bloom stage. The content of nitrogen was higher at flower initiation stage (2.55 per cent). There was no statistical difference with respect to contents of P, K, Ca, Mg and S at various stages of observation.

Total soluble sugars (%)

Total soluble sugar content ranged from 11.62 per cent to 21.05 per cent. The total soluble sugar content increased from 45 DAP (16.31 per cent) to flower bud initiation stage (21.05 per cent) and thereafter decreased with maturity. At 50 per cent flowering stage the total soluble sugar content was 12.80 per cent and at full bloom stage it was 11.62 per cent.

Reducing sugars (%)

Reducing sugar content ranged between 7.52 per cent and 12.16 per cent. The lowest content was at full bloom stage. Similar to total soluble sugar reducing sugar content also increased from 45 DAP, reached peak at flower bud initiation stage and thereafter decreased (10.03, 12.06, 8.03 and 7.52 per cent, respectively at 45 DAP, flower bud initiation stage, 50 per cent flowering and at full bloom stage).

Non reducing sugars (%)

Non reducing sugars also followed same trend of total soluble sugars and reducing sugars with maximum content at flower bud initiation stage. The range of non reducing sugars was 4.08 per cent to 8.93 per cent (6.15, 8.93, 4.76 and 4.08, respectively at 45 DAP, flower bud initiation stage, 50 per cent flowering and at full bloom stage).

Total energy (Kcal)

Significant variation was observed total energy content of stevia leaves based on their stage of harvesting. The total energy ranged from 27.37 Kcal to 32.12 Kcal. The lowest energy was at 45 DAP (27.37 Kcal), reached peak at flower bud initiation (32.12 Kcal) and decreased thereafter (29.86 Kcal at 50 per cent flowering and 29.10 Kcal at full bloom stage).

Total steviol glycoside content (%)

The highest steviol glycoside content of 7.36 per cent was observed at flower bud initiation stage. The glycoside content decreased with increase in maturity. At 45 DAP the content was 6.82 per cent. At 50 per cent flowering stage and at full bloom stage the content of steviol glycosides were 5.20 and 4.71 per cent, respectively.

Table 29. Effect of stages of harvest on phytochemical parameters of stevia leaves

Treatments	Total carbohydrates (%)	Total fat (%)	Crude fibre (%)	Moisture content (%)	Total protein (%)	Total energy (Kcal)	Total mineral content (%)	Total soluble sugars (%)	Reducing Sugars (%)	Non- reducing sugars (%)
45 DAP	0.62	0.44	5.56	15.64	5.24	27.37	13.29	16.31	10.03	6.15
Flower bud initiation	0.73	0.56	6.17	15.58	6.04	32.12	15.08	21.05	12.16	8.93
50 % flowering	0.69	0.47	7.26	11.45	5.73	29.86	16.17	12.80	8.03	4.76
Full bloom stage	0.70	0.42	7.79	11.30	5.63	29.10	16.82	11.62	7.52	4.08
CD(0.05)	0.027	0.032	0.084	0.383	0.075	0.092	0.069	0.074	0.095	0.284

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulphur (%)	Steviol glycosides (%)
45 DAP	2.10	0.17	0.40	0.35	0.25	0.13	6.82
Flower initiation	2.55	0.18	0.45	0.38	0.26	0.15	7.36
50% flowering	2.35	0.22	0.43	0.40	0.46	0.17	5.20
Full bloom stage	2.28	0.23	0.42	0.41	0.42	0.18	4.71
CD(0.05)	0.147	NS	NS	NS	NS	NS	0.12

4.2.2 Phytochemical parameters of stevia leaves under different growing conditions

Phytochemical parameters such as total carbohydrates, total fat, crude fibre, total protein, total soluble sugars, reducing sugars, non reducing sugars, total mineral content, content of N, P, K, Ca, Mg, S, total steviol glycoside content, moisture content and total energy of stevia leaves in open and shaded growing conditions at 45 DAP, flower bud initiation, 50 per cent flowering and at full bloom stage are presented in tables 30, 31, 32 and 33, respectively.

Total carbohydrates (%)

At all stages of observation higher content of total carbohydrates was observed under shaded growing condition. However, significant difference was observed only at 45 DAP. At this stage total carbohydrate content under open condition was 0.64 per cent as compared to 0.73 per cent under shade (Table 30). At flower bud initiation stage carbohydrate content under open condition and shade were 0.70 and 0.80 per cent, respectively (Table 31). At 50 per cent flowering stage the content decreased to 0.65 per cent and 0.72 per cent, respectively under open and shade (Table 32). At full bloom stage the contents were 0.65 per cent and 0.75 per cent, respectively under open and shaded growing conditions (Table 33).

Total fat (%)

Total fat content of stevia leaves did not show any significant difference under open and shaded condition. However, at all stages of observation higher values were observed under open condition 0.48, 0.60, 0.52 and 0.46 per cent, respectively at 45 DAP, flower bud initiation, 50 per cent flowering and at full bloom stage under open conditions, and 0.40. 0.48, 0.43 and 0.40 per cent, respectively at 45 DAP, flower bud initiation, 50 per cent flowering and at full bloom stage under shade conditions.

Crude fibre (%)

Significant variation was observed in crude fibre content of leaves under open and shade. At all stages of observation, higher crude fibre content was observed under open condition (5.12, 6.05, 7.12 and 7.86 per cent, respectively at 45 DAP, flower bud initiation, 50 per cent flowering and at full bloom stage and 4.95, 5.20, 6.45 and 6.79 per cent, respectively at 45 DAP, flower bud initiation, 50 per cent flowering and at full bloom stage under shaded conditions.

Moisture content (%)

Higher moisture content was observed under shade. At 45 DAP the moisture content under open condition was 14.37 per cent and under shade it was 16.85 per cent. At flower bud initiation stage, the moisture content decreased to 12.92 per cent and 15.30 per cent, respectively under open and shade. Moisture content at 50 per cent flowering stage was 10.56 per cent under open and 12.33 per cent under shade. During full bloom stage moisture content under open condition was 10.21 per cent and under shade was 11.89 per cent.

Total protein (%)

At all stages of observation higher protein content was observed under open condition. The protein content was 6.11, 7.15, 6.42 and 6.37 per cent, respectively at 45 DAP, flower bud initiation, 50 per cent flowering and at full bloom stage under open condition. Under shade corresponding values were 4.37, 4.94, 5.02 and 4.88 per cent.

Mineral content (%)

In general, growing stevia under open condition resulted in higher total mineral content. Total mineral content at 45 DAP was 14.22 and 12.37 per cent, respectively at open and shade. The total mineral content at flower bud initiation was 16.60 per cent under open and 13.56 per cent under shade.

At 50 per cent flowering stage, it was 17.08 per cent and 15.24 per cent and at full bloom stage total mineral content was 17.86 under open and 15.72 per cent under shade. Regarding contents of individual elements, effect of growing conditions on N, P, K, Ca, Mg and S contents was non significant at all stages of observation.

Total soluble sugars (%)

Total soluble sugar content was higher under open condition at all stages of observation. Under open condition the contents of total soluble sugars were 17.08, 22.76, 13.39 and 7.98 per cent, respectively at 45 DAP, flower bud initiation, 50 per cent flowering and at full bloom stages. Under shaded condition the values were 15.53, 19.31, 12.28 and 11.11, respectively at different stages of observation.

Reducing sugars (%)

As in the case of total soluble sugars, content of reducing sugar was also found higher under open condition. The reducing sugar content at 45 DAP was 10.41 and 9.58 per cent under open and shaded growing condition. At flower bud initiation stage the content increased to 13.06 and 11.20 per cent,respectively under open and shade. At 50 per cent flowering and at full bloom stage the contents were 8.43 and 7.72 per cent under open and 7.98 and 7.13 per cent under shade.

Non reducing sugars (%)

Data on non reducing sugars were significant at 45 DAP and at flower bud initiation stages with higher values under open growing condition. At 45 DAP and at flower bud initiation stages the non reducing sugar content under open condition was 6.67 per cent and 9.70 per cent, respectively. While under shaded condition the values were 5.95 per cent and 8.11 per cent, respectively.

Total energy (Kcal)

Significant variation was observed in total energy content of stevia leaves based on their growing condition. At all stages of observation higher total energy was computed for open condition. At 45 DAP the total energy under open condition was 32.64 Kcal as compared to 24.0 under shade. At flower bud initiation stage the total energy under open condition was 36.80 kcal and under shade it was 27.28 Kcal. At 50 per cent flowering and at full bloom stage the total energy under open condition was 32.96 Kcal and 32.22 Kcal, respectively. Under shaded condition it was 26.83 Kcal and 25.92Kcal, respectively at 50 per cent flowering and at full bloom stage.

Total steviol glycoside content (%)

Open growing condition resulted in higher concentration of total steviol glycosides. At all stages of observation, the data was significant. The steviol glycoside content under open growing condition at 45 DAP, flower bud initiation stage, 50 per cent flowering stage and full bloom stages were 7.72, 8.55, 5.73 and 5.02 per cent, respectively. Glycoside contents of 5.91, 6.16, 4.68 and 4.42 per cent, respectively were recorded at 45 DAP, flower bud initiation stage, 50 per cent flowering stage and full bloom stages under shaded condition.

Table 30. Effect of growing condition on phytochemical parameters of stevia leaves at 45 DAP

Treatments	Total carbohydrates (%)	Total fat (%)	Crude fibre (%)	Moisture content (%)	Total protein (%)	Total energy (Kcal)	Total mineral content (%)	Total soluble sugars (%)	Reducing Sugars (%)	Non-reducing sugars (%)
Open	0.64	0.48	5.12	14.37	6.11	32.64	14.22	17.08	10.41	6.67
Shade	0.73	0.40	4.95	16.85	4.37	24.0	12.37	15.53	9.58	5.95
CD (0.05)	0.05	NS	0.14	0.31	0.25	0.27	0.48	0.29	0.06	0.13

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulphur (%)	Steviol glycosides (%)
Open	2.2	0.18	0.41	0.37	0.24	0.14	7.72
Shade	2.1	0.17	0.39	0.32	0.23	0.11	5.91
CD (0.05)	NS	NS	NS	NS	NS	NS	0.048

Table 31. Effect of growing condition on phytochemical parameters of stevia leaves at flower bud initiation stage

Treatments	Total carbohydrates (%)	Total fat (%)	Crude fibre (%)	Moisture content (%)	Total protein (%)	Total energy (Kcal)	Total mineral content (%)	Total soluble sugars (%)	Reducing Sugars (%)	Non-reducing sugars (%)
Open	0.70	0.60	6.05	12.92	7.15	36.8	16.60	22.76	13.06	9.70
Shade	0.80	0.48	5.20	15.30	4.94	27.28	13.56	19.31	11.20	8.11
CD (0.05)	NS	NS	0.88	1.30	0.867	2.56	1.85	2.08	2.47	0.89

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulphur (%)	Steviol glycosides (%)
Open	2.6	0.20	0.46	0.40	0.26	0.18	8.55
Shade	2.4	0.17	0.44	0.35	0.25	0.14	6.16
CD (0.05)	NS	NS	NS	NS	NS	NS	1.19

Table 32. Effect of growing condition on phytochemical parameters of stevia leaves at 50 per cent flowering stage

Treatments	Total carbohydrates (%)	Total fat (%)	Crude fibre (%)	Moisture content (%)	Total protein (%)	Total energy (Kcal)	Total mineral content (%)	Total soluble sugars (%)	Reducing Sugars (%)	Non-reducing sugars (%)
Open	0.65	0.52	7.12	10.56	6.42	32.96	17.08	13.39	8.43	4.96
Shade	0.72	0.43	6.45	12.33	5.02	26.83	15.24	12.28	7.72	4.56
CD (0.05)	NS	NS	0.89	1.02	0.97	2.48	1.07	0.99	0.51	NS

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulphur (%)	Steviol glycosides (%)
Open	2.5	0.23	0.44	0.42	0.45	0.20	5.73
Shade	2.2	0.21	0.41	0.38	0.46	0.18	4.68
CD (0.05)	NS	NS	NS	NS	NS	NS	0.82

Table 33. Effect of growing condition on phytochemical parameters of stevia leaves at full bloom stage

Treatments	Total carbohydrates (%)	Total fat (%)	Crude fibre (%)	Moisture content (%)	Total protein (%)	Total energy (Kcal)	Total mineral content (%)	Total soluble sugars (%)	Reducing Sugars (%)	Non-reducing sugars (%)
Open	0.65	0.46	7.86	10.21	6.37	32.22	17.86	12.19	7.98	4.21
Shade	0.70	0.40	6.79	11.89	4.88	25.92	15.72	11.11	7.13	3.98
CD (0.05)	NS	NS	1.00	0.98	1.24	3.25	1.09	0.78	0.64	NS

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulphur (%)	Steviol glycosides (%)
Open	2.5	0.21	0.41	0.44	0.41	0.18	5.02
Shade	2.2	0.23	0.44	0.40	0.43	0.22	4.42
CD (0.05)	NS	NS	NS	NS	NS	NS	0.42

5. DISCUSSION

5. DISCUSSION

A field experiment entitled “Environmental influences on yield and quality of stevia (*Stevia rebaudiana* Bertoni)” was conducted in the Department of Agronomy, College of Agriculture, Vellanikkara during the year 2020. The key findings of the experiment presented in the previous chapter are discussed in this chapter based on available literature.

