EVALUATION OF STRESS INDUCED CHANGES IN SELECTED METABOLITES IN SOME VARIETIES OF GINGER

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By

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CERTIFICATE

This is to certify that the Ph.D. thesis entitled **"EVALUATION OF STRESS INDUCED CHANGES IN SELECTED METABOLITES IN SOME VARIETIES OF GINGER"** is an authentic record of the original research work accomplished by **Ms. Neena A.** under my supervision and guidance at the Centre for Post Graduate Studies and Research in Botany, St. Joseph's College (Autonomous) Devagiri, Calicut, Kerala and that no part of this thesis has been published earlier for the award of any other degree or diploma. Also certified that the contents in the thesis are subjected to **Plagiarism Check** using the software **Ouriginal** and that no text or data is reproduced from other's work.

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DECLARATION

I, **Neena A,** do hereby declare that this Ph.D. thesis entitled **"EVALUATION OF STRESS INDUCED CHANGES IN SELECTED METABOLITES IN SOME VARIETIES OF GINGER"** is the summary of the research work carried out by me under the supervision of **Dr. Binu Thomas,** Assistant Professor, Department of Botany, St. Joseph's College (Autonomous) Devagiri, Calicut, Kerala in partial fulfilment of the requirement for the award of the **Degree of Doctor of Philosophy in Botany** under the faculty of **Science** of the **University of Calicut**. I also declare that no part of this thesis has been submitted by me for the award of any other degree or diploma, and it represents original work done by me.

St. Joseph's College, Devagiri

Neena A.

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ABBREVIATIONS

ACN	: Acetonitrile
AlCl3	: Aluminium Chloride
amu	: Atomic Mass Unit
ASTA	: American Spice Trade Association
C	: Control
CAS	: Chemical Abstracts Service
CH3	: Methyl group
Chl-a	: Chlorophyll- a
Chl-b	: Chlorophyll-b
CO2	: Carbon dioxide
cv	: Cultivar
D	: Drought
DMSO	: Dimethyl Sulfoxide
DPPH	: 2,2-Diphenyl-1-picrylhydrazyl.
DW	: Distilled Water
et al.	: et alia (and others)
eV	: Electron volt
F-C	: Folin-Ciocalteu
g	: gram
GA	: Gallic acid
GAE	: Gallic acid equivalent
GC-MS	: Gas Chromatography Mass Spectrometry
H_2O	: Water
HPLC	: High Performance Liquid Chromatography
HPTLC	: High Performance Thin Layer Chromatography
ICAR	: Indian Council of Agricultural Research
IISR	: Indian Institute of Spices Research
KDa	: Kilo Dalton

kg	: Kilogram
M	: Molar
M.W.	: Molecular Weight
MeOH	: Methanol
mg	: Milligram
min	: minutes
ml	: Milli litre
MS	: Mass Spectrometry
NaOH	: Sodium Hydroxide
NBPGR	: National Bureau of Plant Genetic Resources
NIST-14	: National Institute of Standards and Technology 14
NVA	: 8-Methyl-N-vanillyl-trans-6-nonenamide
nm	: Nanometer
PAL	: Phenylalanine Ammonia Lyase
PDA	: Photo diode array
PK TY	: Peak Type
ppm	: Parts per million
QE	: Quercetin
QE/g	: Quercetin equivalent per gram
R.T.	: Retention time
Rf	: Retention factor
rpm	: Rotations per minute
SA	: Salicylic Acid
TLC	: Thin Layer Chromatography
TPC	: Total Phenolic Content
UV	: Ultraviolet
v/v	: Volume by volume
viz.	: namely
$ZnSO_4$ $7H_2O$	: Zinc Sulphate Heptahydrate

µl	: Micro litre
6- G	: 6- Gingerol
8-G	: 8-gingerol
10-G	: 10-gingerol

Spices have been the heart and the soul of Indian economy since ages. Ginger is one of the most widely used spices in the world. It has been utilized in Asian cuisine for more than a millennium. Despite being used in cooking; ginger is thought to have a variety of health benefits. As a result, it is widely used as an herbal cure as well as a spice or flavoring component. Ginger was one of the first raw herbal medications that may have gone into several Ayurveda formulations, making it the most popular medication in the Indian medical system. In ancient literature, ginger is referred to as "*An aromatic of Paradise*," and it has a long history as a spice. Rhizomes or the modified underground stem of ginger, are used as a spice to flavor a wide variety of cuisines and food products all over the world. Additionally, it is utilized in medications, specifically conventional medications (Lawrece, 1984; Selvan *et al.*, 2002). It was first used in Europe at least 2,000 years ago. In Indian medical traditions, ginger is referred to be "great medicine" and "universal cure,". It is a magnificent spice with an unmatched variety of uses. The basis of the Indian system of medicine, or Ayurveda, is the knowledge provided in the original ancient Indian texts "*Susrutha Samhitha*" and "*Charaka Samhitha*" on the use of ginger. Chinese and Sanskrit literature from later ages detailed the use of this plant (Ravindran & Babu, 2016).

Ginger which is a member of Zingiberaceae family is well known for its use in ethno medicine and Ayurveda. It is considered as the most widely consumed spices in the world. Ketones especially gingerols gives the spicy aroma to the ginger. This spice is cultivated all over the humid regions with India as the largest producer. Indians and Chinese consider this as a tonic root for over 5000 years to treat many ailments. Since time immemorial ginger has been cultivated in India for both fresh vegetable and as dried spice. The zingiberacean family is a tropical group especially abundant in indo-malasian region and consists of more than 1200 plant species with over 53 genera (Dhanik *et al.*, 2017).

1.1 Origin and history of ginger

It is believed that ginger has been originated in India. The names '*Zingiber*' itself support this belief. It is originated from the Sanskrit word '*Singibere*' later '*Zingiberi*' in Greek and from that the generic name Zingiber has come. Ginger was an important trade in India and was exported from India to Rome over 2000 years ago because of its high medicinal property. Even after the fall of Roman Empire with Arab merchants the Ginger continued to occupy its position as highly sought commodity in Europe. During thirteenth

and fourteenth century the value of ginger was calculated as equal to the value of a sheep. In England Queen Elizabeth-I is attributed with the invention of Ginger bread, which became a famous Christmas treat (Pruthi, 1993).

Ginger is consumed throughout the world as a prime spice because of its pungency and aroma. Both fresh and dried form of ginger is used in medicinal and culinary purposes (Akhila & Tewari, 1984). The main part used is the rhizome and is mainly used in the form of paste, dried form of ginger is used. Fresh ginger is used as a vegetable. Western countries use ginger in puddings, soups, cakes, pickles, beer, bread and wine. The vast application of ginger in a wide range of culinary purposes is mainly because of its aroma and pungency. The unique properties of flavor and pungency of ginger arise from its essential oil composition and the oleoresin content. Both oleoresin and essential oil gives ginger its unique flavor and aroma which is used in several food products such as beverages and in many pharmaceutical formulations. Ginger can be found in different food combinations of daily food diet of human, that is it indicates that considerable amount of ginger is taken as a food supplement along with other dietary food of human. Besides its importance in culinary purposes both essential oil and oleoresin has got importance in the field of medicine too, from digestion problems to cancer treatments (O'Hara *et al.,* 1998).

Indians consider ginger as a '*Mahaaushadha*' (the great medicine) and is a part of traditional medicinal practices in all the ginger growing countries. It is widely used in traditional oriental medicine for common cold, digestive disorder and rheumatism (Surh *et al.,*1999). Ayurveda, Chinese and traditional medicinal systems recommended ginger as remedy for numerous ailments including nausea, motion sickness, nausea related to pregnancy, vomiting, loss of appetite, stomach cramps, diarrhea, heart burn, colic, flatulence, indigestion, common cold, influenza, cough, arthritis, rheumatic disorders, migraine headaches, cardiac palpitations, hypertension and impotence. It is reported to exhibit stimulant, aphrodisiac, aromatic and carminative properties when taken internally. While behaving as a sialagogue when chewed, and a rubefacient when applied externally. (Kritikar & Basu, 1975; Nadkarni & Nadkarni, 1976). In addition, ginger is found to be an effective against tumor growth, rheumatism and migraine and is active as an antioxidant in the body. There are many reviews cited indicating various functions of ginger. Pharmacological and clinical effects of ginger was given by Chrubasik *et al.* (2005).

Grzanna *et al.* (2005) reported ginger as an anti-inflammatory agent. Shukla & Singh (2007) also reported the cancer prevention properties of crude drug. Some of the phytochemical, pharmacological and toxicological properties of ginger was given by Ali *et al.* (2008). Banerjee *et al,* 2011 & Chakraborty *et al,* 2012 also gives an insight over the greater potency of gingerol on the treatment of various diseases including colorectal cancer too.

1.2 Ginger: *Zingiber officinale* Rosc. (Zingiberaceae) a botanical description.

Ginger (*Z. officinale* Rosc.) is an herbaceous, perennial plant of about 1m in height with many fibrous roots, aerial shoot with leaves and branched rhizome. The flowers occur as a dense spike and are fragile consisting of several overlapping scales on an elongated stalk. Each single flower possesses three yellowish - orange petals with an additional purplish lip like structure. The rhizomes are thick lobbed, branched with structures with spicy scent (Ravindran *et al.*, 2005).

1.3 Cultivation and production of ginger

Ginger is one of the important spices cultivated in several parts of the world. The major ginger producing countries include India, China, Japan, Indonesia, Nigeria, Australia and West Indies Islands. Among the ginger produced by the above-mentioned countries Indian and Jamaican ginger is the best quality ginger, followed by the West African variety. Since Chinese ginger is not exported as a fried spice, it is preserved in sugar syrup otherwise converted into '*Candy ginger'* or '*Ginger candy'* It's a low pungency ginger. Certain amount of pungency is there in Japanese ginger but it lacks the characteristic aroma in them. Chinese ginger which possess low pungency and aroma it cannot be used for the essential oil and oleoresin separation (Kandiannan *et al*., 1996).

India is the foremost producer and consumer of ginger in the world. Out of the states Kerala contributes one third of the ginger production. Out of the total ginger produced 30% of them are converted in to dried ginger (*Chukku* in Malayalam) 50% of them are used as fresh material and rest as seed material (Nair, 2019). Major share of dried ginger is produced in Kerala and is exported. Calicut and Cochin ginger are the two major Indian varieties of ginger in the world market (Bag, 2018).

1.4 Chemistry of ginger

The phytochemistry of compounds reported from Zingiberacean family including *Zingiber officinale* was given by Pancharoen *et al.* (2000). The aroma of ginger is represented by the steam distilled Volatile oil and it ranges from 1.0% to 2.5 % in dried rhizomes from different countries and has different compositional varieties too. This compositional variety may in turn is a result of different parameters. Factors such as maturity of rhizomes, geographical regions, fresh or dried rhizomes and conditions of drying process (Vernin & Parkanyi, 2016).

Ginger oil contains a variety of compounds. It contains high amount of Sesquiterpene hydrocarbons especially Zingiberene. Non-volatile compounds especially gingerols contribute to the pungent principles of ginger. These have significant contribution towards medicinal properties of ginger too. Structurally gingerols are related to Capscicinoids from chilly which give them their pungency (Gaikwad *et al*., 2017).

The potential health benefit of ginger is attributed to gingerol and shogaol. These are compounds which are bioactive. Shogaol which is present in ginger act as a potential anti coughing agent. While gingerol has got analgesic and anti-inflammatory properties. 6 gingerol inhibits the formation of new blood vessels thus by acting against tumors. Gingerol is also used to treat or found effective against nausea which occur during pungency and chemotherapy (Hassan & Hassan, 2018; Shahrajabian *et al.,* 2019).

Flavor, aroma and pungency of ginger are due to the presence of zingerone and Zingiberene. Zingiberene is present in higher quantity than zingerone. 30% of the essential oil contains zingiberene. Fresh ginger's pungency comes from gingerol while its flavor comes from zingiberene (Ashraf *et al.*,2017).