5.1 Effect of growing condition and planting dates on growth and yield of *Stevia rebaudiana*

5.1.1 Plant height

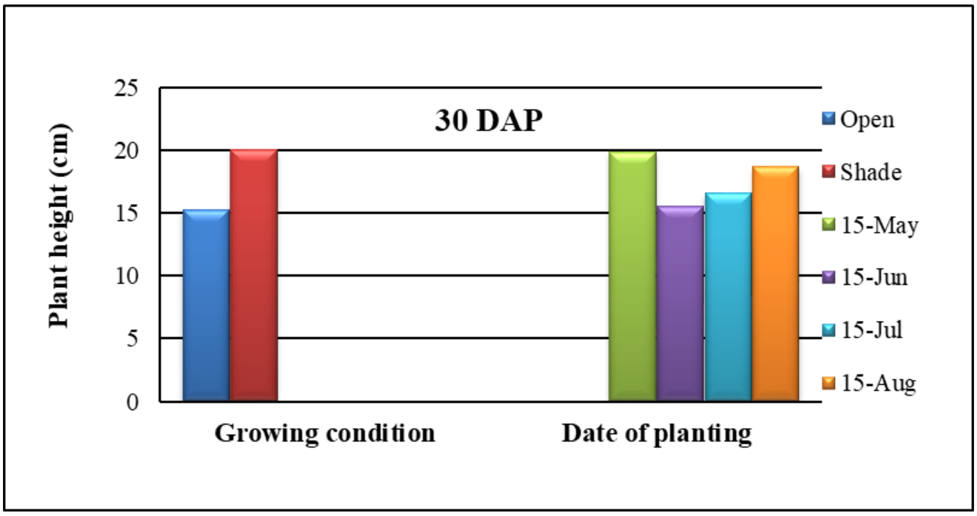
In general, crop planted under 50 per cent shaded condition recorded the tallest plants when compared to open condition, and the plant heights observed were 20.08 cm, 28.20 cm and 32.22 cm at 30 DAP, 60 DAP and at harvest, respectively (Table 3, Fig. 4). Many scientists reported increase in plant height under shaded condition (Moniruzzaman *et al*, 2014; Rakesh *et al*., 2012). As per Boardman (1977), when plants were grown under shaded condition, they developed elongated stems with large intercellular spaces due to the enhanced activity of auxins.

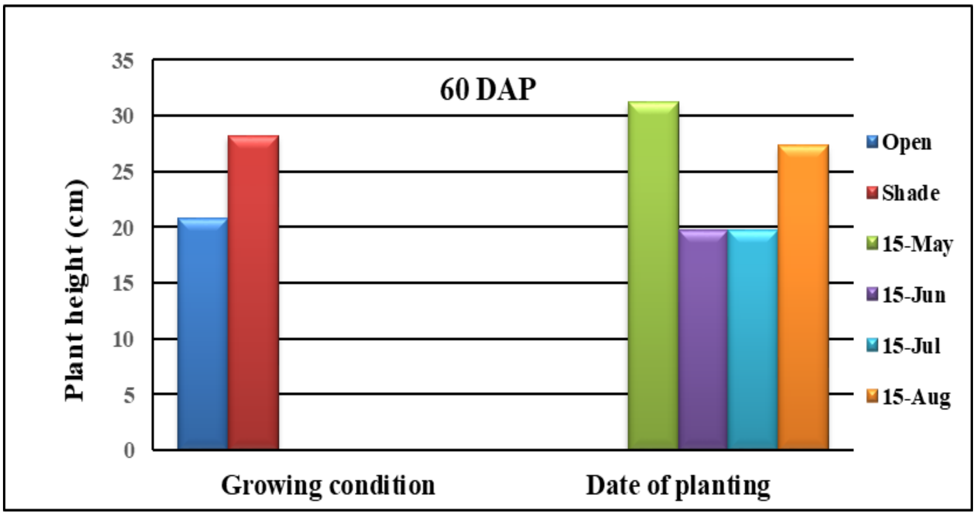
Sowing or planting time is an important non-monetary agronomic input that plays an important role in enhancing crop yield and quality by influencing morphological development, biomass production and metabolism. A slight change in the sowing/planting period leads to significant changes in the associated meteorological parameters and, consequently, the yield and quality of the crop. In this experiment also, significant variation in plant heights was observed at different stages of observation due to changes in dates of planting (Table3 and Fig. 4). May planted crop resulted in taller plants at 30 DAP, 60 DAP and at harvest. Mordechai *et al*. (2013) also reported April and May as the optimal time for planting stevia in an open field.

5.1.2 Number of branches per plant

The results clearly indicate influence of open growing as condition on increasing number of branches as compared to shaded conditions at 30 DAP, 60 DAP, and at harvest. The high temperature under open condition might have favored the formation of more lateral buds, resulting in a greater number of branches, whereas the shaded environment favored apical dominance and increased plant height. In addition, the maximum and minimum temperatures were higher in the open condition than in the shade condition. A positive association with the number of branches and the maximum temperature was noticed in this study (Table 4 and Fig. 5).

Crop planted in May recorded higher number of branches at 30 DAP (2.36). At 60 DAP also, higher number of branches was observed in May planting (8.60) and was on par with August planting (7.08). At harvest, May planting recorded higher number of branches (9.43) and was on par with August planting (7.96). Variations in number of both primary and secondary branches in stevia due to difference in planting dates were reported by Khan *et al*. (2012). According to them, maximum number of branches were observed when planting was done in April and they attributed it to warm environmental conditions like clear sunshine and high temperature during the month of April.





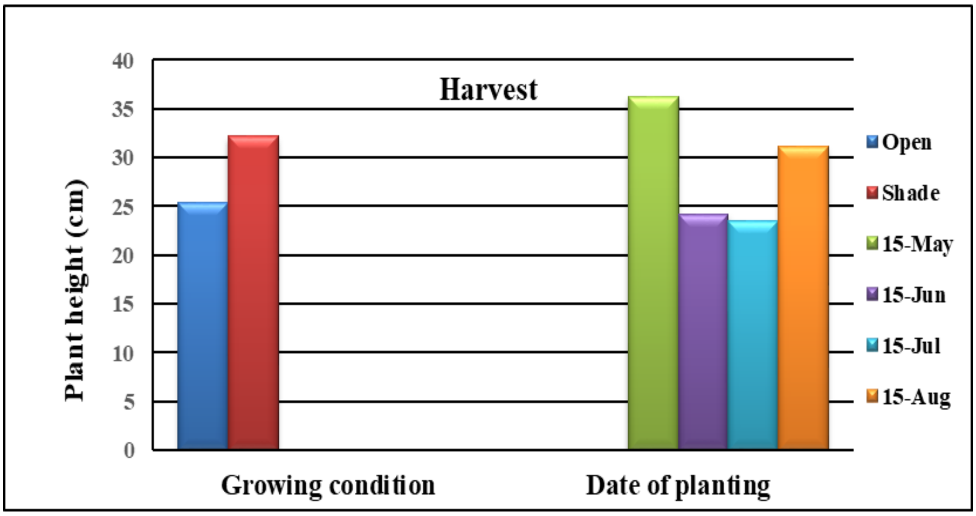


Fig .4. Effect of growing condition and date of planting on plant height at different growth stages of stevia *(Stevia rebaudiana)*

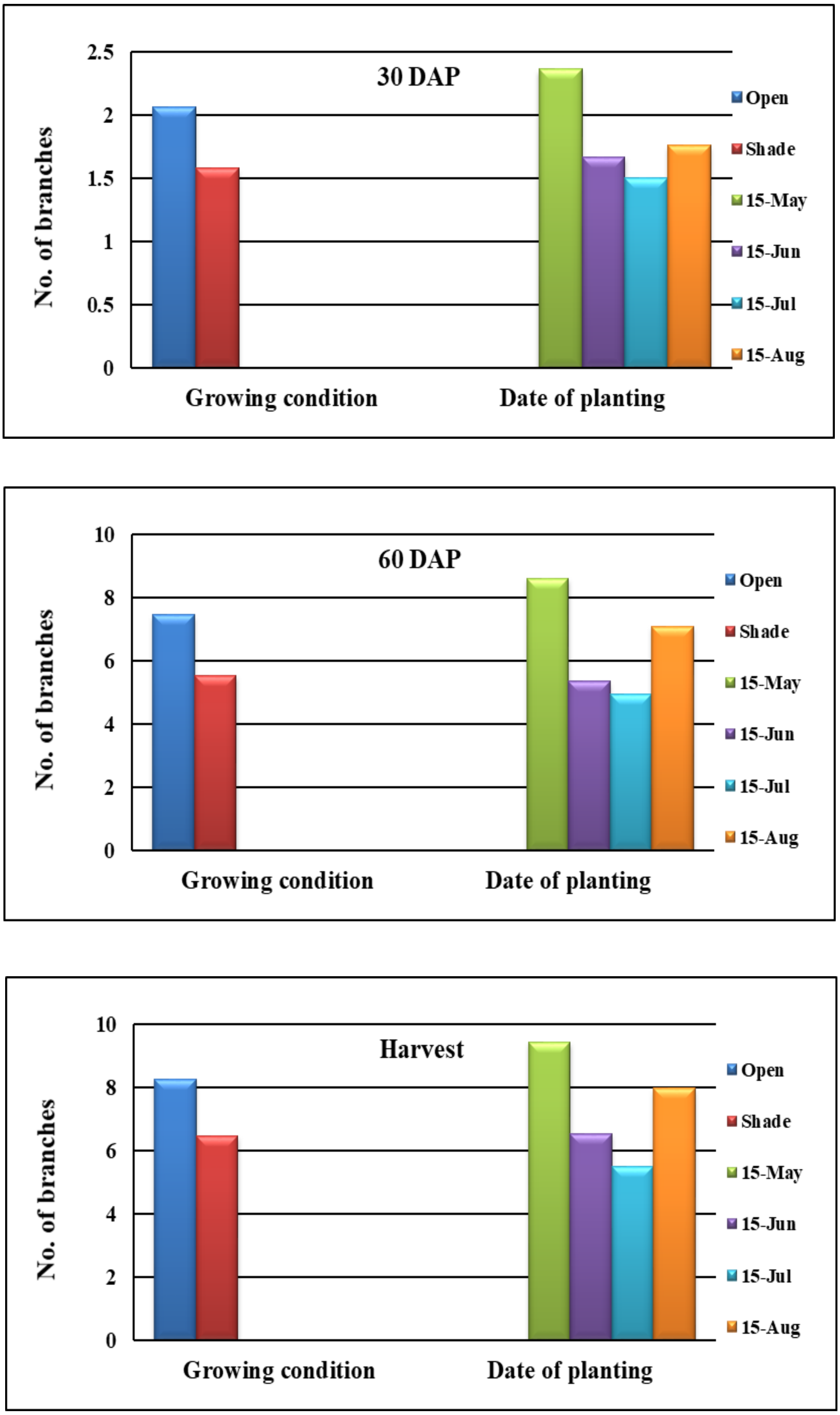


Fig. 5. Effect of growing condition and date of planting on number of branches at different growth stages of stevia *(Stevia rebaudiana)*

5.1.3 Number of leaves per plant at 30 DAP, 60 DAP and at harvest

Higher number of leaves was noticed under open condition as compared to the shaded condition during entire crop period (Table 5 and Fig. 6). It was observed in all planting dates that open conditions favored stevia leaf production over shaded conditions. As in other biometric parameters, for number of leaves also there was a positive association with the maximum temperature.

Significant variation was observed for number of leaves with different dates of planting. The higher number of leaves was obtained in May planting (50.84, 164.83 and 315.32, respectively at 30 DAP, 60 DAP and at harvest). Ramesh *et al.* (2006) reported that growing stevia under long day conditions provided better opportunity to enhance the leaf mass. According to them, the optimal time for planting stevia in an open field was in months of April and May. According to Mordechai *et al.* (2013), stevia planting in January-March and in October decreased crop biomass whereas planting in April and May resulted in higher yield and higher number of leaves.

5.1.4 Leaf weight at 30 DAP, 60 DAP and at harvest

Higher fresh (Table 6 and Fig. 7) and dry weights (Table 7) of leaves were noticed under open condition compared to the shaded condition during the entire crop period and for all dates of planting. A difference of 11.5 g/plant in fresh weight was observed by changing only the growing condition.

Among dates of planting, May planting resulted in higher fresh and dry weights. It was followed by planting in August. June and July planting resulted in lower weights. The difference in fresh weights among crops planted in May and July at harvest stage was 37.54 g/plant. Khan *et al*. (2012) reported higher fresh weight of leaves in stevia crop planted during April and May.

Variation in fresh and dry weight of leaves due to changes in planting dates could be attributed to variations in weather parameters experienced by the crop during their various life stages.

5.1.5 Herbage yield at 30 DAP, 60 DAP and at harvest

At all growth stages of stevia and also under all planting dates of planting higher herbage yield was noticed under open condition compared to the shaded condition (Table 8 and Fig. 8). Open condition resulted in fresh herbage yield of 155.44 g/plant as compared to 131.41 g/plant under shade at final harvest stage. The yield improvement under open growing condition was 18.29 per cent.

Significant variation was observed for fresh herbage yield with different dates of planting. The herbage yield followed the order May>August>July>June. The better herbage yield in May planted crop could be attributed with favorable weather parameters experienced by the crop, and the greater height, number of branches, leaves and leaf weight of the may planted crop. Strong positive correlation was observed with fresh biomass yield and maximum temperature, whereas the correlation between rain fall and after noon relative humidity was negative (Table 28). The month of June and July received rain fall of 103.15 mm and 145.74 mm, respectively as compared to 26.76 mm in May and 128.20 mm in August. Heavy rain coupled with cloudy weather during planting and initial establishment stage might have adversely affected the growth and development of the crop. As per Ramesh *et al.* (2006) stevia is highly sensitive to the day length and it requires 12-16 h of sunlight.

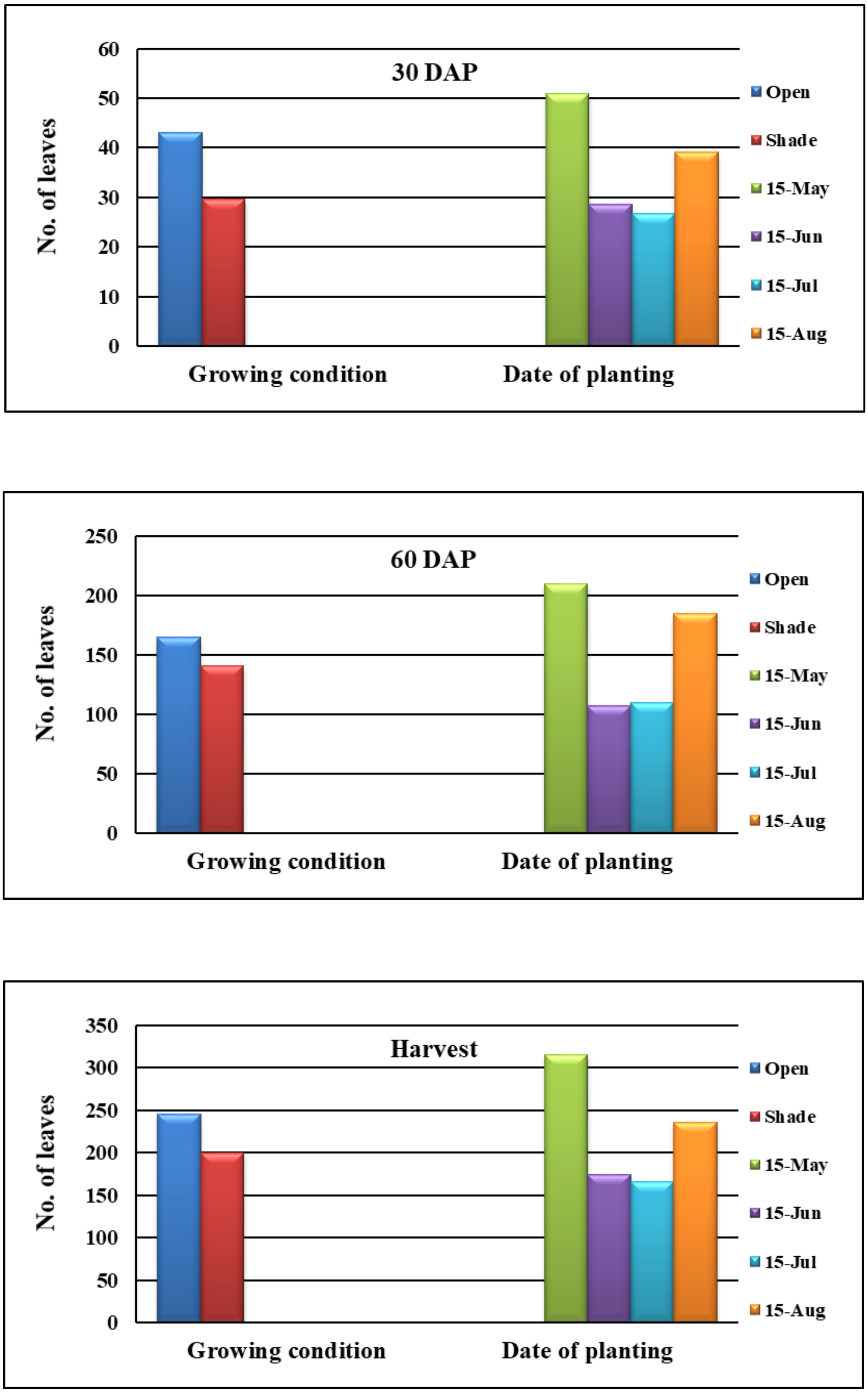


Fig. 6. Effect of growing condition and date of planting on number of leaves at different growth stages of stevia *(Stevia rebaudiana)*

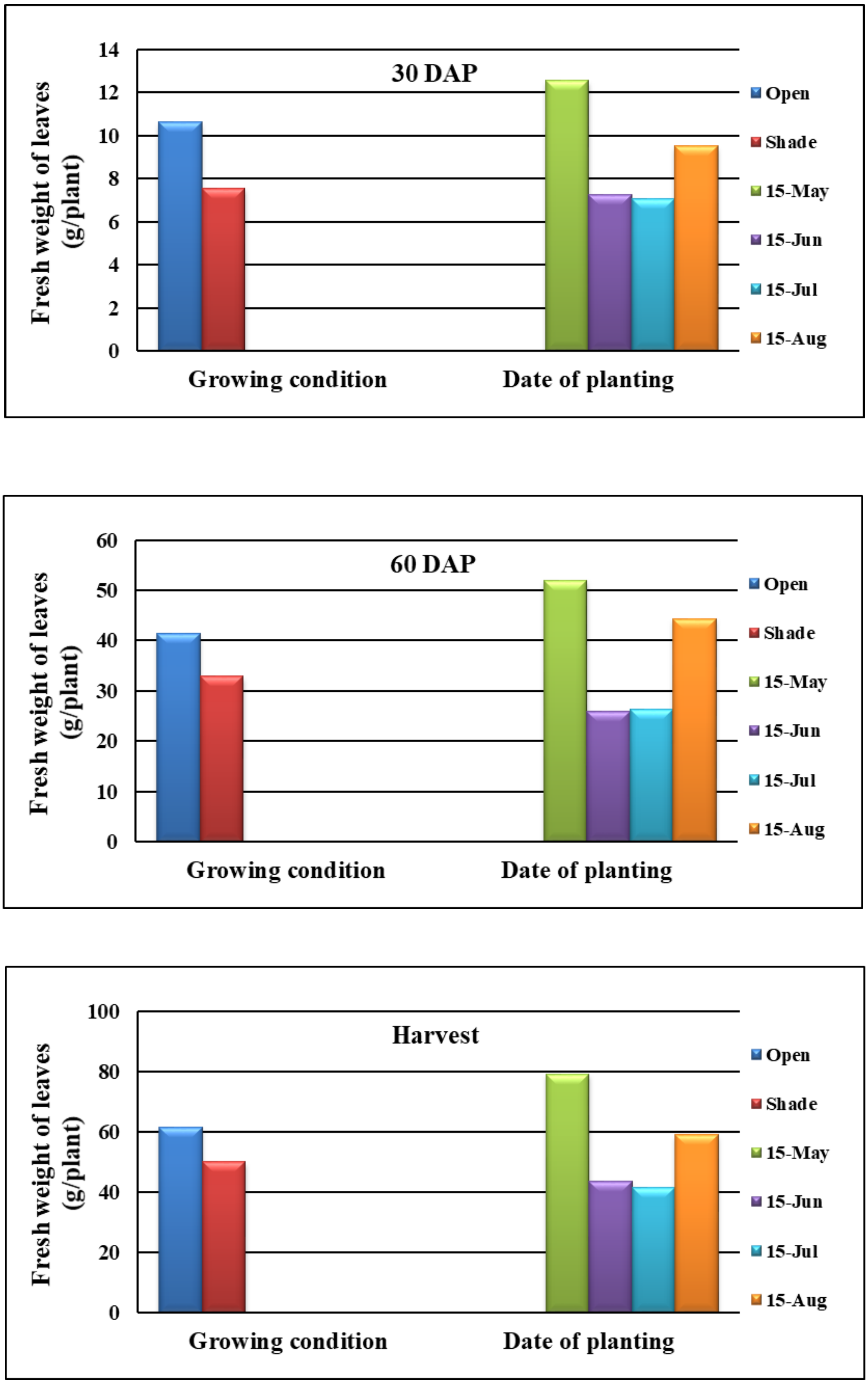


Fig. 7. Effect of growing condition and date of planting on fresh weight of leaves at different growth stages of stevia *(Stevia rebaudiana)*

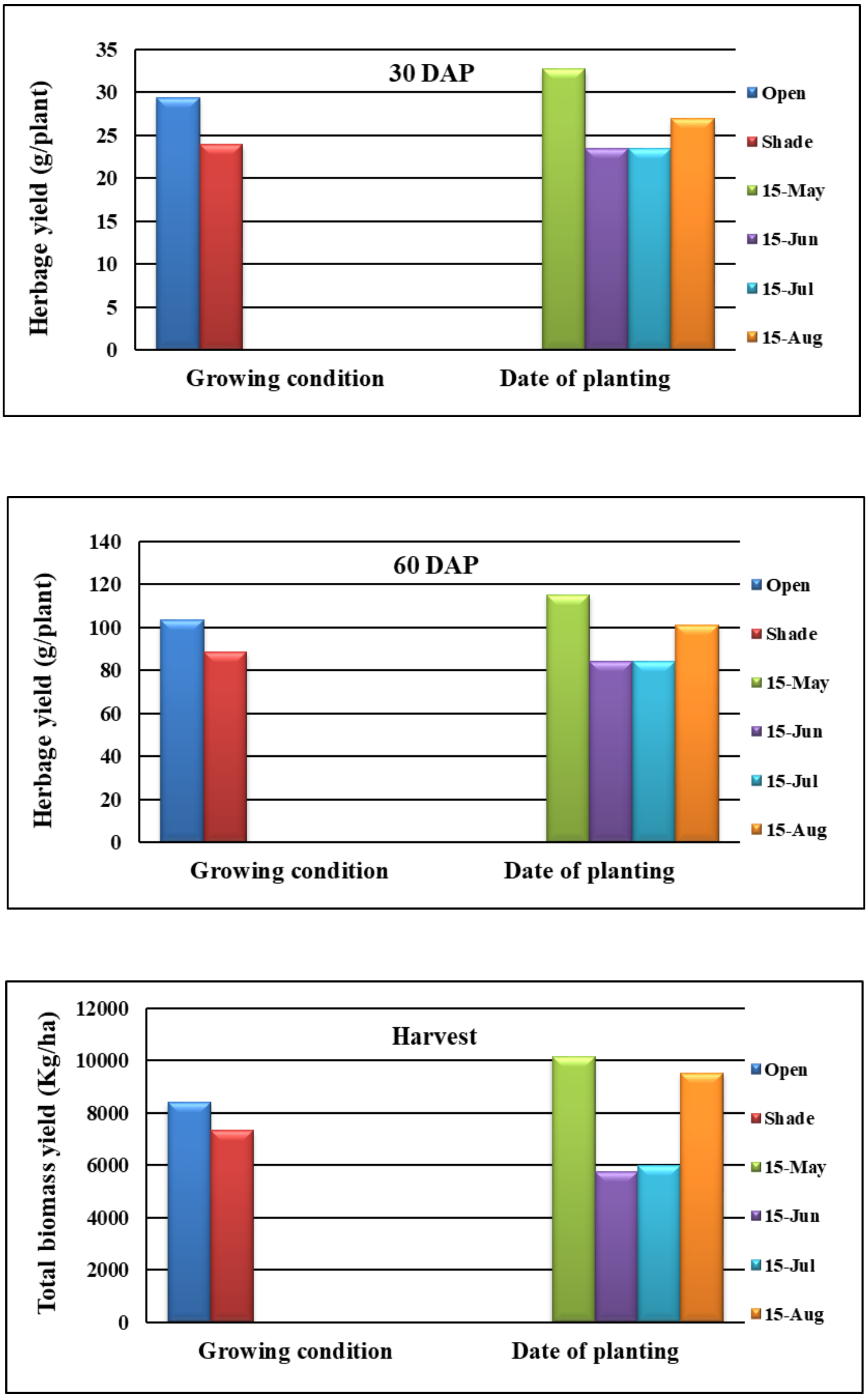


Fig. 8. Effect of growing condition and date of planting on herbage yield at different growth stages of stevia *(Stevia rebaudiana)*

5.1.6 Total biomass yield

As like all other parameters assessed total biomass yield was also found highest under open growing condition and in May planting (Table 11 and Fig. 9). Just by altering shade level, a biomass improvement of 1078 kg on a hectare basis was observed. Date of planting also significantly influenced the total biomass yield of stevia and followed the trend of herbage yield. May planting recorded the highest yield of 10161 kg/ha and was on par with August planting (9540 kg/ha). A yield reduction of 75.84 per cent as compared to May planting was noticed in June planted crop. June planted crop received 103.15 mm rain fall during its seedling stage and a negative correlation between total biomass and rain fall was observed. Better production of biomass in May planted crop under open growing condition could be correlated with availability of ample sunlight, favourable temperature and low relative humidity experienced by the crop in the early establishment stage. Allam *et al.* (2001) observed remarkable increase in stevia yield during the summer (May- August) due to favourable climatic conditions, such as temperature and length and intensity of photoperiod, as compared to winter.

5.1.7 Steviol glycoside content

Data on the steviol glycoside content clearly revealed the significant influence of environmental conditions on enhancing quality of a crop (Fig. 10). The highest steviol glycoside content was obtained under open growing condition (7.57 per cent) compared to shade (5.09 per cent). According to Tateo *et al*. (1998), environmental and agronomic factors had significant influence on stevioside production in stevia. Kumari *et al.* (2016) studied the effect of growing conditions (open field and shade) on quality of stevia and reported higher rebaudioside-A content (7.00 per cent) in open field grown plants compared to plants grown in shaded condition.

Planting dates also showed significant influence on steviol glycoside content. Crop planted in May showed the highest steviol glycoside content. When planting was delayed by one month significant reduction in stevioside content was observed (9.32 per cent in May as compared to 4.89 per cent in June). As per Madan *et al*. (2010), the stevia plant is highly sensitive to day length. Stevia requires12-16 hours of sunlight per day to maximize stevioside accumulation in the leaves. Megeji *et al*. (2005) and Tavarini and Angelini (2013) also reported variations in stevioside production based on time of planting and harvest.