1.5 Plant responses to environmental stress

In a competitive growing environment, the ability of sessile plants to recognize, integrate, and respond to biotic and abiotic environmental variables by continuously adjusting physiology and metabolism to optimize growth and reproduction in an ever-changing environment is a key factor underlying plant resistance. This ability is made possible by the cross-tolerance phenomenon, in which exposure to a multitude stress results in increased tolerance in plant, when comparing with solitary stress (Pastori & Foyer, 2002;

Mittler, 2006). When many pressures are present, their combined effects might be hidden, boosting both the quantity and quality of output (Foyer *et al.*, 2016).

Very limited information is available on the varietal evolution of ginger in terms of morphological and phytochemical parameters in response to stresses, especially to foliar sprays and its combinations. If any foliar spray of chemical compound can alter the negative impact of drought stress in ginger it can be a breakthrough in the improvement of this commercial crop. To date much less focus has been placed on the integrated response of plants to multiple stresses typically encountered under field conditions, however fundamental knowledge is now sufficiently advanced to tackle these questions. In the present study an attempt was made to assess the impact of solitary stress and comparing it with combination stresses for morphological parameters and secondary metabolite content.

Objectives of the study

A comparative analysis of two cultivars of ginger in terms morphology and production secondary metabolites under various solitary as well as combined stress treatments was the main aim of this study. The major objectives of the present study includes;

- Preliminary phytochemical studies of selected ginger varieties.
- Develop chemical fingerprints of selected ginger varieties using various chromatographic techniques.
- To investigate the influence of stress signals on morphological characters and secondary metabolite production in selected ginger varieties.
- Comparative quantification of selected secondary metabolites in certain ginger varieties using chromatographic techniques.

Spices are an essential part of human diet from the ancient past. Besides its uses in food, spices are also appreciated for their medicinal properties. The future and the potential of nation are involved in the control of spice trade (Parry,1969). One of the well-known rhizomatous oriental spices, ginger (*Zingiber officinale* Rosc.) of the Zingiberaceae family, is grown across the world, primarily in tropical and subtropical regions for use in food and medicine preparation. Ginger can be used to make a variety of high-value goods, such as oleoresin and essential oil. Significant export potential exists for less fibre in the global market. Food, drink and pharmaceutical products use the essential oil and oleoresin as flavorings. Although India produces 33.9 percent of the world's ginger, the primary challenge to growing ginger is the shift in agro climatic areas, which increases the likelihood of significant variance in secondary metabolites (Ronya *et al.,* 2020)

Zingiber officinale commonly known as Ginger is used as medicine in herbal traditions of Asian, Indian and Arabian cultures (Altman & Marcussen, 2001). After Pepper, ginger was the commonest and the most valuable of all spices during the period of 13^{th} and 14^{th} century. It costs nearly 7 scrolling per pound (Mahindru, 1982). Since more than 2500 years it has been widely used to treat different ailments like cold, nausea, head ache etc. (Sharma *et al.,* 1997). It is also extensively used in western parts as a part of herbal medicine practice for the treatment of different conditions like rheumatological diseases, muscular discomfort and arthritis (Bordia *et al.,*1997, Langner *et al.,* 1998).

In certain countries like Africa several tribes namely *Savaras* use this for their religious functions such as marriages (Mahindru, 1982). They also point out that '*Green ginger'* the preserved ginger in syrup form was also used to import to the western world during the middle ages. The Western Herbalist John Gerard writes in his herbal (1633) that "ginger is right good with meat in sauces" and says that this spice is "of an eating and digesting quality" (Parry, 1969). During these times English tavern keepers used ground ginger on the top of the beer or and then stir in to the drink with a red-hot poker (Rosengarten, 1969). The Greek '*Zingiberi*' and the Latin *Zingiber* came from the Sanskrit word '*Sringvera*' (Rosengarten, 1969; Purseglove *et al.,* 1981). The different names of ginger in different languages in India are *Adrak* (Hindi, Punjabi and Urdu), *Inji* (Tamil), *Inchi* (Malayalam), *Shunti* (Kannada), *Ada* (Bengali, oriya), *Allamu, Sonthi* (Telungu), *Adu* (Gujarati), *Ale* (Marathi) (Ravindran *et al.*, 2005).

2.1 *Zingiber officinale* Rosc.

Humans have used ginger (*Zingiber officinale* Rosc.) as a crop for both medicinal and culinary purposes. Sanskrit refers to ginger as Sringavera, from which the term *Zingiberi* may have developed and been used in Greek, eventually evolving into the Latin title *Zingiber*. It is a member of the economically significant plant family Zingiberaceae, which is primarily found in tropical regions of Africa, Asia and America with a predominance in Southeast Asia. Only in India is the family Zingiberaceae described with 22 genera and roughly 178 species (Jain & Prakash, 1995), compared to the family's projected 1400 species under 52 genera (Burtt & Smith, 1972).

Since ancient times, several traditional medicine systems have used members of the Zingiberaceae family for their medicinal benefits and many species are also employed in Ayurveda, Unani and Homoeopathic medical practices. According to Jantan *et al.* (2003), the medicinal and aromatic qualities of Indian Zingiberaceae members are mostly beneficial in the preparation of food, spices, medications, dyes and perfumes. Additionally, they are effective in the treatment of headache, cuts, sores, diarrhea, dysentery, peptic ulcers, fractures, colds and flu (Langner *et al.,* 1998).

2.2 History of ginger in India

'*Indian science of Life'*, the Ayurveda with more than 5000 years of classical times reported the importance of ginger in the field of medicine. It is known as '*Mahaaushadi*' in Ayurveda. Van Rheede (1692) first documented ginger as '*inschi'* (*Inchi*) in *Hortus Malabaricus* which is considered as the first printed account of plants of Malabar costs of India.

During the past period Ginger was not given much importance. It was named *Mahabheshaj*, *Maha aushadi* meaning good remedy and the great cure. People believed that this spice is a god given medicament for all most all ailments. It is due to this belief ginger occupied a place in ancient Ayurvedic texts like Charaka (*Charaka samhita*) and Susruth (*Susrutha samhita*). *Ashtangahridayam* an ancient Ayurvedic texts by Vaghabatta, also emphasize on its medicinal values and there is a clear recommendation of ginger along with other herbs for the treatment of certain diseases like elephantiasis, gout, indigestion *etc.*

Linschotten (1596), observed that ginger grew in many parts but the best and better quality one was the one which grew along the costs of Malabar. Detailed description of about ginger and turmeric practices prevalent in the 19th century of India was given by Ridley (1912).

Ginger is considered to be a native of South East Asia (Purseglove *et al.,* 1981). History says that during the period of scurvy outbreak ginger plants grown in pots were carried on the long sea voyages between China and South East Asia (Rosengarten, 1969).

2.3 Origin and geographical distribution

Since ancient times, it has been believed that ginger may have its origins in Southeast Asia. According to reports, ginger is also grown in India and China (Bailey, 1949; Purseglove, 1981).

Geographically, tropical and subtropical nations are home to *Zingiber officinale*. India, China, Jamaica, Nigeria, Bangladesh, Mauritius, Sierra Leone, Brazil, Fiji, Philippines, Taiwan, Japan, Ghana, Costa Rica, Sri Lanka, Malaysia, Thailand, Solomon Islands, Trinidad, Uganda, Tobago and Guatemala are the primary producers of ginger (Kampe, 2018).

2.4 Taxonomic description of the plant

Ginger is a perennial herb that grows to a height of 30 to 100 cm and has leafy aerial shoots (pseudo stems) that are harvested annually. Depending on the genotype or cultivar, the physical attributes of ginger plants tend to vary mostly at rhizome structures and plant height. The narrow, oblong, lanceolate, simple, alternating leaves have a thin texture and rather short. These leaves are present as scale leaves in rhizomes, as foliage leaves in aerial stem and sheath leaves at distal part of rhizome (Holttum, 1950). Numerous scale leaves on the nodes of the underground stem are shielded and protected by axillary buds.

Ginger's inflorescence rises directly from the rhizome. The length of the radical ranges from 15 to 25 cm. Spike is often round or cylindrical, approximately 4-7x 2-3 cm long, and the scape is typically thick or slender, erect, short or long. Its bracts are relatively large, 2.5 x 1.5–2 cm, oval or elliptic, green with a darker membranous edge, usually mucronate and turning brightly colored (yellow or red), typically fleshy, and closely imbricating with apices free, and margins plane (Purseglove *et al.,* 1981). Typically, the blooms are tiny, bracteolate, bisexual and zygomorphic. Flowering is not a typical trait of

plants, especially ginger plants, since it varies at different geographic locations and is impacted by diverse climatic conditions under which it is grown. Likewise, the fleshy roots, which grow from the mother rhizome close to the lower nodes as well as from the primary fingers, are thicker and milky white in color. Roots help in the absorption of nutrients and water (Parthasarathy *et al.*, 2012).

2.5 Cultivation of ginger

Ginger is best grown in regions with a predominantly tropical or subtropical environment. Ginger needs a warm, humid climate, moderate rainfall and plenty of shade during the growing season to produce healthy, disease-free root. Dry weather is crucial, even in the months before harvesting. The best recommended soils for ginger cultivation are lateritic loam or red loam, sandy or clay loam. It can be grown in partial shade at higher altitudes (Pruthi, 1993).

2.6 Varieties and production of ginger

There are a lot of commercial varieties of ginger. The Cochin ginger (Kerala, India) is the largest of all, well scraped and high quantity of starch. On the other hand, the Nigerian ginger is lesser in size, darker and having high pungent taste. Humid and warm atmosphere is the advisable condition for the cultivation of ginger (Utpala *et al.,* 2011). In different parts of India, many cultivars of ginger are grown. Some are given suggested names, while others are given local names in honor of the places where they are grown or collected. Jamaica, Himachal, Thingpuri, Tura, Karakkal, Maran (Ravindran *et al.,* 2005). Some of the well-known cultivars include Rio-de-Janeiro, Varada, Himgiri, Kunduli local, Ernad Chernad, Kerakkal, Gurubthan, Poona, Shilong, Suprabha, Suruchi, Suravi, and Waynad local.

India is one of the world's leading producers of ginger, accounting for approximately 33.9 percent of global production each year from an area of approximately 165,000 hectares (Factfish, 2016). Every state in India grows ginger, with Kerala having the highest area of cultivation (approximately 19 percent of the total), followed by Odisha (17 percent), West Bengal (12 percent), Meghalaya (12 percent) and Arunachal Pradesh (6 percent). Himachal Pradesh, Mizoram, Manipur, Assam, Sikkim, Madhya Pradesh, Andhra Pradesh, Maharashtra, Gujarat, Karnataka, Rajasthan, Tamil Nadu, Bihar and Jammu & Kashmir are just a few of the states that are also expanding the cultivation of ginger

(Ravindran & Babu, 2016). According to HerbaZest (2018), ginger is a significant economic crop that is grown for commercial purposes throughout South and Southeast Asia, tropical regions of Africa, Latin America and Caribbean regions. Although Asia is where fresh ginger is most frequently consumed, North America and Western Europe have also seen an increase in demand for ginger. China and Thailand are the other two largest producers of ginger, even though India now leads in this regard.

Although there are many local varieties being grown in India, there are only 75 identified varieties of ginger along with two exotic cultivars (Rio-de-Janeiro and China) (Rattan, 1988). Suprabha, Suruchi, Suravi, SG-666, IISR, Varada and other enhanced cultivars have been released from these research facilities through selection, among others. These selections are based on traits including disease resistance, low crude fibre content, high oleoresin content, high essential oil content, and excellent dry ginger recovery. National Research Centre for Spices (ICAR-IISR), Calicut and National Bureau of Plant Genetic Resources (NBPGR), New Delhi, have both undertaken various sorts of characterization and documentation of ginger germplasm; nearly all of their regional and base centres are situated at Trichur and Shillong.