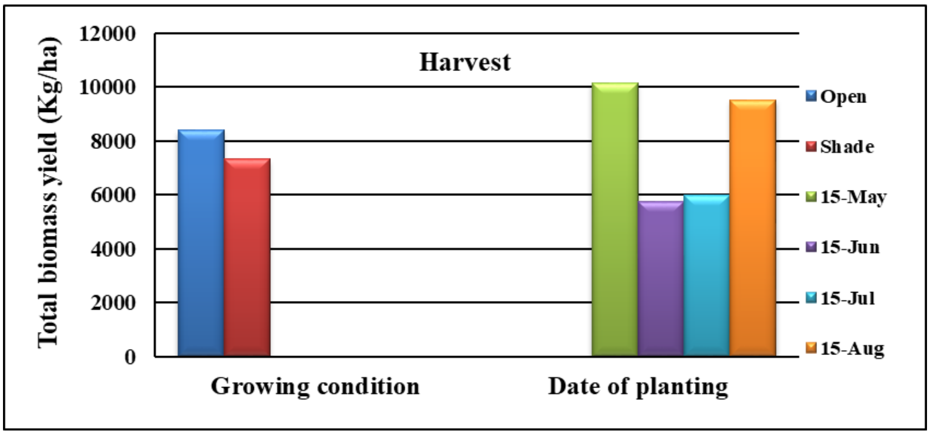


Fig. 9.Effect of growing condition and date of planting on total biomass at different growth stages of stevia *(Stevia rebaudiana)*

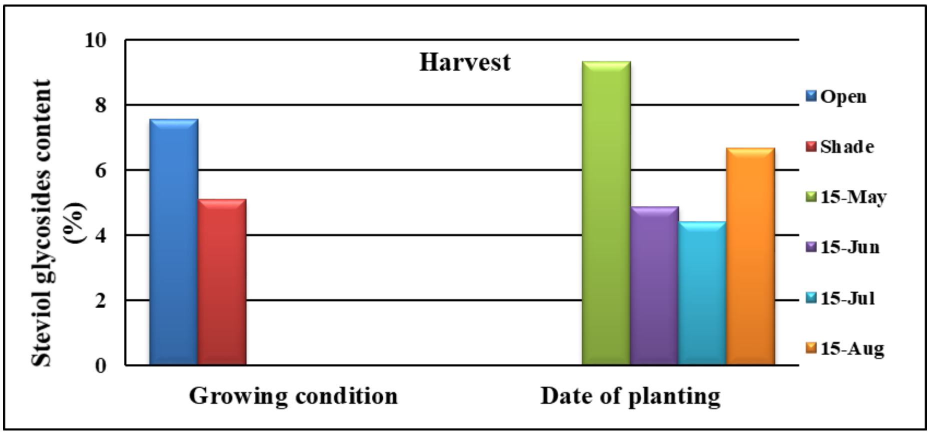


Fig. 10.Effect of growing condition and date of planting on steviol glycoside content of stevia *(Stevia rebaudiana)*

5.2 Crop weather correlation observed during the experiment

The correlation between important morphological, yield and quality characters and mean monthly weather and microclimatic parameters like temperature (maximum and minimum) relative humidity (forenoon and afternoon), rainfall and bright sunshine hour were worked out and are presented in Table 28.

The results indicated positive correlation of maximum and minimum temperatures with plant height, number of leaves, leaf weight, biomass yield and steviol glycoside content. According to Kumar *et al.* (2012), temperature plays a vital role in almost all biological processes of crop plants and it is one of the most important climatic factors affecting the growth, development and yield of the crops. Positive correlation between growth, yield and quality of stevia was reported by many scientists (Singh *et al*, 2014; Clemente *et al.*, 2021).

The correlation of afternoon relative humidity and rainfall with plant height, number of leaves, leaf weight, biomass yield and steviol glycoside contents were negative. Tavarini and Angelini (2013) reported negative relation between rain fall and quality of stevia. Orozco (2020) also suggested heavy rainfall as the main reason for low relative growth rate in stevia.

5.3 Phytochemical evaluation of stevia leaves at different growth stages

5.3.1 Effect of stage of harvesting on phytochemical constituents

Phytochemical constituents present in medicinal plants are the material basis of their therapeutic effects. They are also important indicators for assessing the quality of medicinal materials. However, the synthesis and accumulation of these phytochemicals are very complex and are influenced by many internal and external factors. Weather parameters, geographical location, season, growth stage, harvesting time and method, post harvest handling and processing are some of the factors which decide quality of medicinal plant materials. Among them growing condition is the one which greatly controls plant growth and development, both in terms of and quality and quantity (Shahak*et al*., 2004). Plant ontogeny or growth stage had close relation with accumulation of phytochemicals (Dhar*et al*., 2006; Verma*et al*., 2013) and hence harvesting at correct stage is very important for harnessing maximum therapeutic property.

Phytochemical parameters such as total carbohydrates and total fat (Fig. 11), crude fibre and total protein (Fig. 12), total energy of stevia leaves (Fig. 13) and total energy of stevia leaves (Fig. 13), total mineral content, total soluble sugars and total steviol glycoside content (Fig. 14) and reducing sugars, non reducing sugars, content of N, P, K, Ca, Mg, S, moisture content at 45 DAP, flower bud initiation stage (65 DAP), 50 per cent flowering stage (80 DAP) and full bloom stage (95 DAP)] were analyzed and the data was presented in Table 30, 31, 32 and 33, respectively. Proximate and nutritional analysis revealed stevia as a sweet herb rich in nutritional compounds like protein, fiber, sugars, primary and secondary elements. Several workers reported stevia as a low calorie sugar crop (Choudhary, 1999; Abou-Arab, 2010). In the present study the calorific value computed for the fresh stevia leaves ranged from 27.37 Kcal to 31.12 Kcal with maximum value at flower bud initiation stage.

All the parameters studied except crude fiber and total mineral contents showed higher values at flower bud initiation stage. The total carbohydrates, fat, protein, soluble sugars, reducing sugars, non reducing sugars, steviol glycoside content, moisture content and total energy of fresh stevia leaves increased from 45 DAP to flower bud initiation stage and decreased thereafter. However, crude fiber and total mineral contents showed an increasing trend from 45 DAP to full bloom stage. Variations in phyto chemical constituents according to crop stage of stevia were also reported by Ibrahim *et al*. (2015) and Esra*et al*. (2018).

Being a sugar yielding crop, most important parameter deciding quality of stevia is its sugar content. In the present study the content of total soluble sugars ranged from 11.62 to 21.05 per cent. The sugar content in leaves showed increasing trend from seedling stage to flower bud initiation stage. After flowering the sugar content decreased. Same trend was observed for both reducing and non reducing sugars and also for steviol glycosides. Steviol glycosides (SGs) are secondary metabolites particularly tetracyclic diterpenoids. They are present mainly in the leaves, small quantities in the stem, and very less in the roots (Brandle and Rosa, 1992). Biosynthesis of SGs takes place in the leaves, from where it is translocated to various plant parts. Maximum concentration of glycosides occurs in leaves before they get translocated into other plant parts. Sumida (1980) reported maximum content of steviol glycosides in the leaves of stevia just before flowering.

Based on preliminary phyto chemical examination the optimum stage of harvesting of stevia can be recommended as at the time of flower bud initiation. Further delay in harvesting led to reduction in sugar content and increase in fiber content. Guerrero *et al*. (2018) and Samadpourrigani *et al*. (2019) also reported the optimal cutting time for better quality in stevia as at the pre-flowering stage.

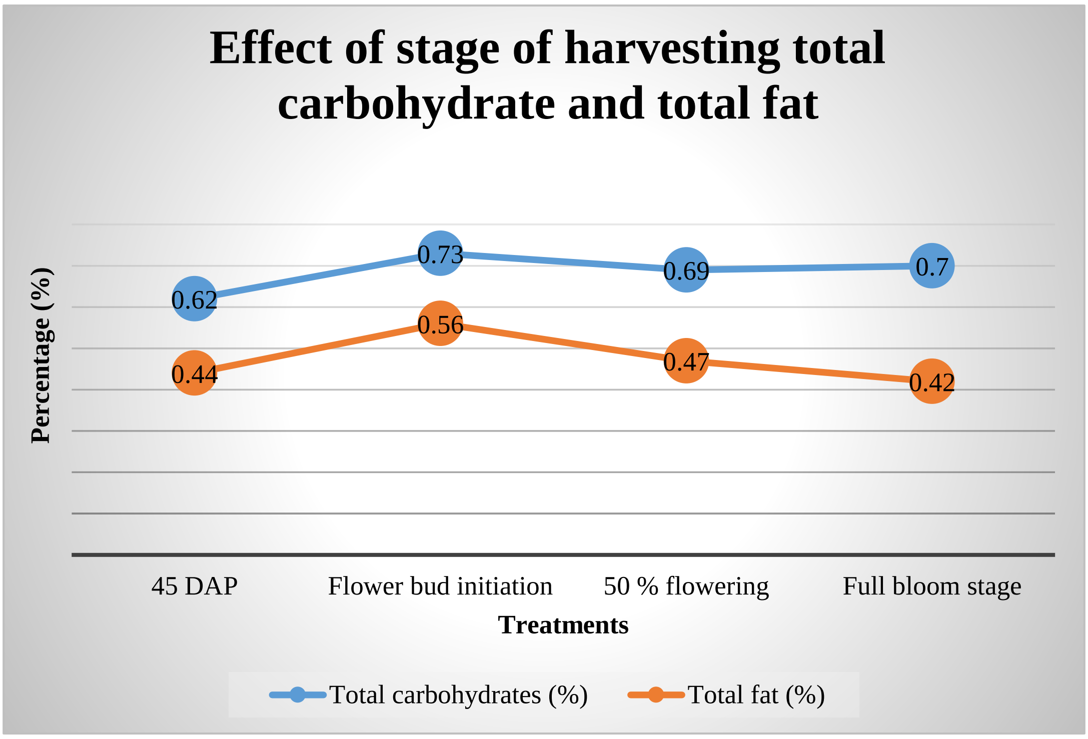


Fig. 11. Effect of stage of harvesting on total carbohydrate and total fat

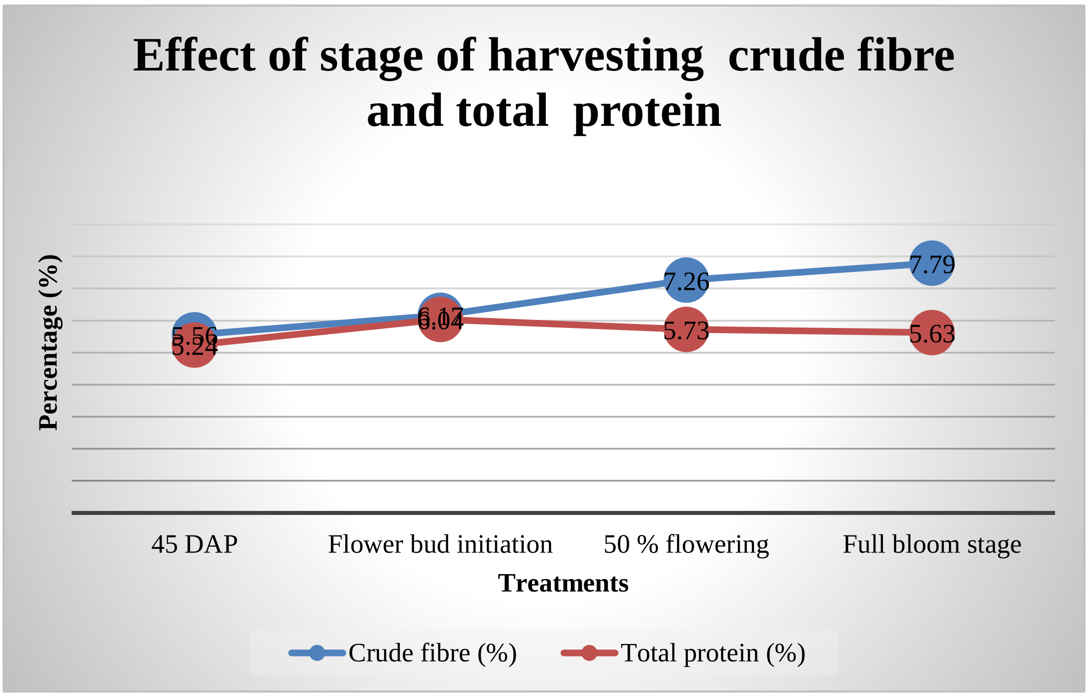


Fig. 12. Effect of stage of harvesting on crude fibre and total protein

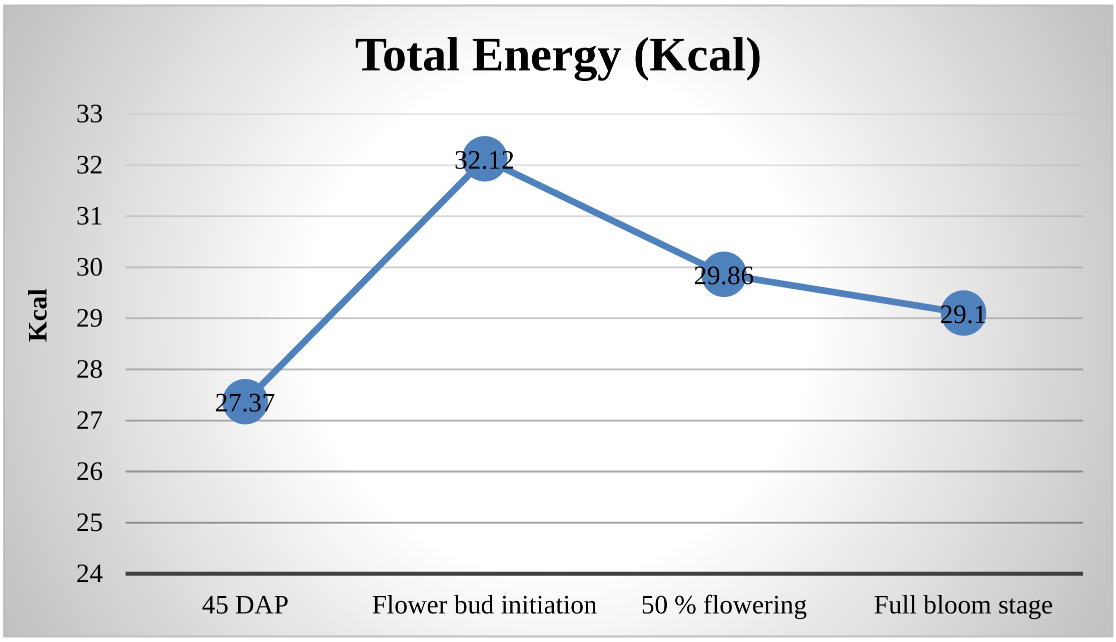


Fig. 13. Effect of stage of harvesting on total energy

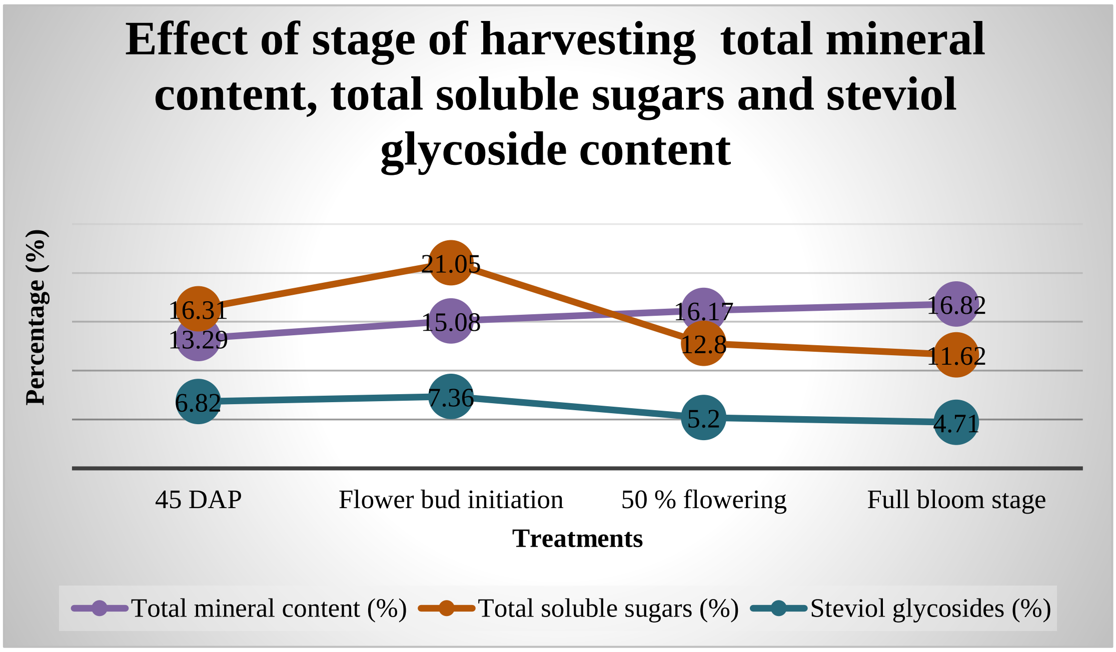


Fig. 14. Effect of stage of harvesting on total mineral content, total soluble sugars and steviol glycoside content