2.7 Economic importance of ginger

Ginger is a classic spice in all illustrious cultures in the world. Many Asian cuisines are regarded as not being complete without its distinctive aroma and sweet-spicy flavour. In addition, ginger is frequently used in Indian curries and various seasonal desserts, including the traditional gingerbread. It has become the most widely used dietary condiment due to its relevance in international cuisine. While eating fresh, raw ginger is undoubtedly the most common way to consume this herb worldwide, powdered or ground ginger is frequently employed as a food additive and flavouring enhancer. The Asian root can also be found candied, crystallised, pickled, or preserved (HerbaZest, 2018).

2.8 Use of ginger as medicine

Ginger has a long history of traditional applications and is regarded as one of the world's most therapeutic herbs thanks to its medicinal properties. In addition to being an anti-inflammatory and painkiller, ginger is particularly good against nausea and vomiting (Mashhadi *et al.*, 2013). When combined with other herbs, such as chamomile, which is frequently used to treat upset stomach and other gastrointestinal ailments but whose

claimed anti emetic effects appear to be moderate in comparison to ginger, ginger's qualities rise dramatically. The well-known anti-nausea effects of ginger can be supplemented by chamomile in a hot infusion, which is reputedly more potent than dimenhydrinate at reducing motion sickness and pregnancy-related nausea. However, ginger's gastro-protective ability when taken as a juice to relieve the stomach pain brought on by gastritis enables bowel motions (Haniadka *et al.,* 2013).

Additionally, ginger has had a significant cultural impact on folk medicine. Ginger is thought to have been manufactured in Asia for over 5,000 years to treat a wide range of common maladies, such as nausea, vomiting, pain, and inflammation. Many of ginger's medicinal benefits have been verified by modern research, and now, many medical professionals advise using ginger as a common household treatment. In addition to its use in the culinary and pharmaceutical industries, ginger is also used in the cosmetic industry. It is used in fragrances, lotions, and balms as a skin-conditioning agent and as an ingredient for scent. It is obvious that ginger deserves a special place among the spices of considerable culinary, economic and social value all over the world because of its diverse uses and health advantages (Herbazest, 2018).

Since ancient times, ginger has been widely used as a commercially important spice as well as a good medicine. Because of its medicinal properties it was well known in Europe from 9^{th} century and in England from 10^{th} century (Sasidharan & Menon, 2010). Ginger is believed to have direct effect on gastrointestinal system to reduce nausea. Hence it is used to prevent nausea which results from motion sickness, surgery and chemotherapy (Langner *et al.,* 1998). Apart from these, ginger is always used to get relieve from problems that occur during pregnancy like nausea, morning sickness, vomiting, etc. (Prasad & Tyagi 2015).

In Indian Ayurveda ginger is highly suggested, because of its property to help in food digestion (Ali & Gilani, 2007). Apart from all these, ginger is used as a potential medicine to treat different types of pains that occurs due to arthritis, muscle soreness, menstrual pain, chest and back pain (Shukla & Singh, 2007). Because of the warming effect of ginger, it has been used for the treatment of flu and cold (Qidwai *et al.,* 2003). Its pungency and odor are mainly exploited in making foods, beverages, soaps and cosmetics (Alam, 2013). Ginger has been used for thousands of years for the treatment of a variety of

ailments ranging from common cold to cancer. Srinivasan, (2011) suggested that the active compounds of ginger has properties to suppress the growth and can induce apoptosis of different types of cancer like breast, cervical, oral, renal, prostrate, gastric, pancreatic, brain and liver.

2.9 Phytochemical analysis of ginger

Plants naturally contain chemical substances called phytochemicals that give them their colour and other organoleptic characteristics. Several active phytocompounds that have been extracted and characterized have high activity profiles and are currently needed in industrial use (Mandal *et al.,* 2007). The fact that phytocompound demands are rising suggests that plant secondary metabolites are important for both human health and nutritional value (Hertog *et al.,* 1993). Through phytochemical screening, several active components have been identified that have free radical scavenging activities, such as flavonoids, alkaloids, steroids, tannins, saponins, etc. (Larson, 1988; Parekh & Chanda, 2007; Soni & Sosa, 2013; Benariba *et al.*, 2013).

Phytochemicals are the chemical compounds which are formed on the plants as a result of normal metabolic processes. These include alkaloids, flavonoids, coumarins, glycosides, phenols, tannins, polysaccharides, terpenes and terpenoides (Okwu, 2004). Apart from these secondary metabolites it also contains other chemical compounds too. There are only about 12,000 secondary metabolites has been isolated so far which is only about 10% of the total, which indicates that plants have got limitless ability to synthesize aromatic substances mainly secondary metabolites.

According to Harborne (1973) phytochemical evaluation for pharmacologically potent drugs from plant species involves the following steps: Taxonomic authentication of plant sample, Extraction of the plant material, Separation and isolation of the plant constituents, Characterization of the isolated and purified compounds, Investigation of the biosynthetic pathways to particular compounds, Quantitative and qualitative analyses and evaluation of biological activities.

The ginger rhizome is defined by its distinctive flavour and fragrance due to the presence of its phytochemical properties, i.e., large concentrations of essential oil and oleoresin. The essential oil's flavour is a result of a combination of several chemical elements (monoterpenoids and sesquiterpenoids) that are present. According to Edris

(2007), Shukla &Singh (2007), Liu *et al*. (2012), ginger essential oil has a wide range of possible pharmacological effects, including antioxidant, antidiabetic, and anticancer properties.

Additionally, ginger oleoresin is a combination of several pungent components (gingerols and shogaols), with [6]-gingerol being a key bioactive element (Pawar *et al.,* 2011; Ravi Kiran *et al.,* 2013). It also has anticancer (Surh, 1999), antiangiogenic (Kim *et al.,* 2005), anti-inflammatory, antiemetic and cardiotonic qualities that are utilized in a variety of medications to treat hyperlipidemia, platelet aggregation, thrombosis, inflammation, coughing and memory disorders (Ficker *et al.,* 2003; Young *et al.,* 2005; Abdel-Aziz *et al.,* 2006). Due to the great drug producing potential of these secondary metabolites of ginger, oleoresin and oil, there is a significant demand for ginger on the global market (Subudhi, 2015). Their greater economic feasibility and exportability, rhizomes with reduced fibre content are seen as being more suitable for industrial usage (Sarwat *et al.,* 2012).

The secondary metabolite content of the fresh rhizome is influenced by the growth circumstances, cultivar type and harvest maturity stage. However, some of the components alter as dried ginger is prepared for postharvest and stored. Young, sensitive rhizomes that are 5 to 7 months old after planting are ideal for making preserved ginger at the start of the harvesting season since they are less fibrous and have a milder pungency at that time. According to reports, the oil content of Australian ginger increased from 1.8 to 4.4 percent on a dry weight basis throughout the harvesting season, but it remained steady at 0.4 percent on a green weight basis. As a result, in Australia, the mid-season ginger harvest was preferred for the extraction of essential oil and oleoresin, whereas the late-season crop was preferred for the production of dried spice (Kaushal *et al.,* 2017).

The highest proportion of ginger oil concentration was reportedly present in India between 215- and 260-days following plantation (Purseglove *et al.,* 1981). As the growing season unfolds, the pungent components and essential oil concentrations reach their peak levels during the maturity stage (9 months after planting) and then rapidly decline as the fibre content rises further. Phytochemicals may differ depending on where they were produced. According to the source and stage of the rhizome, the characteristics of ginger essential oil that contribute to scent range from 1.0 to 3.0 percent (Govindarajan, 1982; Van

Beek *et al.,* 1987; Ekundayo *et al.,* 1988). Depending upon the seasonal variations, oleoresin, which contributes to pungency, ranges from 4.0 to 8 percent (El-Gengaihi & Wahba, 1994; El-Ghorab *et al.,* 2010). Even while the dried ginger's crude fibre content (measured on a dry weight basis) can reach 10%, commercial dried gingers typically have a range of 1.5 to 6%. (Natarajan *et al.,* 1972).

The primary ingredients in ginger products are oleoresin and essential oil, which provide the scent and pungent properties. As a result, the raw material, solvent and extraction conditions all have an equal impact on the yield and the individual components of oil and oleoresin. The phytochemical characteristics of dried spices are also impacted by the subsequent preparation and storage processes because some essential oils are lost, leading to a flattening of flavour and aroma, while the quality of the oleoresin deteriorates and is particularly susceptible to losing pungency. When using either substance for the flavouring of processed foods or pharmacological properties, heat treatment of the spice also causes the oleoresin and essential oil to degrade (Babu *et al.,* 2017).

There are a large number of secondary metabolites reported from *Zingiber officinale* which impart medicinal property to it (Mallikarjuna *et al.,* 2008). The major phytochemical groups include essential oils, flavanoids, phenolic compounds, saponins, glycosides, steroids, terpenoids and tannins (Dhanik *et al.,* 2017). Gingerol and Phellandrene are the chief constituents of ginger essential oil and are mainly responsible for the aroma and flavor (Babu *et al.,* 2017).

Neutraceuticals are the phytochemicals which has the capacity to induce the nutritional value besides acting as a medicine. Due to this property of phytochemicals plants with higher quantity of beneficial phytochemicals play a vital role in improving the health of an individual (Nasri *et al.,* 2014). Different techniques have been used for the separation of phytochemicals present in ginger. Thin layer chromatography, column chromatography and flash chromatography together with different solvent system differing in polarity showed satisfactory separation of gingerol (Zarate *et al.,*1992).

Studies using different extracting solvent system by comparing the [6]- gingerol content of ginger rhizome and alligator pepper seed showed that GCMS analysis of gingerol using acetone extract showed maximum gingerol content and water extract showed the least in both (Usman *et al.,*2013). The oleoresin fractions from *Zingiber officinale* were isolated

and studied for gingerol content. The ethanol extraction of ginger rhizome followed by phytochemical analysis by TLC and HPTLC. Clear spots have been observed in TLC chromatogram of ethanolic extract (Gaikwad *et al.*,2017).

Ginger essential oil which has got diverse range of application in the field of antimicrobial, stabilizing behavior, anti-carcinogenic, anti-vomiting, anti-ulcer and also got cardio vascular properties. 17 chemical compounds have been isolated from essential oil of ginger. The essential oil contains greater amount of zingiberene (31.79%) with a retention time of about 22.28 minutes. Camphene was found in least quantity while comparing with other compounds in the oil (Kamaliroosta *et al.,* 2013).

2.10 Antioxidant properties of ginger

The antioxidative property of ginger is believed to be the major reason for its use as traditional medicine for various ailments (Aeschbach *et al.,* 1994; Ahmad *et al.,* 2001). There are reports in which ginger can be used to decrease the age-related oxidative stress markers (Topic *et al.,* 2002). High level of antioxidant activity (3.85mmol/100g) is reported in ginger root (Halvorsen *et al.,* 2002).

2.11 Biochemical markers

Biochemical markers have been widely used for the characterization of various plant germplasm (Sarwat *et al.,* 2012). The various bioactive components of ginger play a crucial role in the characterization of different germplasm. The total extract of ginger, also known as the oleoresin which contains all the bioactive components as well as the pungent principles. The two main components which impart the oleoresin its pungency is gingerol and shogaol (Ravindran *et al.,* 2005). The quality comparison of ginger is mainly based on the amount of gingerol and shogaol present in the extract oleoresin. Such a classification was done by Zachariah *et al.,* (1993), who classified 86 ginger accessions in to high, medium and low-quality ginger based on the relative content of the quality Component.

2.12 Stress physiological studies in ginger

Plants are continuously facing with both biotic and abiotic stress, which may cost serious damages to the host plant reducing the productivity. Their responses to the confronting stresses are diverse and complex. During the course of evolution plants have developed various mechanisms to adapt themselves to the changing environment. Biotic

and abiotic stress disturbs plant metabolism resulting heavy physiological costs (Heil, 2002 & Swarbrick, 2006).

The concept of stress was put forward by the scientist Hans Selye in 1936, he describes stress as any un favorable changes and environmental constraints that affect the plant which cause severe damage to them. In stresses, abiotic stress has got prime importance based on the damage and caused by them they are the reason for major losses in the field. And the reduction can lead up to >50% in almost all the plant species (Wang *et al.,* 2003).