5.3.2 Effect of growing condition on phytochemical constituents

Stevia plants grown under open and 50 per cent shade were analyzed for their phytochemical constituents at 45 DAP, flower bud initiation stage, 50 per cent flowering stage and at harvest. At all stages of observation variations in phytochemical constituents were observed in stevia leaves grown under open and shaded condition (Tables 29, 30, 31, 32, 33 and Fig.15, 16, 17, 18). Meteorological conditions, such as sunshine, temperature, rainfall etc. significantly influenced the growth of medicinal plants in terms of dry matter production and accumulation of active constituents (Hang *et al.*, 2005).

Parameters like total fat, crude fiber, total protein, total mineral content, total soluble sugars, reducing, non reducing sugars and total steviol glycosides were observed as higher under open growing condition. Kumar *et al*. (2012) also reported positive influence of open growing condition on increasing total steviol glycosides content in stevia. Significant variation was not observed for total carbohydrates and fat contents.

In the field experiment significant interaction between growing condition and planting dates were observed on plant height, number of branches, number of leaves, fresh leaf weight, dry leaf weight and total biomass yield at different growth stages of stevia. The crop planted in the month of May under open condition recorded better yield and yield parameters, followed by planting in August under open condition. The highest glycoside content was observed in a May planted crop grown under open condition.

Higher quantities of total soluble sugars and steviol glycosides were observed when the crop was harvested at flower bud initiation stage. So harvesting stevia at flower bud initiation can be suggested for obtaining higher quality parameters.

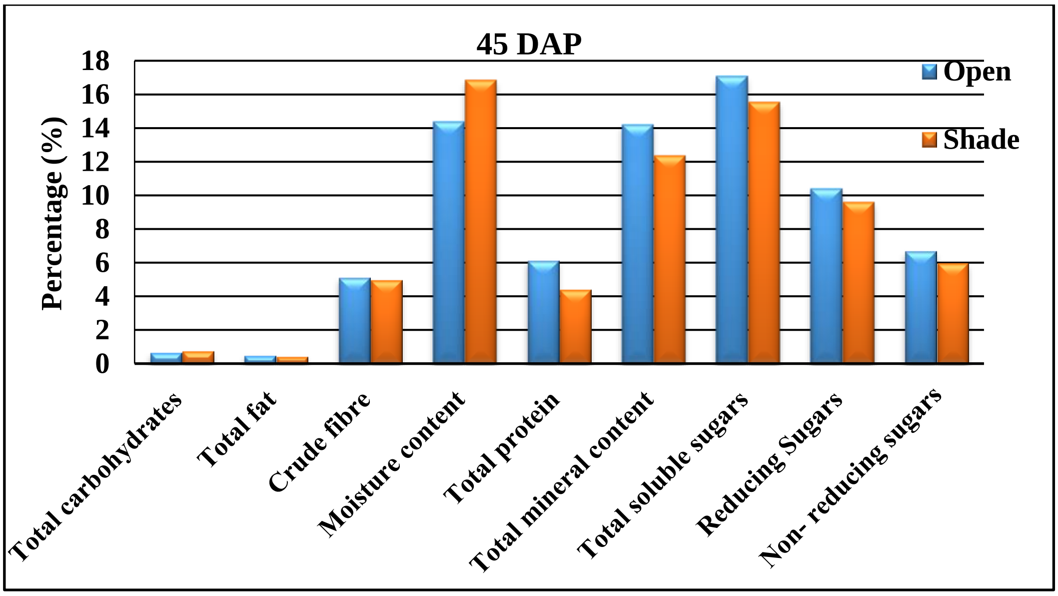


Fig. 15. Effect of growing conditions on phytochemical constituents at 45 DAP

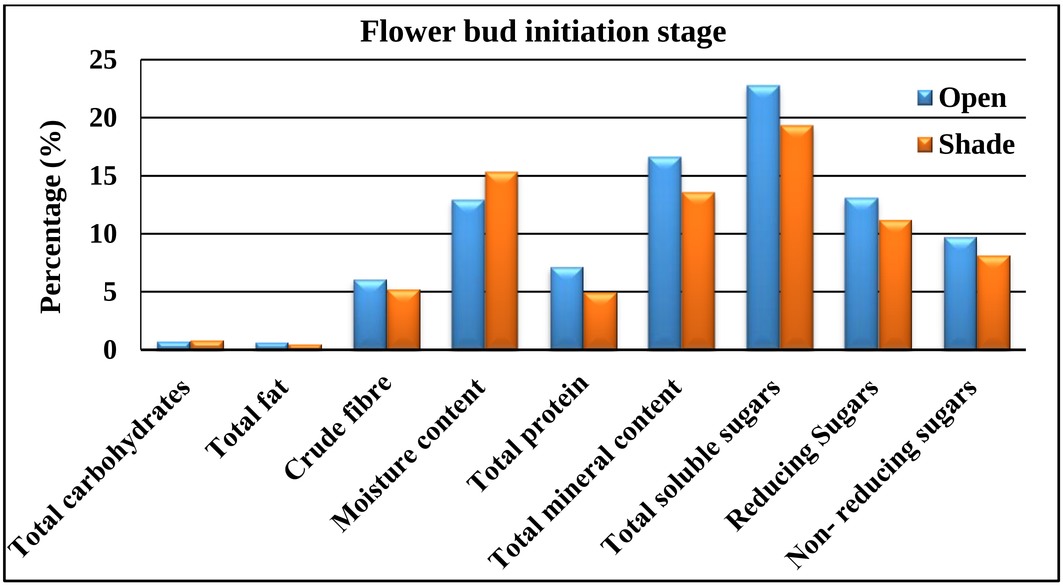


Fig. 16. Effect of growing conditions on phytochemical constituents at flower bud initiation stage

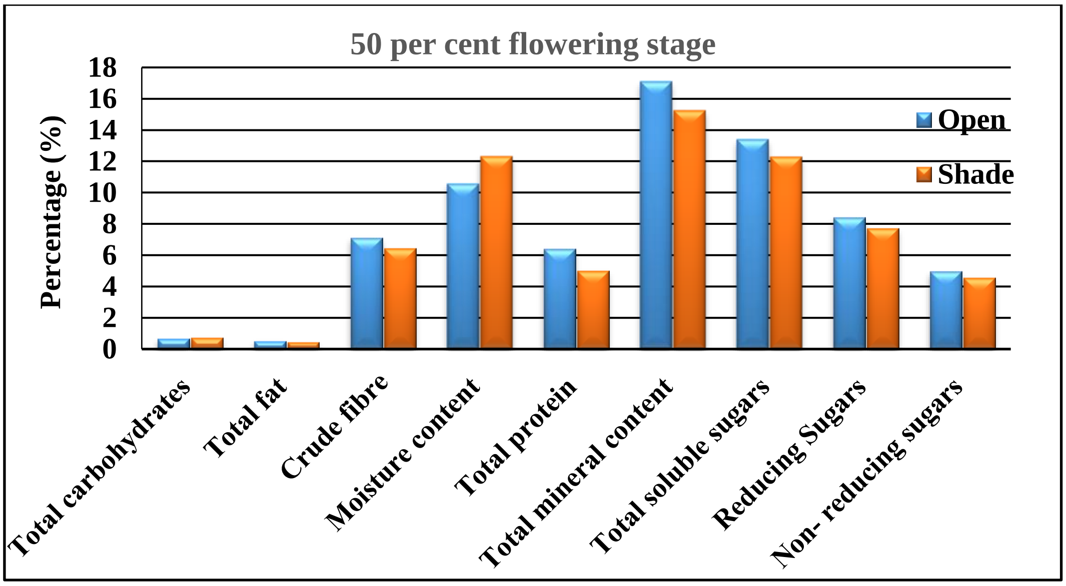


Fig. 17. Effect of growing conditions on phytochemical constituents at 50 per cent flowering

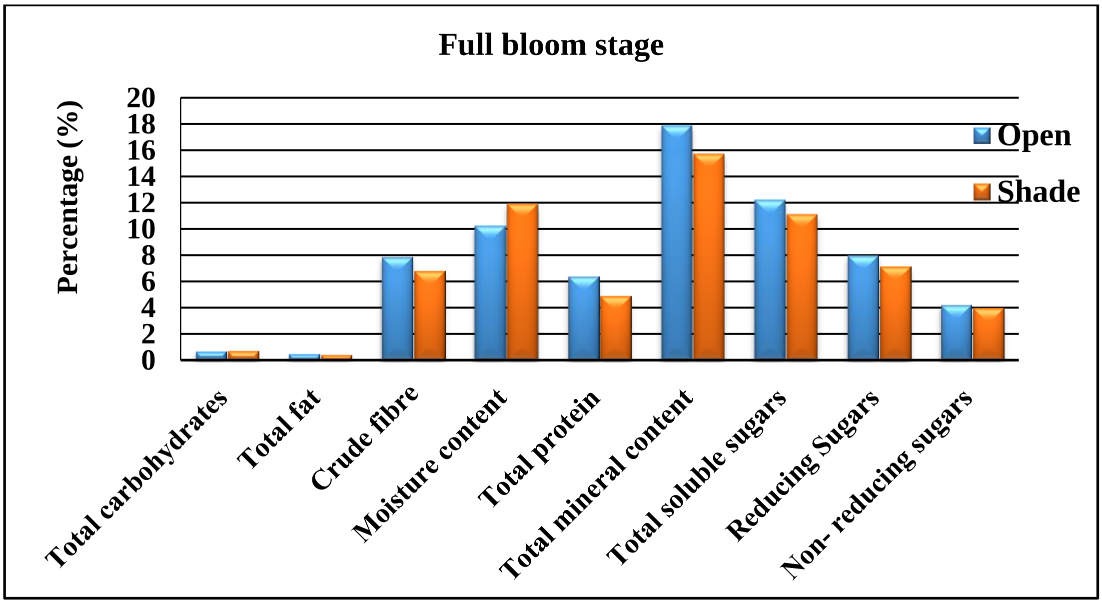


Fig.18. Effect of growing conditions on phytochemical constituents at full bloom stage

6. SUMMARY

6. SUMMARY

A study entitled "Environmental influence on yield and quality of stevia (*Stevia rebaudiana* Bertoni)" was conducted in the Department of Agronomy, College of Agriculture, Vellanikkara during the year 2020. The study was conducted as two sets of experiments, one for assessing effect of growing condition and planting dates on yield and quality of stevia and other for assessing the effect of stage of harvesting on phytochemical parameters of stevia. The first experiment was conducted as a field study laid out in split plot design with three replications. The main plot treatments consisted of two growing conditions viz., open and 50 per cent shade and the sub plot treatment consisted of four dates of planting viz., May15th, June 15th, July 15th, and August15th. Biometric characteristics, weather parameters, physiological and biochemical characters of plants were recorded. Experiment was a pot culture designed in completely randomized design (CRD). The significant findings of the research are summarized and presented below.

Effect of growing condition

- Growing condition significantly influenced plant height of stevia at different growth stages. Throughout the study period, taller plants were observed under shaded condition.

- Open condition resulted in higher number of leaves, fresh leaf weight and dry leaf weight.

- Under open condition, fresh biomass yield and herbage yield were higher.

- Plants grown in the open condition exhibited higher CGR at initial stages and at harvest.

- Weather observations like maximum and minimum temperatures, relative humidity, rainfall, and bright sunshine hours varied considerably under open and shaded growing conditions.

- In open condition, maximum and minimum temperatures were higher, whereas relative humidity was higher in shaded condition.
- The total glycoside content of the crop was higher in the open planted condition.

Effect of date of planting

- Plant height followed the trend May>August>June>July at different growth stages.
- During crop growth the number of branches was found to be higher in May planted crop and was comparable with the August planted crop.
- Number of leaves was found to be higher in May planted crop at 30 DAP and 60 DAP and it was on par with the August planting, while at harvest stage, May planted crop recorded more number of leaves.
- At 30 DAP and 60 DAP, the fresh leaf weight in the May planted crop was higher and was on par with the August planted crop. At harvest stage, the May planted crop had the highest fresh weight of leaves.
- At 30 DAP and 60 DAP, the dry weight of leaves in the May planted crop was on par with the August planted crop, while at harvest stage, the May planted crop had the highest dry weight.
- During initial stages May planted crop recorded the highest herbage yield. At 60 DAP and at harvest stage the herbage yield of May planted crop was on par with the August planted crop.
- May planted crop have the higher total biomass yield and it was comparable to the August planted crop.

- Weather parameters experienced by the crop altered significantly due to changes in dates of planting. Higher temperature was experienced by the May and August planted crops. June and July planted crop faced heavy showers during their planting and early establishment phase. Afternoon R.H was higher in June. May and August planted crops received more bright sunshine hours.