Stress factors can be broadly classified in to two; biotic and abiotic stress, biotic stress is stress that any disruption caused by living agent and abiotic stress is that any distortion to the plant caused by non-living agent (Atkinson & Urwin, 2012). Literature on abiotic stress related work is limited, though there are a number of literatures available for biotic stress on ginger. Ginger is essentially a cash crop which requires well performed rain fall and acidic soil conditions. So, salinity in soil and drought are the major stress factors which reduce the yield of ginger (Vivek *et al.,* 2013).

Performance of different ginger varieties under shade net conditions were studied and found that vegetative parameters like maximum plant height, number of tillers per plant, leaf area per plant, leaf area index were highest in the Suprabha variety at 30, 60, 90, 120, 150, and 180 days after planting followed by Himachal, Pundibri and Jalsingapara local. And thus, concluded that Suprabha, Himachal, Pundibhari and Jalsingapara local showed better performance and are found suitable for cultivation under shade net conditions in Andhra pradhesh (Babu *et al.,* 2017).

2.13 Drought stress

According to Boken (2005), there are more than 150 interpretations for the term "drought," which is frequently classified as a meteorological, hydrological, socioeconomic and agricultural drought (Smith & Pethley, 2009).

Meteorological drought is characterized by rainfall that is below or absent compared to the normal. Inadequate monsoon rainfall, extreme heat and evaporation, unseasonal rains and fog or snowfall are the main causes. Hydrological drought: When a meteorological drought persists, it reduces the amount of water that is stored and flows naturally through streams. Socioeconomic drought: It links the demand for and supply of precipitation.

Rainfall depends on the weather. Long-lasting droughts harm a region's economy and sociopolitical climate.

Agricultural drought is characterized by insufficient precipitation and decreasing soil moisture throughout the agricultural growing season, which cause crop stress and wilting and either cause crop destruction or result in low crop yields. Depending on how susceptible they are to drought stress, field crops are classified into three classes. Tolerant crops include wheat, sorghum, barley, alfalfa and oats. Moderate crops include corn, soybean, sunflower, peas and beans. Drought-sensitive crops include potato, rice and tomato (Zoltán *et al.*, 2007). Plants in the tolerant group can evade water stress and those in the intermediate group can to some extent withstand drought stress. As a result of deforestation, overgrazing, and industrialization, the "*Sahel drought*," which affects biomass yield, is observed (Held *et al.*, 2005).

The arable regions of the world have been classified according to the presence of stress factors. 26 percent of the usable land is affected by drought, 20 percent by mineral stress, 15 percent by cold and freezing stress and 29 percent by other stress causes. Only 10% of the earth's usable field is unaffected by stress factors (Blum & Jordan, 1985). Our planet has a surface area of about 1400 million km^3, of which about 9000-14,000 km^3 are suitable for human use as freshwater. Freshwater is necessary for the growth of plants and various agricultural processes absorb around 80% of the freshwater that is available (Jury & Vaux, 2005). Only approximately 16 percent of the world's agricultural land is irrigated, with roughly 80 percent of it being rain-fed (McCarthy *et al.*, 2001). More than 50% of water is lost and less than 45% is accessible for agriculture due to inefficient water use. The United States had an economic loss of around $1 billion in 2009 as a result of variables related to the availability of water (Anderson *et al.*, 2009).

Increased consumption of meat also has an impact on agricultural land use because it increases the need for grains for animal feed, which indirectly raises water consumption (Eckardt *et al.*, 2009). Freshwater accessibility is a basic necessity for plant growth and survival. Water deficit conditions that restrict growth and yield have an impact on a variety of physiological and biochemical processes in plants, including translocation, respiration, photosynthesis, nutrient metabolism, ion uptake, decreased chlorophyll and carotenoid

content, carbohydrates, protein biosynthesis and growth promoters (Bray, 1997; Massacci *et al.,* 2008; Blum, 2009).

Transpiration: When there is a lack of water, the water content of the soil decreases. As a result, plants' access to water is impacted. Plants are typically exposed to significant sun radiation while they are under drought stress. Leaf temperature is maintained through transpiration (Nobel, 1999). Although it may not be instantly evident, increased respiration is said to be a necessary component of plant defense against drought stress (Slot *et al.,* 2008).

Photosynthesis: At various phases, drought impacts photosynthesis, a key metabolic activity for crop development and output (Pieters & Souki 2005; Praxedes *et al.,* 2006), mostly because closure of stomata (Cornic, 2000). The damage to photosynthesis machinery depends on the length and severity of drought (Zhang & Kirkham, 1996). Numerous plants, including sunflower (Manivannan *et al.,* 2007; Kiani *et al.,* 2008), *Vaccinium myrtillus* (Tahkokorpi *et al.,* 2007), cotton (Massacci *et al.,* 2008), *Catharanthus roseus* (Jaleel *et al.,* 2008a), and others have shown decreased chlorophyll content under drought stress. Under water stress conditions, all photosynthesis-related cellular components and pathways result in damage, including the thylakoid's ETC, the stomata's failure to regulate CO_2 and the reduction carbon cycle, an increase in lipid peroxidation, a disturbance of water equilibrium shown by damaged chloroplast membranes, distorted and enlarged lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al.,* 1981).

Plant height: When there is a water shortage, plant development is slowed down because the elongating cells cannot get enough water, which impairs mitosis and could speed up leaf senescence (Nonami, 1998; Bhatt & Rao, 2005; Hussain *et al.,* 2008). When plants are under drought stress, their height might drop by up to 25%. (Wu *et al.,* 2008). Rice is a submerged crop that is more subject to drought stress than any other crop (Jaleel *et al.,* 2008a). Potato (Heuer & Nadler, 1995), Parsley (Petropoulos *et al.,* 2008), Soybean (Specht *et al.,* 2001; Zhang *et al.,* 2004), Okra (Sankar *et al.,* 2008) and Cowpea all exhibit reduced stem length under drought condition (Manivannan *et al.,* 2007).

Leaves and root system: As soil water content is depleted, leaf number per plant, individual size and survival is affected (Rucker *et al.,* 1995) along with reduction of water

content and closure of stomata (Jaleel *et al.*,2008c). The "leaf area adjustment" that plants go through in response to a water shortage speeds up senescence and the abscission of the older leaves. Under drought stress, dwarf plants with smaller leaves are seen as they develop vegetatively. Due to reduced nitrogen fixation by root nodule bacteria, legumes' leaf area and leaf weight decrease during droughts (Knapp & Smith 1988). Numerous plants, including Populus (Wullschleger *et al.,* 2005), Soybean (Zhang *et al.,* 2004) and others, exhibit reduced leaf growth when exposed to water scarcity (Farooq *et al.,* 2009).

A robust root system is advantageous during drought stress and quick root elongation draws water from deeper soil layers (Reid & Renquist, 1997). Since shoots are more sensitive to growth inhibition than roots are, a rise in the root-shoot ratio is seen (Wu & Cosgrove, 2000). Under drought stress, periwinkle (Jaleel *et al.,* 2008b) and sunflower (Tahir *et al.,* 2002) showed increased root growth, whereas Populus showed a decreased dry root weight (Wullschleger *et al.,* 2009). The degree of plant adaptation to stress is determined by root-shoot signaling through the xylem. Abscisic Acid (ABA), Ethylene, Cytokinins, Malate and others are some crucial elements of this process. Stomata close as a result of this signaling, which is a significant adaptation. Water deficit and salinity-induced osmotic stress are controlled by ABA dependent and independent mechanisms. Genetic research suggests that there is no distinct boundary between these two routes and that they interact with one another (Knight & Knight, 2001; Xiong & Zhu, 2001).

Yield: Reports of decreased plant productivity due to drought include Wheat (Dickin & Wright, 2008), Oranges (Wu *et al.,* 2008), Common beans and Green gram (Webber *et al.,* 2006), Soybeans (Specht *et al.,* 2001), Sunflowers (Reddy *et al.*,2004), Parsley (Petropoulos *et al.,* 2008), Maize (Monneveux *et al.,* 2006). Under situations of water deficiency, it has been observed that phospholipid and glycolipid, linolenic acid and percentage oil content decrease, whereas steryl ester content increases (Pham Thi *et al.,* 1990). The seed output and seed weight are decreased during the flowering and grain filling stages. Even at the bud initiation stage, a water shortage is damaging and eventually lowers output (Prabhudeva *et al.,* 1998; Chiatante *et al.,* 1999). Productivity is reduced due to decrease in the yield components such as seeds per unit area, grain size, fewer pods and lower grain number (Specht *et al.,* 2001; Dickin &Wright, 2008).

Secondary metabolites: Under drought stress, various medicinal plants produce more secondary metabolites, such as Artemisinin in *Artemisia* leaf tissues (Charles *et al.*, 1993), Hyperforin in St. John's wort leaves (Zobayed *et al.*, 2007), and Ajmalicin in Periwinkle roots (Jaleel *et al.*, 2009).

Plants' reactions to drought stress are complicated and varied, as evidenced by the instantaneous modification of a protein's phosphorylation status within minutes, followed by a modulation of gene expression. It depends on a variety of factors, including the genotype of the plant, the length and severity of the drought, the age and stage of plant development, the kind of organ and cell and the subcellular compartment of the plant. This knowledge aids in our comprehension of the tactics used by plants to tolerate and avoid stress. Through a decrease in the rate of ROS generation, an acceleration of the rate of ROS scavenging, an increase in the rate of recovery of damaged cellular components and other methods, plants attempt to restore the cellular homeostasis (osmotic and ionic equilibrium) of the cell and control the damages. Under biotic and abiotic stressors, trichomes protect leaves from various harmful effects. They serve as a barrier for insects, shield plants from solar damage and lessen water evaporation (Du *et al.*, 2009). Another mechanism for maintaining cellular turgor is the buildup of waxes on the surface of leaves (Sanchez *et al.*, 2001). In general, there are two strategies to maintain cellular turgor: by adjusting osmotic pressure and by altering the volumetric size of the cells.

Cell contraction: is described as a reduction in cell size caused by shrinking coupled with elastic adjustment of cell walls in response to a plant's resistance (Fan *et al.*, 1994). Cell contraction under drought stress is seen in Cassava plants as turgor maintenance (Alves & Setter, 2004)

Flavonoids: All plant parts, including pollen, leaves and floral components, as well as glycosides that accumulate in the vacuole, contain flavonoids. Among all osmolytes, flavonoids are the most bioactive plant secondary metabolites with a wide range of functions, including ROS scavenging through locating and neutralizing radicals, pigmentation of seeds, flowers and fruits, UV light protection, defense against phytopathogens, acting as signal molecules in plant-microbe interactions, and significance in pollen germination and plant fertility (Olsen *et al.*, 2010). Increased accumulation of

flavonoids is described in response to metal toxicity, nutritional shortage, injury and water deficit (Winkel-Shirley, 2002).

Antioxidant Mechanism: Plants have a sophisticated network of scavenging systems and these mechanisms utilize ROS as mediators of signal transduction (Mittler *et al.,* 2004; Bailey- Serres & Mittler, 2006). Antioxidants (redox buffer) control the expression of genes associated with biotic and abiotic stressors as well as the regulation of cellular ROS homeostasis. According to research on antioxidants, they can defend against low temperature, water shortage and salt stress (Iannelli *et al.,* 2002; de Azevedo Neto *et al.,* 2006).

Phenolics: Plant phenolics (also known as aromatic chemicals) are secondary metabolites that have a phenyl ring and one or more acidic hydroxyl groups attached. Flavonoids, lignin and tannins are significant groups of phenols. Phenolic chemicals protect plants from biotic and abiotic stressors while promoting plant growth and development (Morris *et al.,* 2000). Low soil fertility, drought, waterlogging, high and low temperatures, high light intensity, UV radiations, etc. have all been linked to increased production of phenolic chemicals (Ashraf *et al.,* 1994; Delalonde *et al.,* 1996; Mole *et al.,* 1988; Close & McArthur, 2002; Ali & Abbas, 2003).