- From planting to 30 DAP and 30 to 60 DAP, crop planted in May had considerably greater CGR. CGR was found to be highest in May planted crops at harvest. RGR was found to higher in May planted crop at initial stages. At 30-60 DAP it was almost equal in August and May planted crops. During harvesting stage there was no significant effect of planting dates on growth indices.

- The total glycoside content of the crop was highest in the May planted crop followed by August planting.

Effect of interaction between growing condition and date of planting

- Significant interaction between growing condition and planting dates was observed on plant height, number of branches, number of leaves, fresh leaf weight, dry leaf weight and total biomass yield at different growth stages of stevia.

- The crop planted in the month of May under open condition recorded better yield and yield parameters, followed by planting in August under open condition.

- The plant height was higher in the May planted crop under shaded condition.

- The highest glycoside content was observed in May planted crop grown under open conditions.

Crop-weather relations

- Maximum temperature had positive correlation with the plant height, number of leaves, fresh leaf weight, total biomass yield and total glycoside content.

- The minimum temperature recorded positive correlation with number of leaves and fresh leaf weight.

- Afternoon relative humidity and rainfall had negative correlation with the plant height, number of leaves, fresh leaf weight, total biomass yield and total glycoside content.

Effect of growing conditions on phytochemical constituents

- Significant variations were observed for phytochemical constituents of stevia due to changes in growing condition.

- At all stages of observation higher content of total carbohydrates was observed under shaded condition.

- Total fat content of stevia leaves did not show any significant difference under open and shaded condition. However, at all stages of observation higher values were observed under open condition.

- At all stages of observation, higher crude fibre content was observed under open condition.

- Higher moisture content was observed under shaded condition.

- At all stages of observation higher protein content was observed under open condition.

- Crop growing under open condition resulted in higher total mineral content.

- Total soluble sugar, reducing and non reducing sugars were higher under open condition.

- At all stages of observation higher total energy was computed for open condition.

- Open growing condition resulted in higher concentration of total steviol glycosides.

- Nitrogen content in open conditions was slightly higher when compared to the shaded conditions.

- Significant variations were not observed for contents of P, K, Ca, Mg and S.

Effect of stage of harvesting on phytochemical constituents

- Total carbohydrate content was higher when harvested at flower bud initiation stage. After that a slight reduction in carbohydrate content was observed in stevia leaves.

- Total fat content was higher when harvest was done at flower bud initiation stage. After flower bud initiation stage a gradual reduction in fat content was observed.

- The highest content of crude fibre was observed in the crop harvested at full bloom stage followed by 50 per cent flowering stage

- The moisture content was maximum when harvested at 45 DAP. Moisture content reduced with increase in crop maturity.

- The highest content of total protein was observed in the crop harvested at full bloom stage. Total protein content in leaves increased on harvested from 45 DAP to flower bud initiation stage and thereafter it decreased.

- Total soluble sugar content was higher on harvesting at flower bud initiation stage. The total soluble sugar content increased when harvest was done from 45 DAP to flower bud initiation stage and thereafter decreased with maturity.

- Like total soluble sugar, reducing sugar and nonreducing sugar contents also increased on harvesting from 45 DAP, reached peak on harvesting at flower bud initiation stage and thereafter decreased.

- Total energy of crop increased on harvesting from 45 DAP, reached a peak at flower bud initiation stage and decreased thereafter.

- The highest steviol glycoside content was observed in the crop harvested at flower bud initiation stage. The glycoside content decreased with increase in maturity.

7. REFERENCES

REFERENCES

Abdalbasit, M., Gasmalla, A., Yang, R., Musa, A., Xiao, H., and Zhang, W. 2014. Physico-chemical assessment and Rebauidioside A. productively of natural sweeteners (*Stevia rebaudiana*). *J. Food Nutr. Res.* 5: 209-214.

Abdulameer, D. A., Osman, M. B., and Sulaiman, Z. 2018. Assessment of *Stevia rebaudiana* Bertoni genotypes via morpho-agronomic traits under two light conditions. *Am. J. Plant Sci.* 9: 1403-1413.

Abou-Arab, A. E., Abou-Arab, A. A., and Abu-Salem, M. F. 2010. Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* Bertoni plant. *Afr.J.Food Sci.*4: 269-281.

Ahmad, J., Khan, I., Johnson, S. K., Alam, I., and Din, Z. 2018. Effect of incorporating stevia and moringa in cookies on postprandial glycemia, appetite, palatability, and gastrointestinal well-being. *J. Am. Coll. Nutr.* 37(2): 133-139.

Aladakatti, Y. R., Palled, Y. B., Chetti, M. B., Halikatti, S. I., Alagundagi, S. C., Patil, P. L., Patil, V. C., and Janawade, A. D. 2012. Effect of irrigation schedule and planting geometry on growth and yield of stevia (*Stevia rebaudiana* Bertoni). *Karnataka J. Agric. Sci*. 25: 30-35.

Allam, A. I., Nassar, A. M., and Besheite, S. Y. 2001. Nitrogen fertilizer requirement of *Stevia rabaudiana* Bertoni under Egyptian condition. *Egypt. J. Agric. Res.* 79: 1005-1018.

Angelini, L. G., Martini, A., Passera, B., and Tavarini, S. 2016. Cultivation of *Stevia rebaudiana* Bertoni and associated challenges. In: Merillon; J. M. and Ramawat, K. G. *Sweeteners, Pharmacology, Biotechnology and Applications*. Reference Series in Phytochemistry. Springer, New York, United States. 652p.

AOAC [Association of Official Analytical Chemists]. 1975. Official and Tentative Methods of Analysis (12th Ed.) Association of official analytical chemists, Washington, D. C. 1094p.

Asp, N. G., Johansson, C. G., Hallmer, H., and Siljestroem, M. 1983. Rapid enzymic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem.* 31(3): 476-482.

Atteh, J. O., Onagbesan, O. M., Tona, K., Decuypere, E., Geuns, J. M. C., and Buyse, J. 2008. Evaluation of supplementary stevia (*Stevia rebaudiana*, bertoni) leaves and stevioside in broiler diets: effects on feed intake, nutrient metabolism, blood parameters and growth performance. *J. Anim. Physiol. Anim. Nutr.* 92(6): 640-649.

Atteh, J., Onagbesan, O., Tona, K., Buyse, J., Decuypere, E., and Geuns, J. 2011. Potential use of *Stevia rebaudiana* in animal feeds. *Arch. Zootec*. 60:133-136.

Balwinder, S., Jaspreet, S., and Jaspreet, S. 2014. Agro-production, processing and utilization of *Stevia rebaudiana* as natural sweetener. *J. Food Agric. Environ.* 1: 20128-20131.

Barathi, N. 2003. Stevia the calorie free natural sweetener. *Nat. Prod. Rad*. 2(4): 120-122.

Beneford, D. J., DiNovi, M., and Schlatter, J. 2006. Steviol glycosides. safety evaluation of certain food additives. In: WHO Food Additive Series. World Health Organization, Geneva: PP. 117-143.

Benhmimou, A., Ibrizl, M., Chaouki, A. F., Fatima, G., Ahmed. D., and Mounira. 2017. Effects of planting density and harvesting time on productivity of natural sweetener plant (*Stevia rebaudiana* Bertoni.) in Larache Region. *Int. Res. J. Plant Sci.* 7(4): 83-89.

Bian, Y. M. 1981. Studies on *Stevia rebaudiana* a new sweet-tasting plant refining stevioside and determination of its concentration. (Chinese). *Plant Physiol. Commun.* 3: 15-17.

Blackman, V. H. 1919. The compound interest law and plant growth. *An. Bot*. 33: 353-360.

Boardman, N. K. 1977. Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Physiol.* 28: 355-377.

Bondarev, N., Reshetnyak, O., and Nosov, A. 2003. Effect of nutrient media composition on development of *Stevia rebaudiana* shoots cultivated in the roller bioreactor and their production of steviol glycosides. *Plant Sci.* 165: 845-850.

Brandle, J. E. and Rosa, N. 1992. Heritability for yield, leaf: stem ratio and stevioside content estimated from a landrace cultivar of *Stevia rebaudiana*. *Can. J. Plant Sci.* 72: 1263-1266.

Brandle, J. E., Starratt, A. N., and Gijzen, M. 1998. *Stevia rebaudiana*: its agricultural, biological, and chemical properties. *Can. J. Plant Sci.* 78(4): 527-536.

Bray, R. H. and Kurtz, L. T. 1945. Determination of total organic and available forms of phosphorus in soils. *Soil Sci*. 59: 39-45.

Carakostas. M. C., Curry. L. L., Boileau. A. C., and Brusick, D. J. 2008. Overview: the history, technical function and safety of *Stevia rebaudioside* A, a naturally occurring steviol glycoside, for use in food and beverages. *Food Chem. Toxicol.* 46: 1-10.

Carneiro, J. W. P., Muniz, A. S., and Guedes, T. A. 1997. Greenhouse bedding plant production of *Stevia rebaudiana*. *Can. J. Plant Sci*., 77(3): 473- 474.

Ceunen, S., Werbrouck, S., and Genus, M. C. 2012. Stimulation of steviol glycoside accumulation in *Stevia rebaudiana* by red LED light. *J. Plant Physiol.* 169(7): 749-752.

Chalapathi, M. V., Timmegowda, S., Rama Krishna Prama, V., and Prasad, T. G. 1997. Natural non calorie sweetener stevia (*Stevia rebaudiana*Bertoni): a future crop of India. *Crop Res.* 14: 347-350.

Chan, P., Linson. B., Chen, Y., Liu, J., Hsieh, M., and Cheng, J. 2000. A double blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. *J. clin. Pharmacol.* 50: 215-220.

Chatsudthipong, V. and Muanprasat, C. 2009. Stevioside and related compounds: therapeutic benefits beyond sweetness. *Pharmacol.Ther.* 121: 41-54.

Chesnin, L. and Yien, C. H., 1951. Turbidimetric determination of available sulfates. *Soil Sci. Soc. Am. J.* 15(C): 149-151.

Choudhary, K. and Bandyopadhyay, N. 1999. Preliminary studies on the inorganic constituents of some indigenous hyperglycaemic herbs on oral glucose tolerance test. *J. Ethnopharmacol*. 64:179-184.

Clemente, C., Angelini, L. G., Ascrizzi, R., and Tavarini, S. 2021. *Stevia rebaudiana* (Bertoni) as a multifunctional and sustainable crop for the Mediterranean climate. *Agric.* 11(2): 123. Available :DOI: 10.3390/ agriculture11020123

Columbus, M. 1997. The Cultivation of Stevia, 'Nature's Sweetener'. Food and Rural Affairs Factsheet. Ontario Ministry of Agriculture [homepage].Available: http://www.omafra.gov.on.ca/english/ crops/ facts/stevia.htm.

Das, K., Dang, R., and Gupta, N. 2009. Comparative antimicrobial potential of different extracts of leaves of *Stevia rebaudiana* Bert. *Int. J. Nat. Eng. Sci.* 3(1): 65-68.

Dhar, A. K., Sharma, P. R., and Agarwal, S. G. 2006. Dynamics of essential oil biosynthesis in relation to inflorescence and glandular ontogeny in *Salvia sclarea*. *Flav. Frag. J.* 21: 817-821.

Dogra, K. S., Chauhan, S., and Jalal, J. S. 2015. Assessment of Indian medicinal plants for the treatment of asthma.*J. Med. Plants Res*. 9(32): 851-862.

Donalisio, M. G., Duarte, F. R., and Souza, C. J. 1982. Estevia (*Stevia rebaudiana). Agronomico.* 34: 65-68.

Dwivedi, R. S. 1999. Unnurtured and untapped super sweet non sacchariferous plant species in India. *Curr. Sci.* 76: 1454-1461.

Ermakov, E. I. and Kochetov, A. A. 1996. Specificities of the growth and development of stevia plants under different controllable light conditions. *Russ. J. Agric. Sci.* 1: 9-11.

Esra, U., Ozyigit, Y., Eruygur. N., Güven, D., and Kutuk, N. 2018. The effect of the plant age and growth period on the nutritional substance, chlorophyll and steviol glycoside rates in stevia (*Stevia rebaudiana* Bertoni) Leaves. *Commun. Soil Sci. Plant Anal.* 49(1): 1-12.

Farooqi, A. A. and Sreeramu, B. S. 2004. *Cultivation of Medicinal and Aromatic Crops*. Universities Press, University of Agricultural Sciences, Bangalore, India, 344p.

Fini, A., Brunetti, C., Loreto, F., Centritto, M., Ferrini, F., and Tattini, M. 2017. Isoprene responses and functions in plants challenged by environmental pressures associated to climate change. *Front. Plant Sci.*Available : https://doi.org/10.3389/fpls.2017.01281.

Frederico, A. P., Ruas, P. M., Marin, M. A., Fuas. C. F., and Nakajima. J. N. 1996. Chromosome studies in some Stevia Cav. Compositae species from Southern Brazil. *Braz. J. Genet.* 19: 605-609.

Geuns, M. C. 2003. Stevioside. *Phytochem*. 64(5): 913-921.

Goettemoeller, J. and Ching, A. 1999. Seed germination in *Stevia rebaudiana*. In: Janick, J. (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. pp. 510-511.

Gopinath, P. P., Parsad, R., Joseph, B., and Adarsh, V. S. 2020. GRAPES: General Rshiny Based Analysis Platform Empowered by Statistics. https://www.kaugrapes.com/home. Version 1.0.0. DOI: 10.5281/zenodo.4923220.

Goyal, S. K., Samsher, and Goyal, R. K. 2010. Stevia (*Stevia rebaudiana*) a Bio-sweetener. *Int. J. Food Sci. Nutri.* 61: 1-10.

Grashoff, J. 1972. A systematic study of the North and Central American species of Stevia. PhD Dissertation. University of Texas, Austin.

Guerrero, A. B., EmeterioL. S., Domeno, I., and Irigoyen, I. 2018. Steviol glycoside content dynamics during the growth cycle of *Stevia rebaudiana* Bertoni. *Am. J. Plant Sci.* 9(4): 892-901.

Hang, S., Barriuso, E., and Houot, S. 2005. Atrazine behavior in the different pedological horizons of two Argentinean non-till soil profiles. *Weed Res*. 45: 130-139.