2.14 Foliar spray of Salicylic Acid

Salicylic Acid (SA): Plants' physical defenses against various physical stresses are limited. They use defense and signaling techniques to defend themselves from diverse environmental pressures. Numerous substances, including Nitric Oxide, Salicylic Acid, Ethylene and Hydrogen Peroxide, among others, are said to protect plants from various stresses when applied exogenously (Zhu, 2002; Wahid *et al.,* 2007). Salicylic Acid (SA), a phenolic chemical, is named after the "willow tree" (‘*Salix’* in Latin), where it was first discovered. Italian chemist Raffaele Piria gave the substance the name "*Acide Salicylice*" (1938). SA was the most popular medicine in the world in 1878 and was created in Germany (Raskin *et al.,* 1990). The amount of naturally occurring SA is reported to be approximately 1µg/g fresh mass as observed in Barley, Rice, Soybean and Crabgrass (Raskin *et al.,* 1990). SA has melting point 157–159^{o}C, pH-2.4, pKa-2.98 (Raskin, 1992).

Biosynthesis of SA: SA is biosynthesized through the shikimate-phenyl propanoid pathway (Sticher *et al.,* 1997). There are two different ways to make SA and they differ

when the aromatic ring is hydroxylated. The enzyme Phenylalanine Ammonia Lyase (PAL), which catalyses the conversion of Phenylalanine into Cinnamic Acid, is involved in the first stages of SA biosynthesis. Then, in one of the first documented processes in pea seedlings, Cinnamic Acid undergoes a 2-hydroxylation to produce o-coumaric acid, which is then decarboxylated to Salicylic Acid (Russell & Conn, 1967; Alibert *et al.,* 1972). Another pathway, reported from rice and tobacco, involves the decarboxylation of the side chain of CA to form Benzoic Acid that undergoes a 2- hydroxylation to form Salicylic Acid (Yalpani *et al.,* 1993; Silverman *et al.,* 1995). It has also been reported that bacteria can produce SA via a third pathway from Shikimic Acid via Chorismic and Isochorismic Acid (Wildermuth *et al.,* 2001; Wildermuth, 2006). According to studies done on Parsley (Thulke & Conrath, 1998), Tomato (Ding *et al.,* 2002), exogenous application of SA and its derivatives causes the development of PAL (Fraissinet-Tachet *et al.,* 1998).

Physiological role of SA in Plants: SA is crucial for a variety of plant processes, including photosynthesis, growth, water relations, stomatal regulation, nutrient uptake, thermogenesis, flower induction, ethylene biosynthesis and protein kinase synthesis, which controls cell division, differentiation and morphogenesis (Zhang & Liu, 2001; Halim *et al.,* 2006). SA controls gravitropism and prevents fruit from ripening (Srivastava & Dwivedi, 2000). Plants are protected from oxidative damage by SA treatment, which is linked to an increase in antioxidant enzyme activity and a decrease in the level of SA treatment provides a protection to the plants from oxidative damage and is associated with an increase in antioxidant enzyme activity and a decrease in the level of ROS and lipid peroxidation (Shi & Zhu, 2008; Shahrtash *et al.,* 2011; Kundu *et al.,* 2011, 2012).

Role of SA in plants under stress condition: Different plants have been reported to respond favorably to SA when they are subjected to pathogen attack and cold stress (Korkmaz, 2005; Loake & Grant, 2007), ozone and ultraviolet (UV) light (Ervin *et al.,* 2004; Mahdavian *et al.,* 2008; Bandurska & Cieslak, 2013), heat stress (Larkindale & Knight, 2002; Larkindale *et al.,* 2005), chilling and drought (El-Tayeb, 2005; Freeman *et al.,* 2005; El-Tayeb *et al.,* 2006; Liu *et al.,* 2012), salt and osmotic stresses (Yusuf *et al.,* 2010; Palma *et al.,* 2009; Khan *et al.,* 2010), heavy metal stress (El-Tayeb, 2005; Freeman *et al.,* 2005; El-Tayeb *et al.,* 2006; Liu *et al.,* 2012),

It is crucial to note that exogenous application of SA cannot provide protection for all crop plants against biotic and abiotic stresses and that its efficacy is also determined by a number of variables, including the application method, concentration, species and stage of plant development etc. (Nemeth *et al.,* 2002; Stevens *et al.,* 2006; Joseph *et al.,* 2010).

Furthermore, SA treatment has been shown to reduce drought tolerance in *Zea mays* (Nemeth *et al.,* 2002) and *Arabidopsis thaliana* (Borsani *et al.,* 2001), primarily as a result of membrane damage that results in electrolytic leakage and generation of ROS that negatively impact the photosynthetic apparatus. There are various methods of applying SA, including soaking seeds before planting, adding to hydroponic solutions and tissue culture media, irrigating and spraying with SA solution etc. are used to protect plants against biotic and biotic stresses (Sakhanokho & Kelley, 2009).

Plants cope up with drought stress by two major molecular and cellular responses. (i) Accumulation of various osmolytes such as proline, glutamate and sugars (Mannitol, Sorbitol and trehalose), which play a key role in preventing membrane disintegration and activation of drought induced enzymes (Mahajan & Tuteja 2005), (ii) Induction of a large number of drought responsive genes and specific protective proteins under drought tolerance (Reddy *et al.,* 2004; Zang & Komatsu, 2007). The drought stress related transcripts and proteins were induced for drought tolerance. A large body of literature reviewed drought stress responsive genes and specific protective proteins are mostly involved in signal transduction and activation or regulation of transcription, antioxidants and ROS scavengers (Cui *et al.,* 2007).

Salicylic acid has an important role in tolerance of some environmental stresses such as heat, salts and drought stress (El- Tayeb, 2005). Salicylic acid and its related compounds are reported to induce remarkable effects on various biological properties of plants. These compounds act in variable manner, either by activating or by inhibiting certain responses (Raskin, 1992). The effect of exogenous application of salicylic acid is different. Different levels of acetyl salicylic acid seem to be affected on the leaves of the plants of *Phaseolus vulgaris,* it prevents the opening of stomata in the epidermal strips of *Commelina communis* (Larqué-Saavedra, 1979).

Salicylic Acid is reported to induce alternative oxidase enzyme activity in mitochondria that are involved in stress alleviation purposes (Vanlerberghe & Mclntosh

1997). Lower concentrations of (Salicylic Acid less than 1mM) are reported to be beneficial for the plant growth (Rivas-san Vicente & Plasencia, 2011). There are reports about increase and decrease (Kiddle *et al.,* 1994), in some secondary metabolites of the plant by Salicylic Acid was also reported. Salicylic acid is also one of the most easily accessible plant growth regulators which are also effective in some other forms such as acetyl Salicylic Acid and Methyl Salicylate (Raskin., 1992). Also, there are reports about interaction between Salicylic Acid and other natural stress management compounds.

Dimethyl Sulfoxide (DMSO), an organosulfur molecule, having two carbon-sulfur linkages in its chemical structure (Zhu *et al.* 2013). This lignin-based, highly polar solvent is principally used as a solvent in the production of synthetic fibres (Hearon, 1957). It is a colourless liquid that boils at a high temperature, is mobile and has an extreme polarity. The majority of organic solvents, including alcohols, ketones, chlorinated solvents and aromatics, are miscible with it as well as water (Parker, 1965). Due to DMSO's unique properties, some reactions have been seen to happen noticeably quicker in DMSO than in normal solvents.

2.15 Foliar spray of Zinc Sulphate

Zn is required for the function of enzymes including dehydrogenases, aldolases, isomerases, transphosphorylases, RNA and DNA polymerases (Lacerda *et al.,* 2018). Additionally, it contributes to tryptophan production, cell division, membrane structure maintenance, photosynthesis and functions as a regulatory cofactor in protein synthesis (Lacerda *et al.,* 2018; Marschner, 2011). Zinc is required for the n protein biosynthesis and carbohydrate metabolism and it plays an important role in gene expression related to environmental stresses (Haslett *et al.*,2001, Fageria *et al.,* 2002). Although Zn is necessary for plants to have a healthy metabolism, its effectiveness depends on how well it is absorbed and transported (Doolette *et al.,* 2018) Zn Sulphate ($ZnSO_4$) or EDTA-Zn chelate are applied to the ground and leaves in traditional farming methods. But the effectiveness is poor (Read *et al.,* 2019).

2.16 Stress crosstalk in plants

Nearly all environmental stresses have corresponding consequences and reactions, including a reduction in photosynthetic activity and growth, hormonal changes, oxidative damage and the accumulation of stress-related proteins (Aroca *et al.,* 2018). Root water

uptake ability also contributes significantly to preventing stress-related growth loss during dehydration in addition to stomatal closure (Christmann *et al.*, 2007). In field settings, heat and drought stress frequently coexist, making research on their combined response essential, especially in semi-arid and drought-affected areas (Raja *et al.*, 2020). Numerous researches have looked at the impact of combined heat and drought stress on the growth and production of different Grasses, Barley, Maize and Sorghum (Oskabe *et al.*, 2014).

2.17 Chemical constituents of ginger

Chemical analysis of ginger revealed that it contains over 400 different compounds. Its major constituents include carbohydrates (50-70%), lipids (3-8%), terpenes and phenolic compounds (Grzana *et al.*, 2005). Chemical compounds can be identified by different types of chromatographic techniques like gas chromatography-mass spectrometry, gas chromatography with flame ionization detection, high performance liquid chromatography, and liquid chromatography mass spectrometry. Volatile and low molecular weight compounds can be identified by Gas chromatography and it will help in the identification of polar compounds. Zingiberene, Bisabolene, Curcumene, Sesquiphellandrene and Farnesene are examples of important terpene chemicals. Additionally, phenolic substances like gingerol, paradol and shogaol exist. The percentage of gingerols and shogaols is greater (Prasad & Tyagi, 2015). Zingiberene and bisabolene are the main aromatic components and gingerols and shogaols are the main pungent principles (Tyler, 1994). Other gingerol or shogaol-related chemicals that have been found in the ginger rhizome in amounts of 1 to 10 percent include 6-paradol, 1-dehydro gingerdione, and 10-gingerdione, as well as 4-, 6-, 8-, and 10-gingerdiols and diaryl heptanoids (Govindarajan & Connell 1983; Ali *et al.*, 2008). Volatile oils including shogaol and gingerols are the source of the distinctive flavor and odor (Harold, 2004).

Ginger includes oleoresins and essential oils, which give it a strong, sour flavor (Beristain *et al.*,2019). Ginger's most significant medicinal constituents are divided into non-volatile and volatile constituents (López *et al.*, 2017; Syafitri *et al.*,2018). The sensory properties of ginger were caused by three major classes of chemicals found in the volatile oils: monoterpenoids, sesquiterpenoids and aldehydes (Yeh *et al.*, 2014; Beristain *et al.*, 2019). The aroma is brought on by the sesquiterpene derivatives (-)-zingiberene, (+)-curcumene, (-)-sesquiphelandrene, and -bisabolene (Syafitri *et al.*, 2018). Sabinene has a

strong, oily-peppery, and slightly pungent spicy taste, while camphene has a terpeney camphoraceous flavour. Curcumene smells and tastes like turmeric and has a somewhat pungent bitter flavour. In contrast to -farnesene, which has a very light, sweet, and warm aroma, zingiberene has a warm, woody, and lasting aroma. Geranial and neral are frequently used chemical with a powerful lemon scent (Yeh *et al.*,2014).

The flavor's pungent quality is caused by non-volatile phenylpropanoid-derived substances, including gingerols, shogaols, paradols and zingerone (Mbaveng & Kuete, 2017, Syafitri *et al.*, 2018, Beristain *et al.,* 2019). Eugenol, zingerone, trans-6- shogaol and geraniol are the major chemicals in oléoresins produced from different solvents (Beristain *et al.,* 2019). The substances that give ginger its spiciness have been referred to as gingerols (Syafitri *et al.,* 2018). The warm, pungent sensation in the mouth is caused by gingerone, which was created from gingerols during drying or boiling (Mbaveng & Kuete, 2017). Many of the plant's pharmacological properties have also been noted. The chemical elements discovered in ginger rhizome included Iron, Calcium, Vitamin C and beta-carotene are among the nutrients included in the powdered ginger sample's nutritional makeup. Essential oils, terpenes (including zingiberene, beta-bisabolene, .alpha.-farnesene, .beta.-sesquiphellandrene and .alpha.-curcumene), and phenol compounds are the primary components of ginger rhizome (gingerol, shogaol, paradols etc.) (Gomes *et al.,* 2016).

2.18 Volatile components of ginger

The odor of the ginger is mainly due to the volatile oil composition. The approximate percentage is about 1-3%. The aroma, quality, its composition and physico chemical characteristics depends on the raw materials and the total distillation time (Mathew *et al.,* 1973). Ginger volatile oil contains more than 50 compounds, mainly consisting of monoterpenoids and sesquiterpenoids. The major monoterpenoids are β phellandrene, cinerol, geraniol, geranial, citral, terpineol and borneol. The major sesquiterpenoids are bisabolene, .alpha.-Farnasene, curcumene and zingiberene. On drying ginger, the components responsible for odour in ginger oil are decreased (Evans, 2009).

2.19 The phenolic compounds of ginger

Since the 19th century, researchers have looked into all of the phenolic chemicals that give ginger its pungency. By isolating and purifying the phenolic compounds in ginger using chromatography, the structure of nearly all of these compounds has been determined.

They are further verified through characterization using spectroscopic techniques. The key ingredients that give ginger its pungency are phenolic compounds. The primary ingredient in fresh ginger is gingerol, but the primary ingredient in dry ginger is shogaol. At the activated aromatic nucleus, gingerols, shogaols and paradols are structurally quite similar with just minor side chain variations (Connell & Sutherland,1969).

While shogaol has an - unsaturated ketone, gingerols indicate a hydroxyl carbon moiety. The alkyl side chain of the paradols solely contains a ketone group. Gingerols are dehydrogenated into shogaols, which are then hydrogenated into paradols (Jolad *et al.*, 2005, Wohlmuth *et al.*, 2005). The most prevalent pungent substances in fresh rhizomes are [n] gingerols. Only trace amounts of shogaols are present in the fresh root; instead, they are mostly present in dried and thermally processed roots, with [6] shogaol being the most prevalent (Jolad *et al.*, 2005).

2.20 Extraction and isolation of phenolic compounds of ginger

The isolation of a certain phenolic compounds from ginger extract is very difficult, since ginger contains many phenolic compounds like [6], [8], [10] gingerols, shogaols, paradols and zingerone. Microwave assisted extraction of phenolic compounds of ginger and optimization of extraction procedure by changing the variables like temperature, time and solvents was done by (Rahath *et al.*, 2013). The bioactive components of ginger have been extracted with appropriate solvent after pre-treatment using enzymes (Nagendra *et al.*, 2013). After treating ginger with .alpha.-amylase, viscozyme, cellulase, pectinase and protease enzymes and later extracted with different solvents.

It has been found that ethanol extract of ginger pretreated with cellulase enzyme has given the maximum yield of phenolic compounds. Some of the methods which was described for the good yield of gingerol are given below.

- With the aid of ultrasound and supercritical CO_2, [6]-gingerol may be extracted from ginger roots. The extraction rate and yield increased when ultrasound was present (Balachandran *et al.*, 2006).
- High-speed counter-current chromatography was adapted as a novel approach for the purification of gingerols from ginger by the extraction and purification of [6]-, [8]-, and [10]-gingerol from a crude extract of ginger is done using a two-phase solvent system, such as light petroleum-ethyl acetate-methanol-water (5:5:6.5:3.5,

v/v/v/v). In this experiment, 200 mg of crude extract is processed in 170 minutes to get 30.2 mg of 6-gingerol, 40.5 mg of 8-gingerol, and 50.5 mg of [10]-gingerol (Zhan *et al.*, 2011).

2.21 Some major uses of gingerol

- In-vitro and in vivo, [6]-gingerol has unique anti-angiogenic effect. Human endothelial cell proliferation induced by VEGF (Vascular Endothelial Growth Factor) is inhibited in vitro by [6]-gingerol, which also causes cell cycle arrest in the G1 phase. (Wang *et al.*, 2003)

  Additionally, it prevents endothelial cells from forming capillary-like tubes in response to VEGF and severely reduces the ability of endothelial cells to sprout in the rat aorta and produce new blood vessels in the mouse cornea in response to VEGF. Tumors and other angiogenesis-dependent disorders may respond well to treatment with [6]-gingerol, which may suppress angiogenesis (Kim *et al.*, 2005).
- The pro-inflammatory cytokine production from macrophages is specifically inhibited by [6]-gingerol, but it has no effect on the activity of the APC or the expression of MHC II and co-stimulatory molecules on the cell surface (Tripathi *et al.*, 2007).
- Antiplatelet therapy is a common method of ischemic heart disease prevention. For many years, aspirin has served as the cornerstone of this anti-platelet therapy. Aspirin, however, has been linked to gastrointestinal bleeding and stomach ulcers, among other side effects. Comparing the effects of gingerol and aspirin on arachidonic acid-induced platelet serotonin release and aggregation in vitro, we find that gingerol suppresses human platelet activation (Koo *et al.*, 2001).
- A function of [6]-gingerol is to impede HIV-1 replication. Gingerol is a viable option for AIDS patients who want to improve their immune systems in a safe and effective way (Lee *et al.*, 2008).
- A two-stage mouse skin carcinogenesis model was used to study [6]-Gingerol's anticancer activity (Park *et al.*, 1998).
- [6]-Gingerol has the potential to be a therapeutic treatment for Alzheimer's disease (Lee *et al.*, 2011).
- [6]-Gingerol has analgesic, anti-inflammatory, and antioxidant properties.

(Reddy and Lokesh, 1992; Ippoushi *et al.,* 2003; Kuo *et al.,* 2005; Young *et al.,* 2005)

- In MDA-MB-231 human breast cancer cell lines, [6]-gingerol reduces cell adhesion, invasion, motility, and MMP-2 and MMP-9 activities (Lee *et al.*, 2008).
- Alpha- amylase can be effectively inhibited naturally by 6-gingerol (Tintu *et al.*, 2012).

2.22 Phenylalanine Ammonia Lyase Estimation

Phenylalanine Ammonia Lyase (PAL) is the first enzyme in the phenyl propanoid pathway. It catalyzes the deamination of phenylalanine to produce trans-Cinnamic Acid and ammonia. The family of enzymes known as aromatic amino acid ammonia-lyases includes PAL. According to Barros *et al.* (2020), it is essential for regulating the movement of carbon from primary metabolism to secondary metabolism. The enzyme's tetramer is made up of 77–83 kDa subunits. The PAL enzyme functions as an antioxidant by converting oxygen radical species (ROS) into phenolic compounds (Tian & Lei, 2006).

2.23 Chlorophyll estimation

In the process of photosynthesis, antenna pigments in the chloroplasts of leaves capture solar energy. Through resonance transfer, the ensuing excitation is then directed to reaction centre pigments, which release electrons and initiate the photochemical reaction. The most significant of these pigments, chlorophylls Chl-a and Chl-b, are therefore practically necessary for the oxygenic conversion of light energy to the chemical energy that powers the biosphere (Wu *et al*., 2008).

The primary biological element in the molecular machinery of photosynthesis is chlorophyll. In general, two techniques are used for pigment estimation. They are destructive and non-destructive techniques which are used to track the amount of chlorophyll in plants. In destructive method, by using organic extraction and spectrophotometric measurement for the estimation of chlorophyll concentration. This method is more reliable and regarded as a standard method. The non-destructive methods are much simpler than destructive methods, but they do not yield the same precise results as the destructive methods (Ali *et al.*, 2008).

The following materials were used and methodologies were adopted to investigate the stress induced changes in selected metabolites in the selected varieties of ginger.

3.1 Collection and maintenance of candidate plant under study.

Zingiber officinale Rosc. cv- Varada and *Zingiber officinale* Rosc. cv- Mahima were used for the present study. Seed rhizomes of both were collected from Indian Institute of Spices Research, Kozhikode (ICAR Unit- IISR Kozhikode) during the period of February 2019 to February 2021. 10 kilograms of both seed rhizomes were purchased. The seed rhizomes were treated with mancozeb (0.3%) and quinalphos (0.0075%) for 30 minutes and stored in a well-ventilated place. One month before planting, the seed rhizomes were cut into small pieces of rhizomes approximately 4-6 g in weight and which contains at least two healthy buds. Bud sprouts were again treated with mancozeb (0.3%) for 30 minutes before planting. Sprouted rhizomes were transferred in to standard grow bags with dimensions of 24 cm x 24 cm x 40 cm containing soil and coco peat in the ratio 2;1 and has given partial shade to grow bags by keeping them inside the shade nets. Healthy and grown ginger rhizomes in the grow bags with two or three tillers were selected. The Present study was conducted in rain-protected green house. The experimental area lies between 11.2588^{o} N, 75.7804^{o} E. Grow bags were maintained in the greenhouse of St. Joseph's College (Autonomous) Devagiri, Kozhikode, Kerala at 28^{o} C. There were about 15 grow bags kept for every single stress treatment. Need based irrigation was done using sprinklers during the initial stages until the commencement of foliar sprays of chemicals and drought experiments by withholding the water supply.

3.2 Preparation of foliar sprays

Salicylic Acid Synonym(s): Salicylic Acid, (2-Hydroxybenzoic Acid) with Linear Formula: 2-(HO) $C_6H_4CO_2$ H; CAS No.69-72-7, Molecular Weight: 138.12 Beilstein No.774890.

Zinc Sulphate Synonyms(s): Zinc sulphate Heptahydrate: Linear Formula: $ZnSO_4 \cdot 7H_2O$, CAS No.7446-20-0. Molecular Weight: 287.56 EC No.231-793-3.

10^{-2} M (0.01) and 10^{-3} (0.001) M of Salicylic Acid solution (SA; 2-hydroxybenzoic acid + 0.02% dimethyl sulfoxide + 100 µl Polyoxyethylenesorbitan monolaurate/ Tween 20, Sigma Chemicals; pH 6.5), Zinc Sulphate with same molar concentrations ($ZnSO_4.7H_2O$ dissolved in distilled water + 100 µl Polyoxyethylenesorbitan monolaurate/

Tween 20, Sigma Chemicals; pH 4). Control plants were sprayed with same solution but without SA and $ZnSO_4$. A trial was also maintained without any foliar application to estimate the number of non-volatile components of the variety under study. Apart from foliar spray drought stress is imposed by withholding water application, at the trials. Plants were sprayed once on the leaves early in the morning and every week until one month.

3.3 Analysis of morphological data

Five plants/ treatments / replication were collected at random and studied for growth characters. Performance of ginger varieties for growth attributes like plant height, number of leaves per plant, number of tillers per plant were evaluated during its growth.

3.4 Sample preparation and extraction

Harvested rhizomes are shade dried. They were powdered separately and from each sample 1 g powder sample was taken and done cold extraction was performed in Methanol. Samples were dissolved in 100 ml Methanol in Standard flask and shaken for 2 hours and kept overnight. For HPLC analysis from surface of the sample 20 ml of supernatant was pipetted to a round bottom flask and evaporated to 2 ml in rotary evaporator (Buchi Rotavapor B-100). This extract is then made up to 5 ml with HPLC methanol in 5ml standard flask. Shaken well and filtered using Syringe filter.

3.5 Qualitative preliminary phytochemical analysis.

To identify the presence of various classes of metabolites, different preliminary analysis was conducted.

3.5.1 Test for Alkaloids

3.5.1.1 ***Mayer's test:*** To a few ml of sample extract, two drops of Mayer's reagent are added along the sides of the tube. Appearance of white creamy precipitate indicates the presence of alkaloids (Tiwari *et al*., 2011).

3.5.1.2 ***Hager's test:*** To a few ml of sample extract, two drops of Hager's reagent are added along the sides of the test tube. Appearance of yellow precipitate indicates the presence of alkaloids (Tiwari *et al*., 2011).