He, A. L., Liu, J., Wang, X. H., Zhang. Q. G., Song. W., and Chen. J. 2019. Soil application of *Trichoderma asperellum* granules promotes growth and resistance to *Fusarium graminearum* in maize. *J. Integr. Agric.* 18: 599-606.

Hossain, M. F., Islam. M. T., Islam, M. A., and Akhtar, S. 2017. Cultivation and uses of Stevia (*Stevia rebaudiana* Bertoni): a review. *Afr. J. Food Agric. Nutr. Dev.* 17: 12745-12757.

Hu, X. G., Bartholomew, B., Nash, R. J., Wilson, F. X., Fleet, G. W., Nakagawa, S., Kato, A., Jia, Y. M., Vanwell, R., and Yu, C. Y. 2010. Synthesis and glycosidase inhibition of the enantiomer of Steviamine, the first example of a new class of indolizidine alkaloid. *Org. Lett*. 2: 2562-2565.

Ibrahim, N. A. S., Gengaihi, S., Reyad, N. S., El-Rigal, and Hamed. M. A. 2015. Hypoglycemic, hypolipidemic andantioxidant potency of the aqueous extract of *Stevia rebaudiana* (Bert.) Leaves. *J. Pharm. Res. Int.* 3(3): 15-32.

Jackson, M. L. 1958. *Soil Chemical Analysis* (Indian Reprint, 1967). Prentice Hall of India, New Delhi, 498p.

JECFA [Joint FAO/WHO Expert Committee on Food Additives]. 2008. Summary and conclusions of the sixty- ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Rome, Italy, pp. 17-26.

Kaplan, B. and Turgut, K. 2019. Improvement of rebaudioside A diterpene glycoside content in *Stevia rebaudiana* Bertoni using clone selection. *Turk. J. Agric. For.* 43(2): 232-240.

Khan, R., Chowdhury, S. H., and Karim, M. M. 2012. Effect of date of planting on the growth and leaf yield of Stevia (*Stevia rebaudiana*). *J. Bangladesh Agric. Univ.* 10(2): 205-210.

Khiraoui, A., Bakha, M., Amchra, F., Ourouadi, S., and Abdelali, B. 2017. Nutritional and biochemical properties of natural sweeteners of six cultivars of *Stevia rebaudiana* Bertoni leaves grown in Morocco. *J. Mater. Environ. Sci.* 8(3): 1015-1022.

Kim, I., Yang, M., Lee, O., and Kang, S. 2011. The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. *Food Sci. Technol.* 44: 1328-1332.

King, R. M. and Robinson. H. 1987. The genera of the Eupatorieae (Asteraceae). Missouri Botanical Garden, National Museum of Natural History, Smithsonian Institution, Washington, 501p.

Kinghorn, A. D. 1987. Biologically active compounds from plants with reputed medicinal and sweetening properties. *J. Nat. Prod.* 50(6): 1009-1024.

Kinghorn, A. D. and Soejarto, D. D. 1985. Current status of Stevioside as a sweetening agent for human use. In: Wagner, H., Hikino, H., Farnsworth, R. (eds) *Economic and Medicinal Plant Research*. Academic Press, London, 52p.

Kirk, R. S., Sawyer, R., and Egan, H. 1991. *Pearson's Composition and Analysis of Foods*. Wiley, New York, 708p.

Kochetov, A. A. 2004. Growth and yield of *Stevia rebaudiana* Bertoni under different conditions of lighting. In: *Agriculture Congress 2004 Innovation Towards Modernized Agriculture*, 4 - 7 October 2004**,** Melaka, Malaysia, pp. 197-199.

Kumar, R., Sharma, S., Ramesh, K., and Singh, B. 2012. Effects of shade regimes and planting geometry on growth, yield and quality of natural sweetener plant stevia (*Stevia rebaudiana* Bertoni) in north western Himalaya. *Arch. Agron. Soil Sci.*59(7): 1-17.

Kumar, R., Sharma, S., Ramesh, K., Prasad, R., Pathania, V. L., Singh, B., and Singh, R. D. 2012. Effect of agro-techniques on the performance of natural sweetener plant–stevia (*Stevia rebaudiana*) under western himalayan conditions. *Indian J. Agron.* 57(1): 74-81.

Kumari, N., Rana, R. C., Sharma, Y. P., and Kumar, S. 2016. Dynamics of steviol glycosides (stevioside and rebaudioside-A) with growth and development of *Stevia rebaudiana* Bertoni. *J. Appl. Nat. Sci.* 8(4): 1953-1958.

Lemus-Mondaca, M., Galvez, A. V., Bravo, L. Z., and Hen, K. A. 2012. *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chem.* 132 (3): 1121-1132.

Lemus-Mondaca, R., Vega-Galvez, A., Rojas, P., Stucken, K., Delporte, C., Valenzuela-Barra, G., Jagus, R.J., Agüero, M.V. and Pasten, A., 2018. Antioxidant, antimicrobial and anti-inflammatory potential of *Stevia rebaudiana* leaves: effect of different drying methods. *J. Appl. Res. Med. Aromat. Plants,* 11: 37-46.

Li, H., Tang, C., and Xu, Z. 2013. The effects of different light qualities on cannot be used (*Brassica napus* L.) plantlet growth and morphogenesis in vitro. *Sci. Hortic. Sci.* 150: 117-124.

Loewus, F. A. 1952. Improvement in anthrone method for determination of carbohydrates. *Anal. Chem.* 24(1): 875-878.

Lopez, V., Perez, S., Vinuesa, A., Zorzetto, C., and Abian, O. 2016. *Stevia rebaudiana* ethanolic extract exerts better antioxidant properties and antiproliferative effects in tumour cells than its diterpene glycoside stevioside. *Food Funct.* 7(4): 2107-2113.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951 Protein measurement with the Folin phenol reagent. *J. biol. Chem*. 193: 265-275.

Madan, S., Ahmad, S., Singh, G. N., Kohli, K., Kumar, Y., and Singh, R. 2010. *Stevia rebaudiana* (Berr.) Bertoni- A review. *Indian J. Nat. Prod. Resour.*1(3): 267-286.

Marcinek, K. and Krejpcio, Z. 2015. *Stevia rebaudiana* bertoni-chemical composition and functional properties. *Acta Sci. Pol. Technol. Aliment.* 14(2): 145-152.

Mastana, P. 2012. Studies on the propagation and harvesting of stevia. M.Sc., (Forestry) thesis. Dr Y.S. Parmar University of Horticulture and Forestry, Naunisolan (HP), India, 75p.

Megeji, N. W., Kumar, J. K., Virendra, S., Kaul, V. K., and Ahuja, P. S. 2005. Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener. *Curr. Sci.* 88(5): 801-804.

Metivier, J. and Viana, A. M. 1979. The effect of long and short day length upon the growth of whole plants and the level of soluble proteins, sugars, and stevioside in leaves of *Stevia rebaudiana* Bert. *J. Exp. Bot.* 30: 1211-1222.

Midmore, D. J. and Rank, A. H. 2002. A new rural industry– Stevia to replace imported chemical sweeteners. A report for the Rural Industries Research and Development Corporation. *RIRDC*, W02/022: 16A.

Mizutani, K. and Tanaka, O. 2002. Use of *Stevia rebaudiana* sweeteners in Japan. In: Kinghorn, A. D. (Ed.), *Stevia, the Genus Stevia. Medicinal and Aromatic Plants Industrial Profile*. Taylor and Francis, London, 19: 178-195.

Mohammad, M., Mohammad, U., Sher, M., Habib, A., and Iqbal, A. 2007. In vitro clonal propagation and biochemical analysis of field established *Stevia rebaudiana* Bertoni. *Pakist. J. Bot*. 39: 2467–2474.

Mohammed, M. H., Meawad, A. A. A., El-Mogy, E. E. A. M. and Abdelkader, M., 2019. Growth, yield components and chemical constituents of Stevia rebaudiana Bert. as affected by humic acid and NPK fertilization rates. *Zagazig J. Agric. Res*. 46(1): 13-26.

Moniruzzaman, M., Rahman, M., Hossain, M. M., and Sirahul, A. J. M. 2014. Response of coriander foliage to different rates and methods of nitrogen application. *Bangladesh J. Agric. Res.* 39(2): 359-371.

Monteiro, W. R., Castro, M. D. R, Viveiros, S. C. M., and Mahlberg, P. G. 2001. Development and some histochemical aspects of foliar glandular trichomes of *stevia rebaudiana*. *Revta Brasil. Bot.* 24(3): 349-357.

Moraes, R. M., Donega, M. A., Cantrell, C. L., and Simone, C. M. 2013. Effect of harvest timing on leaf production and yield of diterpene glycosides in *Stevia rebaudiana* Bert: A specialty perennial crop for Mississippi. *Ind. Crops Prod.* 51: 385-389.

Mordechai, S., Ibdah, M., Fisher, R., and Chaimovitsh, D. 2013. Dynamics of yield components and stevioside production in *stevia rebaudiana* grown under different planting times, plant stands and harvest regime. *Ind. Crops Prod.* 50: 731-736.

Nguyen, V. T., Van Vuong, Q., Bowyer, M. C., Van Altena, I. A., and Scarlett, C. J. 2015. Effects of different drying methods on bioactive compound yield and antioxidant capacity of *Phyllanthus amarus*. *Dry. Technol.* 33(8): 1006-1017.

Nygren, M. and Killomaki, S. 1993. Effect of shading on leaf structure and photosynthesis in young birches. *For. Ecol. Manag.* 7(2): 119-132.

Oddone. B. 1999. How to Grow Stevia. Guarani Botanicals, Inc., Pawcatuck, Connecticut. 30p.

Olsson, K., Carlsen, S., Semmler, A., Simon, E., Mikkelsen, M. D., and Moller, B. L. 2016. Microbial production of next-generation stevia sweeteners. *Microb. Cell Fact*. 15: 207.Available:DOI 10.1186/s12934-016-0609-1

Orazco, A. J., Caballero, E. C., and Navas, J. J. 2020. Growth and development of *Stevia rebaudiana*, in high and low levels of radiation. *Curr. Plant Biol.* 22: 1-5.

Pal, P. K., Kumar, R., Guleria, V., Mahajan, M., Prasad, R., Pathania, V., Gill, B. S., Singh, D., Chand, G., Singh, B., and Singh, R. D. 2015.

Crop-ecology and nutritional variability influence growth and secondary metabolites of *Stevia rebaudiana* Bertoni. *BMC Plant Biol.* 15(1): 1-16.

Pandey, S., 2018. Morphology, chemical composition and therapeutic potential of *Stevia rebaudiana*. *Indo Am. J. Pharm. Sci.* 5(4): 2260-2266.

Panpatil, V. V. and Polasa, K. 2008. Assessment of stevia (*Stevia rebaudiana*) natural sweetener: a review. *Nat. J. Food Sci. Technol.* 45(6): 467-473.

Pant, B. D., Pant, P., and Erban, A. 2015. Identification of primary and secondary metabolites with phosphorus status-dependent abundance in Arabidopsis, and of the transcription factor PHR1 as a major regulator of metabolic changes during phosphorus limitation. *Plant Cell Environ.* 38: 172-187.

Parhi, P. K. and Mohapatra, P. 2013. Preliminary quantitative estimation of the phytoconstituents of *Spermacoce ocymoides* (Burm. F) DC. *Int. J.* 1(9): 903-906.

Piper, C. S. 1942. Soil and Plant Analysis (Asian Reprint, 1996). Hans Publications, Bombay, 368p.

Rakesh, K., Sharma, S., Ramesh, K., and Bikram, S. 2012. Effects of shade regimes and planting geometry on growth, yield and quality of the natural sweetener plant stevia (*Stevia rebaudiana* Bertoni) in north-western Himalaya. *Arch. Agron. Soil Sci.* 59: 963-979.

Ramesh, K., Singh, V., and Megeji, N. W. 2006. Cultivation of Stevia (*Stevia rebaudiana* Bert.) Bertoni): a comprehensive review. *Adv. Agron.* 89: 137-177.

Ranjbar, T., Nekooeian, A. A., Tanideh, N., and Hosseinabadi, K. 2020. A comparison of the effects of Stevia extract and metformin on metabolic syndrome indices in rats fed with a high-fat, high-sucrose diet. *J. Food Biochem.* 44(8): 13242. Available: doi: 10.1111/jfbc. 13242.

Rao, L. J. and Mittra, B. N. 1988. Growth and yield of peanut as influenced by degree and duration of shading. *J. Agron. Crop. Sci.* 160(4): 260-265.

Samadpourrigani, E., Tansi, L. S., and Kafkas, N. E. 2019. The Effects of planting density, cutting stage and cutting number on yield and quality of *Stevia rebaudiana*. *J. Agric. Fac. Gaziosmanpasa Univ*. 36 (1):81-90.

Satpathy, S. and Das, M. 2010. In vitro shoot multiplication in *Stevia rebaudiana* Bert. A medicinally important plant. *Gen. Appl. Plant Physiol*. 36(3): 167-175.

Savita, S. M., Sheela, K., Sunanda. S., Shankar. A. G., Ramakrishna. P., and Sakey. S. 2004. Health implications of *Stevia rebaudiana*. *J. Hum. Ecol.* 15(3): 191-194.

Schmeling. V. G. A. 1967. Edulcorante natural nao calorico. *Boletim do Sanatorio Sao Lucas,* 94: 67-68.

Shaffert, E. E. and Chetobar, A. A. 1994. Structure, topography and ontogeny of *Stevia rebaudiana. Bot. Zhurnal,* 79: 38-48.

Shahak, Y., Gussakovsky, E. E., Cohen, Y., Lurie, S., Stern, R., Kfir, S., Naor, A., Atzmon, I., Doron, I., and Greenblat, Y. 2004. Color nets: a new approach for light manipulation in fruit trees. *Acta. Hortic,* 636: 609-616.

Shuping, C. and Shizhen, S. 1995. Study on storage technique of *Stevia rebaudiana* seed. *Acta. Agron.* 21(1): 102-105.

Singh, A. and Verma, P. P. S. 2015. Survival and growth performance of stevia cutting under different growing media. *J. Med. Plants Stud.* 3(2): 111-113.