3.5.2 Test for Coumarins

3.5.2.1 ***Coumarins test:*** To 2 ml of sample extract, 3 ml 10% Sodium Hydroxide solution is added along the sides of the test tube. Appearance of yellow coloured solution indicates the presence of coumarins (Jayaprakash & Sangeetha, 2015).

3.5.3 Test for Flavonoids

3.5.3.1 ***Alkaline reagent test:*** 2 ml of 2% sodium hydroxide solution was mixed with plant crude extract, intensive yellow colour was formed, which turned colourless on addition of dilute acid (Jaradan *et al*., 2015).

3.5.3.2 ***Lead Acetate test:*** To 2 ml of extract, few drops of lead acetate solution was mixed. Formation of yellow precipitate (Tiwari *et al*., 2011).

3.5.4 Test of Glycosides

3.5.4.1 ***Keller Killiani's test:*** To 2 ml extract Glacial Acetic Acid is added along the sides of the test tubes and one drop of 5% Ferric Chloride solution added. Reddish brown colour appears at the junction of 2 layers and upper layer appears bluish green (Singh & Bag, 2013).

3.5.5. Test of Phenol

3.5.5.1. ***Ferric Chloride test:*** To 2-3 ml of extract a few drops of 5% Ferric Chloride solution was added, presence of deep blue-black colour (Santhi & Sengottuvel, 2016).

3.5.6. Test for Quinones

3.5.6.1. ***Quinones test:*** To 2-3 ml of sample extract 3 ml Hydrochloric Acid is added. Appearance of yellow colour (Harborne, 1999).

3.5.7. Test for Saponins

3.5.7.1. ***Foam test:*** 2 ml sample is taken in a test tube to which 4 ml distilled water is added, mix well and vigorously. Indicates the formation of foam at the top of the sample (Hossain *et al*., 2013).

3.5.8. Test for Steroids

3.5.8.1. ***Salkowski's test:*** To 2 ml of extract 2ml chloroform is added. 2 ml concentrated Sulphuric Acid is added along the sides of the test tube. Chloroform layer appears red colour and acid layer shows greenish yellow fluorescence (Joseph *et al*., 2013).

3.5.9. Test for Tannins

3.5.9.1. ***Braymer's test:*** 2-3 ml extract is diluted by adding 2 ml of distilled water. To which 2-3 drops of 5% Ferric Chloride is added. Appearance of black green or bluish colour (Rishikesh *et al*., 2013).

3.5.10. Test for Terpenes

3.5.10.1. ***Copper acetate test:*** 2 ml extract is dissolved in distilled water, to which 3-4 drops copper acetate solution is added and mixed, results in the production of emerald green (Morsy, 2014).

3.6. Quantitative phytochemical analysis

3.6.1 *Estimation of Antioxidant Activity* (DPPH Assay)

The antioxidant capacity of the extracts from sage under various treatments was evaluated by DPPH assay (Hung *et al.*, 2005). Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. The amount of sample necessary to decrease the absorbance of DPPH (Sigma–Aldrich Co., Steineheim, Germany).

ANTIOXIDANT ACTIVITY

Principle:

DPPH is a nitrogen centered free radical. The color of which changes from violet to yellow on reduction by H+/e- donation. The substances that are able to perform this reaction are called antioxidants.

Reagents:

1. 0.04% DPPH in methanol (freshly prepared)
2. Ascorbic Acid standard-0.1g in 100ml methanol(1mg/ml)
3. Stock-0.5g extract dissolved in 100ml methanol(5mg/ml)

For the comparative purpose 5ml Ascorbic Acid is taken from working standard and added 1 ml DPPH to it. Stock extract is dissolved to get varying concentration and fixed volume is taken from each tube (0.5ml). The volume in all the tubes made up to 5ml with methanol. 4ml methanol was added to control. Added 1ml of 0.04% DPPH to all the tubes and mixed well. Kept in dark for exactly 30min. The absorbance was measured at 517nm.

Calculation:

DPPH radical scavenging capacity (% inhibition)=

(absorbance of control)-(absorbance of sample)/ (absorbance of control)*100

3.6.2. *Estimation of Total Phenolics*

The total phenolic content in the extracts was determined by theFolin–Ciocalteu (Sigma–Aldrich Co., Steineheim, Germany) assay (Singleton & Rossi, 1965). The absorbance of the samples was measured at 650 nm against a reagent blank using a UV–vis

spectrophotometer (Thermoscientific Genesys 50). Gallic Acid (Merck Co., Darmstadt, Germany) equivalent (GAL) was used as the reference.

ESTIMATION OF TOTAL PHENOL

Principle:

Phenols react with phosphomolybdic acid in Folin-Ciocalteu in an alkaline medium and produce blue coloured complex (molybdenum blue)

Reagents:

1. Folin-Ciocalteu reagent (1;1)

2. 10% Na2Co3

3. Stock-100mg Gallic acid in 100ml distilled water.

4. Working standard-10ml stock to 100ml

Procedure:

Pipetted out different aliquots (0.3-3.0 ml) into test tubes, varying concentrations of stock extract was taken. The volume in all tubes was made up to 3ml with Methanol. A tube containing 3ml methanol taken as blank. Added 0.5ml of Folin-Ciocalteu reagent to all the tubes including blank. After 3minutes, added 2ml of Na_2Co_3 solution to each tube. Mixed thoroughly. Placed the tubes in boiling water for exactly 1minute, cooled and centrifuged. Absorbance was measured at 650nm against reagent blank. Prepared a standard curve using different concentrations of Gallic acid.

Calculation:

From the standard curve, the concentration of phenols in the test samples was found out and expressed as mg phenols/100g sample.

3.6.3. *Estimation of Total Flavonoids*

Total flavonoids content in the extracts from sage under various treatments was determined according. Ordonez *et al.*, (2006) based on the formation of a flavonoid–aluminum complex with a maximum absorptivity at 510 nm. The flavonoids content is expressed as mg quercetin (Roth Co., Karlsruhe, Germany) equivalents per gram of each extract on dry basis mg quercetin/100g dry weight.

ESTIMATION OF TOTAL FLAVONOIDS

Principle:

Flavonoids present in the extract formed a charge transfer complex with several heavy metals to give a characteristic colour. In this reaction, the high electropositive nature of Aluminum attracts the atomic nuclei of the aromatic rings in the Flavonoids and creates a charge transfer resonance hybrid. This hybrid is highly stable in the aqueous medium which then interacted to form a pink coloured complex that is spectrophotometrically measured at 510nm.

Reagents:

1. 5% Sodium Nitrite

2. 10% Aluminum Chloride

3. 1M NaOH

4. Stock-100mg Quercetin in 100ml methanol(1mg/ml)

5. Working standard-10ml stock to 100ml(0.1mg/ml)

Procedure:

Total flavonoid content was measured with Aluminum Chloride colorimetric assay. An aliquot of extracts and standard solution of Quercetin (20,40,60,80,100 μ/ml)was taken. Made up to 1ml with distilled water. Then added 0.5ml 5% $NaNO_2$ After 5 minutes,0.5ml 10% $AlCl_3$ was added. At the 6th minute,2 ml of 1M NaOH was 24 added and the total volume was made up to 5 ml with distilled water. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510nm.

Calculation:

From the standard curve, the concentration of flavonoids in the test samples was found out and expressed as mg quercetin/100g dry weight.

3.7 chromatographic Studies (HPLC)

3.7.1 ***Sample preparation and extraction***

Harvested rhizomes are powdered separately and from each sample 1g powder sample was taken and done cold extraction was performed in Methanol. Samples were dissolved in 100 ml Methanol in Standard flask and shaken for 2 hours and kept overnight. From surface of the sample 20 ml of supernatant was pipetted to a round bottom flask, and

evaporated to 2 ml in rotary evaporator (Buchi Rotavapor B-100). This extract is then made up to 5 ml with HPLC methanol in 5ml standard flask. Shaken well and filtered using Syringe filter.

3.7.2 *Preparation of Standard and Instrumentation*

Gingerol reference standard was prepared by dissolving 0.1 g of 6-gingerol in 100 ml HPLC methanol in a 100 ml standard flask. From this reference standard, 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml and 0.8 mg/ml were prepared. Standard curve is plotted for these concentrations using HPLC. The general fingerprints were obtained by HPLC Photo Diode Array (PDA) detector using Shimadzu C10- 10 AS. The mobile phase consists of Acetonitrile and HPLC Water (65;35) in 1% acetic acid, filtered and sonicated for 30 minutes and used. The flow rate was 1ml/minute. The operating temperature was maintained at 23ºC.

HPLC ANALYSIS OF GINGEROL

Instrument: Shimadzu LC 10 AT VP

Column: Purosphere Star-RP C 18 (5µm)

Mobile Phase: ACN: Water with 1% Acetic Acid

Injection Volume: 20µl

Detector Wavelength: 280nm

Flow Rate: 1ml/minute

Method: Estimation of Gingerol (ASTA)

Method: Estimation of Gingerol (International Organisation for Standardisation 1997).

1 gram of dry ginger powder was accurately weighed, transferred to 100 ml standard flask and made up with methanol. It was shaken well for two hours and left standing overnight. Without disturbing the solution, 20 ml of supernatant was pipette out and transferred to a 50 ml round bottom flask and concentrated in vacuo at 40ºC using rotary evaporator. The residue was re suspended in HPLC methanol, transferred to a 5 ml standard flask and made up with HPLC methanol. The extract was filtered using 0.2 µm syringe filter and transferred to a 1.5 ml micro centrifuge tube. 20µl was directly injected to the HPLC.

HPLC CONDITION

The Ginger extracts were analyzed on a HPLC system consisting of Shimadzu LC 10 AT VP using UV detector. A purosphere star RPC 18column (Dimension of 280×4.6 mm, 5µm Particle size) was used. Acetonitrile; Water with 1% Acetic Acid was used as the mobile phase. All solutions were degassed and filtered through a 0.45µm pore size filter (Millipore USA). Separations were affected by an isocratic elution program (65;35) at a flow rate of ml/minute and the run was set for 20 minutes. The detector was set at 280 nm. Quantitation of [6]- gingerol was achieved after comparison with a calibration curve of authentic N- Vanilyl nonanamide. Standard stock solution of NVA was prepared by dissolving 100 mg of NVA in 100 ml of methanol, to get a stock solution containing 1000µg/ml NVA. The stock solutions were diluted to create a five-point standard curve (0.2mg/ml,0.4mg/ml,0.6mg/ml,0.8mg/ml).

$$\% \text{ 6-G} = (A \times K) \div U$$

A- Area of 6-G peak

K- Response factor of standard

C- Concentration of the sample

3.8 PAL Enzyme assay

The procedure by Khan *et al.* (2003), was followed for the estimation of the activity of PAL enzyme in the plant tissue with some modifications.

The enzyme Phenylalanine Ammonia lyase (PAL) plays an important role in plant during pathogen attack. It is a key enzyme involved in the synthesis of phytoalexins, the antimicrobial compound produced by the plant. The enzyme catalyzes deamination of Phenylalanine thus converting it into t-Cinnamic Acid. The crude enzyme extract is incubated with L-Phenylalanine which is the substrate of PAL. During the incubation L-Phenylalanine is converted to t- Cinnamic Acid. The quantity of t- Cinnamic Acid can be calculated by reading the absorbance of the reaction mixture at 290nm. This absorbance is then compared with the standard curve to derive the amount of t- cinnamic acid formed during incubation.

ENZYME ASSAY

2N and 1 N Hcl

Prepare 2 N Hcl by diluting 17.09 ml of concentrated Hcl to 100 ml DW, dilute this solution two times with DW to prepare 1 N Hcl.

Extraction Buffer 50 mM tris Hcl, 14 mM β mercapto ethanol, p^H 8.5

Dissolve 0.606g tris base in 500ml DW and add 980µl 2 mercapto ethanol. Adjust the p^H to 8.5 by 1 N Hcl and make up the volume to 1000ml DW.