Singh, P., Dwivedi, P., and Atri, N. 2014. In vitro shoot multiplication of Stevia and assessment of stevioside content and genetic fidelity of the regenerants. *Sugar Tech.* 16: 430-439.

Singh, S. D. and Rao, G. P. 2005. Stevia: the herbal sugar of 21st century. *Sugar Tech.* 7: 17-24.

Singh, V. and Kaul, V. K. 2005. *Stevia rebaudiana* for income generation. *Vigyan Pragati*. 10-15.

Soejarto, D. D., Compadre, C. M., Medon, P. J., Kamath, S. K., and Kinghorn, A. D. 1983. Potential sweetening agents of plant origin. II. Field search for sweet-tasting Stevia species. *Econ. Bot.* 37(1): 71-79.

Starratt, A. N., Kirby, C. W., Pocs, R., and Brandle, J. E. 2002. Rebaudioside F, A diterpene glycoside from *Stevia rebaudiana*. *Phytochem.* 59: 367-370.

Subbaiah, B. V. and Asija, L. L. K. 1956. A rapid procedure for estimation of available nitrogen in soils. *Curr. Sci.* 25: 259-260.

Sumida, T. 1980. Studies on *Stevia rebaudiana* Bertoni as a new possible crop for sweetening resource in Japan. *J. Cent. Agric.* 31: 1-71.

Taleie, N., Hamidoghli, Y., Rabiei, B., and Hamidoghli, S. 2012. Effects of plant density and transplanting date on herbage, stevioside, phenol and flavonoid yield of *Stevia rebaudiana* Bertoni. *Int. J. Agric. Crop Sci.*, 4(6): 298-302.

Tateo, F., Mariotti, M., Bononi, M., Lubian, E., Martello, S., Cornara, L. 1998. Stevioside content and morphological variability in a population of *Stevia rebaudiana* (Bertoni) from Paraguay. *Ital. J. Food Sci.* 10(3): 261-267.

Tavarini, S. and Angelini, L. G. 2013. *Stevia rebaudiana* Bertoni as a source of bioactive compounds: the effect of harvest time, experimental site and crop age on steviol glycoside content and antioxidant properties. *J. Sci. Food Agric.* 93(9): 2121-2129.

Thomas, W. and Dutcher, R.A., 1924. The colorimetric determination of carbohydrates in plants by the picric acid reduction method I. The estimation of reducing sugars and sucrose1. *J. Am. Chem. Soc*. 46(7): 1662-1669.

Valio, I. F. M. and Rocha, R. F. 1977. Effect of photoperiod and growth regulators on growth and flowering of *stevia rebaudiana*. *Jpn. J. Crop Sci.* 46: 243-248.

Verma, S., Mohanta, T., Revathy, T., Suthindhiran, K. and Jayasri, M. A., 2013. Phytochemical and pharmacological evaluation of selected plants. *Am. J. Biochem. Biotechnol.* 9(3): .291-299.

Watanabe, P. S. and Olsen, S. R. 1965. Test of an ascorbic acid method for determining phosphate in water and NH4HCO3 extracts from soil. *Proc. Soil. Sci. Am*. 29: 677-678.

Watson, D. J. 1952. The physiological basis of variation in yield. *Adv. Agron.* 4: 101-145.

Yadav, A. K., Singh, S., Dhyani, D., and Ahuja, P. S. 2011. A review on the improvement of Stevia. *Can. J. Plant Sci.* 91: 1-27.

Yang, Y. H., Huang, S. Z., and Han, Y. L. 2015. Base substitution mutations in uridine diphosphate-dependent glycosyl transferase 76G1 gene of *Stevia rebaudiana* causes the low levels of rebaudioside A: Mutations in *UGT76G1*, A key gene of steviol glycosides synthesis. *Plant Physiol. Biochem.* 80: 220-225.

Zaidan. L. B. P., Dietrich, S. M. C., and Felippe, G. M. 1980. Effect of photoperiod on flowering and stevioside content in plants of *Stevia rebaudiana*. *Jpn.J.Crop Sci.* 49(3): 569-74.

Environmental influence on yield and quality of stevia

(*Stevia rebaudiana* Bertoni)

By

SOMANATHAM SUHAS
(2018-11-078)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture
(Agronomy)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF AGRONOMY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2022

ABSTRACT

Stevia (*Stevia rebaudiana* Bertoni) is natural sweetener of the Asteraceae family, commonly known as candy leaf, honey leaf, sweet leaf, or sugar leaf. The plant contains steviol glycosides (SVglys), which are of high interest in the human diet as a low calorie and high potency sweetener. As the leaf is the main economic part of stevia, production of more leaf biomass with higher steviol glycosides is the main criteria for crop performance. Since both biomass production and quality are equally important in medicinal plant cultivation, it is necessary to determine optimal growth factors that can ensure high yield and quality.

The present study entitled "Environmental influence on yield and quality of stevia (*Stevia rebaudiana* Bertoni)" was conducted at the Department of Agronomy, College of Agriculture, Vellanikkara during the year 2020. The objective of the study was to determine the influence of growing conditions and planting dates on the biomass production and quality of stevia. The study consisted of a field experiment designed in split plot design with three replications and a pot culture. The aim of field experiment was to find out the effect of growing condition and planting dates on yield and quality of stevia. The main plot treatments consisted of two growing conditions viz., open and 50 per cent shade and the sub plot treatments consisted of four dates of planting viz., May 15th, June 15th, July 15th and August 15th. The pot culture study was designed in completely randomized design (CRD) to evaluate the phytochemical constituents of stevia leaves at different growth stages.

Growth parameters such as plant height, number of branches, fresh leaf weight, dry leaf weight, herbage yield, and total biomass yield were significantly influenced by growing conditions and planting dates. Open condition (8406 kg/ha) and May planting (10162 kg/ha) resulted in the highest leaf yields of stevia. The interaction between growing condition and

planting dates was also significant. May planting under open condition proved to be the best treatment with respect to leaf yield.

Growing conditions and planting dates had a profound influence on physiological and biochemical parameters. May planting under open conditions resulted in higher CGR and RGR values. Planting in May under open condition resulted in the highest steviol glycoside content of 9.05 per cent.

A positive correlation was observed between maximum temperature and plant height, number of leaves, fresh leaf weight, total biomass yield and total glycoside content. Whereas, negative correlation was found with respect to afternoon R.H and rainfall

Pot culture study conducted to assess the variations in phytochemical constituents of stevia leaves at different growth stages and growing conditions revealed variations in parameters such as total carbohydrates, fat, protein, soluble sugars, reducing sugars, non reducing sugars and steviol glycoside contents. Total carbohydrates and moisture content was observed to be higher under shaded growing condition, whereas open condition resulted in higher contents of total fat, crude fibre, protein, total minerals, total soluble sugars, reducing and non reducing sugars, total energy and total steviol glycosides.

Total carbohydrates, total fat, moisture, total soluble sugars, reducing and non reducing sugars and total steviol glycoside contents were higher at flower bud initiation stage. A slight reduction in carbohydrate content was observed towards maturity. The highest content of total protein was observed at full bloom stage. Total energy value increased from 45 DAP, reached a peak at flower bud initiation stage and decreased thereafter.

It can be concluded that open growing condition, planting in the month of May and harvesting at flower bud initiation stage can be suggested for better leaf yield and quality of stevia under Kerala conditions.

സംഗ്രഹം

മലയാളത്തിൽ മധുര തുളസി എന്നറിയപ്പെടുന്ന സ്റ്റീവിയയുടെ വളർച്ചയിലും ഗുണ മേന്മയിലും കാലാവസ്ഥ ഘടകങ്ങളുടെ പങ്ക് എന്ന വിഷയത്തിൽ കാർഷിക സർവകലാശാലയുടെ കോളേജ് ഓഫ് അഗ്രിക്കൾച്ചർ വെള്ളാനിക്കരയിലെ അഗ്രോണോമി വിഭാഗത്തിൽ മെയ് 2020 മുതൽ നവംബർ 2020 വരെ ഒരു പഠനം നടത്തുകയുണ്ടായി.

രണ്ട് വ്യത്യസ്ത തണലുകളിലായി (തുറസ്സായ സ്ഥലം, അമ്പതു ശതമാനം തണലുള്ള സ്ഥലം) മെയ് 15, ജൂൺ 15, ജൂലൈ 15, ഓഗസ്റ്റ് 15 എന്നീ തീയതികളിൽ സ്റ്റീവിയ തൈകൾ നട്ടാണ് കാലാവസ്ഥയുടെ സ്വാധീനം പഠിച്ചത്. സ്പ്ലിറ്റ് പ്ലോട്ട് എന്ന ഡിസൈനിൽ മൂന്ന് റെപ്ലിക്കേഷൻ ഉള്ള പഠനമാണ് നടത്തിയത്. ഒപ്പം സ്റ്റീവിയയുടെ കൃത്യമായ വിളവെടുപ്പ് സമയം മനസ്സിലാക്കുന്നതിനുള്ള പഠനവും നടത്തി. സി ആർ ഡി ഡിസൈനിൽ ചട്ടികളിലായാണ് ഈ പഠനം നടത്തിയത്. സ്റ്റീവിയയുടെ ഇലകൾ നട്ട് 45 ദിവസത്തിന് ശേഷവും, പൂമൊട്ടുകൾ പ്രത്യക്ഷപ്പെടാൻ തുടങ്ങുമ്പോഴും, 50 ശതമാനം മൊട്ടുകൾ വിടർന്നതിനു ശേഷവും, എല്ലാ പൂക്കളും വിടർന്നതിനുശേഷവും വിളവെടുപ്പ് നടത്തി ഇലകളുടെ രാസ ഘടന താരതമ്യ പഠനം നടത്തുകയാണ് ചെയ്തത്.

ചെടികളുടെ ഉയരം, ശാഖകളുടെ എണ്ണം, ഇലകളുടെ ഭാരം, ഉണങ്ങിയ ഇലകളുടെ ഭാരം, മൊത്തം വിളവ് തുടങ്ങിയ ചെടികളുടെ സ്വഭാവസവിശേഷതകൾ വളരുന്ന സാഹചര്യങ്ങളും നടീൽ തീയതികളും ഗണ്യമായി സ്വാധീനിച്ചു. തുറന്ന അവസ്ഥയും (8406 കിലോഗ്രാം/ഹെക്ടർ), മെയ് നടീലും (10162 കിലോഗ്രാം/ഹെക്ടർ) സ്റ്റീവിയയുടെ ഏറ്റവും ഉയർന്ന ഇല വിളവ് ലഭിക്കുന്നതിന് കാരണമായി. വളരുന്ന അവസ്ഥയും നടീൽ തീയതികളും തമ്മിലുള്ള പരസ്പരബന്ധവും പ്രാധാന്യമർഹിക്കുന്നു. ഏറ്റവും നല്ല വിളവ് ലഭിക്കാൻ സ്റ്റീവിയ മെയ് മാസത്തിൽ തുറസായ സ്ഥലങ്ങളിൽ നടുന്നതാണ് നല്ലത് എന്ന് പഠനത്തിൽ നിന്നും വ്യക്തമായി.

സ്റ്റീവിയയുടെ ഗുണ മേന്മ ഘടകമായ സ്റ്റീവിയോസൈഡ് എന്ന രാസ ഘടകം ഏറ്റവും കൂടുതലായി കണ്ടതും മെയ് മാസത്തിൽ തുറസായ

സ്ഥലത്തു നട്ടപ്പോഴാണ്.

അന്തരീക്ഷ താപനിലയുമായി ചെടികളുടെ ഉയരം, ഇലകളുടെ എണ്ണം, ഇലകളുടെ ഭാരം, മൊത്തം വിളവ്, എന്നിവയുടെ പരസ്പരബന്ധം പോസിറ്റീവ് ആണെന്നും അന്തരീക്ഷ ആർദ്രത, മഴ എന്നിവയുമായി ഇവയുടെ ബന്ധം നെഗറ്റീവ് ആണെന്നും പഠനം വ്യക്തമാക്കുന്നു.

തണലിൽ വളർത്തുന്ന ചെടികളിൽ കാർബോഹൈഡ്രേറ്റും ഈർപ്പവും കൂടുതലായി കാണപ്പെട്ടു. തുറന്ന അവസ്ഥയിൽ കൊഴുപ്പ്, നാരുകൾ, പ്രോട്ടീൻ, ധാതുക്കൾ, പഞ്ചസാര, ഊർജ്ജം, സ്റ്റീവിയോൾ ഗ്ലൈക്കോസൈഡുകൾ എന്നിവയുടെ ഉയർന്ന ഉള്ളടക്കം ഉണ്ടായി.

കാർബോഹൈഡ്രേറ്റുകൾ, കൊഴുപ്പ്, ഈർപ്പം, പഞ്ചസാര, സ്റ്റീവിയോൾ ഗ്ലൈക്കോസൈഡ് എന്നിവ പൂമൊട്ടിന്റെ ആരംഭ ഘട്ടത്തിൽ കൂടുതലായിരുന്നു. അതിനുശേഷം കാർബോഹൈഡ്രേറ്റ് ഉള്ളടക്കത്തിൽ നേരിയ കുറവ് നിരീക്ഷിക്കപ്പെട്ടു. പ്രോട്ടീന്റെ ഏറ്റവും ഉയർന്ന തോത് പൂവിടൽ മുഴുവനാകുമ്പോൾ നിരീക്ഷിക്കപ്പെട്ടു. സ്റ്റീവിയയിലെ ഊർജ തോത് പൂമൊട്ട് ആരംഭിക്കുന്ന ഘട്ടത്തിൽ അത്യുന്നതത്തിലെത്തുകയും പിന്നീട് കുറയുകയും ചെയ്തു.

പരീക്ഷണത്തിൽ നിന്ന്, തുറസ്സായ സാഹചര്യം, മെയ് മാസത്തിൽ നടീൽ, പൂമൊട്ടിന്റെ ആരംഭ ഘട്ടത്തിൽ വിളവെടുപ്പ് എന്നിവ ഇലകളുടെ മികച്ച വിളവിനും സ്റ്റീവിയയുടെ ഗുണനിലവാരത്തിനും നിർദ്ദേശിക്കാവുന്നതാണ്.