L- Phenylalanine solution 1mM substrate

Dissolve 165.1 mg of L-Phenylalanine in DW sufficient to make 100ml of solution.

Cinnamic acid (Standard)

Dissolve 10 mg of cinnamic acid in toluene sufficient to make 10 ml of solution containing 1 mg of cinnamic acid per ml. Dilute this stock solution 100 times with toluene to prepare working standard 10µg/ml.

Extraction of enzyme

Prepare crude enzyme extract by homogenizing 1g tissue (Control and stressed separately) in 1ml ice cold extraction buffer in pre-chilled motor and pestle. Kept in ice bucket. Add polyvinyl pyrrolidine at the time of extraction to absorb phenolics, which interfere with spectrophotometric readings centrifuge the extract at 10,000 rpm for a minute and take the clear supernatant for assay.

Prepare the assay mixture (control and stressed) separately in a test tube containing 1ml of 1mM Phenylalanine and 300 ml of enzyme extract and incubate it for 60 minutes at 30^0C. Arrest the reaction by adding 1.5 ml of toluene to it and vortex the tube for 30 seconds. Centrifuge the content at 1000 rpm for 5 minutes and separate the toluene solution containing t- Cinnamic Acid. Read the toluene phase at 290 nm against the toluene blank in spectrophotometer.

Prepare a standard curve of t- Cinnamic Acid by recording the absorbance of following solution at 290 nm against the toluene blank.

Plot the absorbance of these solutions against quantity of t- Cinnamic Acid produced in the assay from this curve and calculate PAL activity in the control and stressed samples from the standard curve.

3.9 Estimation of pigment composition of the leaf

Estimation of chlorophyll and carotenoids were done according to Arnon (1949). Fresh leaves of control as well as treated plants were washed with water and blotted between sheets of filter paper. To estimate chlorophyll and carotenoids 80% acetone was used as extracting medium.

100 mg of fresh leaf sample was weighed in an electronic balance and crushed using mortor and pestle in 20ml of 80% acetone(V/V). Then the homogenate was centrifuged at 5000rpm for 1o minutes and supernatant was collected. The residue was re extracted with 80% acetone and centrifuged. The process was repeated till the pellet become colourless. The final volume of the pooled supernatant was noted. The absorbance was read at 663 nm, 646 nm,750 nm and 470 nm against the solvent blank (80% acetone). Then the quantity of chlorophyll and carotenoids present in the extract was calculated as µg chlorophyll and carotenoids per gram dry weight using the following formula.

$$\text{Chl a µg/g Fresh weight} = \frac{12.69(A663-A750)- 2.69(A646-A750)}{\text{Fresh weight of the sample}} \times \text{Vol}$$

$$\text{Chl b µg/g Fresh weight} = \frac{22.9(A646-A750)- 4.68(A646-A750)}{\text{Fresh weight of the sample}} \times \text{Vol}$$

$$\text{Chl a}_{+b} \text{ µg/g Fresh weight} = \frac{20.12(A646-A750) +8.02(A663-A750)}{\text{Fresh weight of the sample}} \times \text{Vol}$$

$$\text{Carotenoid µg/g Fresh weight} = \frac{1000(A470) + 3.27 (\text{Chl a} - \text{chl b})}{\text{Fresh weight of the sample} \times 229} \times \text{Vol}$$

3.10 Gas Chromatography Mass Spectrometry (GC-MS)

The GC-MS analysis of the hexane fraction of ginger samples were studied to identify the presence of low polar compounds. The hexane extraction of dried powder was reduced to the maximum by the rotary evaporator. All ten samples were then freeze-dried in a Heto Lyolab 3000 lyophilizer (Heto-Helton A/S, Denmark). GC-MS grade hexane was used for the preparation of stock solution. 300 mg of the powdered hexane fraction of the samples were weighed accurately and mixed with 10 ml of GC-MS grade hexane and filtered using Whatman no.1 filter paper to prepare the stock solution for GC-MS analysis.

Apparatus

GC-MS analysis was performed on an Agilent Gas chromatograph series 6850 System fitted with a HP-5 MS fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 μm) coupled with an Agilent 5975C VL-MSD with Triple-Axis detector.

Procedure

1 μL of the diluted samples of ginger were injected automatically and in a split mode. Helium was used as carrier gas at a constant flow mode of 1 mL/min. The injector temperature was set to be at 250ºC. The oven temperature was raised from 60ºC to 130ºC at 5ºC /min, and hold at 130oC for 5 min. The temperature was again raised from 130ºC to 260ºC at 10ºC /min after the hold at130ºC. Mass spectra were recorded over 50-500 amu range. The electron multiplier was set to 1460 eV.

3.11 Identification

The Identification of the components was made by the comparison of their relative retention indices and by the comparison of their mass spectra with those stored in NIST-14 library and CMPR library kottakkal. Comparison of their mass spectra with the literature was also studied. Component relative percentages were calculated based on GC-MS peak areas**.**

4.1 A Comparative Analysis of Plant Height in Response to Stress

Plants are continuously exposed various biotic and abiotic stress. Plants exposed to stress condition exhibit a broad range of morphogenic responses. *Z. officinale* cultivars Varada and Mahima responded significantly to foliar treatments of Salicylic Acid and Zinc Sulphate Heptahydrate when compared to the control plants **(Table 4.1)**. When compared to other foliar sprays and controls, plants that received Salicylic Acid in a 0.01M dosage were relatively tall plants. In combined tests, plants sprayed with both foliar sprays at greater concentrations (0.01M each) were noticeably taller, but not significantly taller than plants sprayed with Salicylic Acid at 0.01M concentration (Solitary application of foliar spray). *Z. officinale* cv-Varada was considerably taller than *Z. officinale* cv-Mahima in both kinds. Drought had a negative impact on height of the plant. The plant got reduced in size when no water was provided to the trials. At the same time samples subjected to foliar sprays in combination with drought stress was significantly taller than the Drought induced plants. This shows that the positive cross tolerance of foliar sprays in combination with drought. The negative impact of drought could have been minimized by the foliar sprays. A bar diagram was prepared **(Fig 4.1)**, and One-way ANOVA has performed for all the trials and Homogeneity by Duncan test using SPSS software, and the results were represented as Mean ± Standard Error (S.E).

Table 4.1 Plant height in response to different stress signals including control in *Z. officinale* cv-Mahima and *Z. officinale* cv- Varada.

Sl.No	Trial	*Z. officinale* cv-Mahima (Cm±S.E)	*Z. officinale* cv-Varada (Cm±S.E)
1	Control	89.98±0.538	95.23±0.66
2	DMSO	77.86±1.27	78.667±1.07
3	SA 0.01	85.26±0.656	88.8±0.577
4	SA 0.001	82.26±1.76	89±1.18
5	SA 0.01+ D	76.88±0.696	84.2667±0.5
6	SA 0.001+ D	74.57±0.254	79±2.22
7	ZnSO4 0.01	73.16±0.2728	78.567±0.296
8	ZnSO4 0.001	76.57±0.3426	72.566±0.88
9	ZnSO4 0.01+D	76.93±0.066	78.666±0.857

10	ZnSO4 0.001+D	78.1±0.5744	81.633±1.85
11	ZnSO4 0.01+ SA 0.01	84.2±0.7023	91.33±1.15
12	ZnSO4 0.001+ SA 0.001	85.36±0.5487	95±1.20
13	ZnSO4 0.01+ SA 0.001	84.5±0.288	89.66±0.33
14	ZnSO4 0.001+ SA 0.01	82.4±0.3	87.33±1.52
15	Drought	68.16±1.5	78±1.13

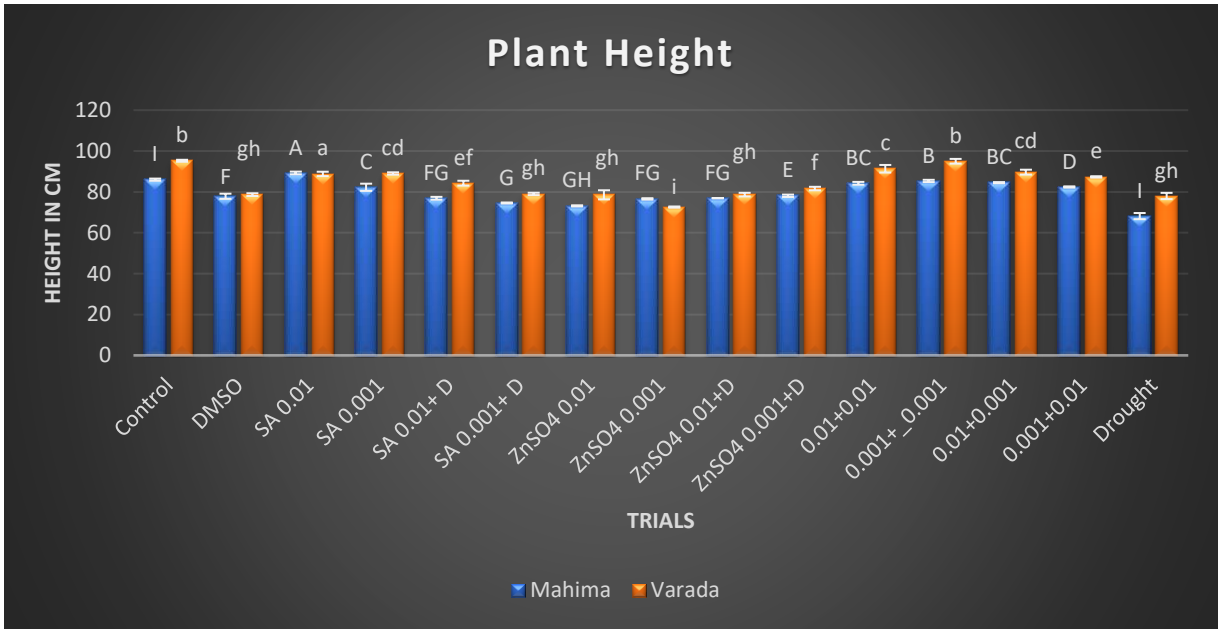

Figure 4.1 Effect of various stress signals on plant height in *Z. officinale* cv- Mahima and *Z. officinale* cv-Varada.

[Height is expressed in Mean± S.E. Bars with the same letters are not statistically different ($p \leq 0.05$). Capital letters is used for *Z. officinale* cv-Mahima and small letters used for *Z. officinale* cv-Varada].

Plant height considerably reduced in response to stress treatments. Control plants were taller than any other trials. At the same time, it is evident from the study that combination trials minimized the negative impacts of drought on the plant height. The combined effect of Salicylic Acid and rhizobium has found to mitigate the negative impacts of drought on plant height in *Pisum sativum* L. was reported by Bashir *et al.* (2020). This agrees with present study that the negative impact of drought on plant height can be minimized by the application Salicylic Acid.

4.2 A Comparative analysis on number of tillers per clump in response to stress signals in *Z. officinale* cv-Mahima and *Z. officinale* cv-Varada

Analysis of number of tillers per clump in response to various stress signals was studied and compared with the control plants. From the analysis it was observed that the number of tillers reduced in every stress when comparing with the control.

Table 4.2 Number of tillers per clump in response to different stress signals including control in *Z. officinale* cv- Mahima and *Z. officinale* cv- Varada.

Sl. No.	Trial	*Z. officinale* cv-Mahima (Nos)	*Z. officinale* cv-Varada (Nos)
1	Control	8	6
2	DMSO	4	2
3	SA 0.01	6	5
4	SA 0.001	4	4
5	SA 0.01+ D	5	3
6	SA 0.001+ D	5	4
7	$ZnSO_4$ 0.01	4	4
8	$ZnSO_4$ 0.001	3	3
9	$ZnSO_4$ 0.01+D	5	4
10	$ZnSO_4$ 0.001+D	4	4
11	$ZnSO_4$ 0.01+ SA 0.01	5	3
12	$ZnSO_4$ 0.001+ SA 0.001	6	5
13	$ZnSO_4$ 0.01+ SA 0.001	5	4
14	$ZnSO_4$ 0.001+ SA 0.01	4	3
15	Drought	3	2

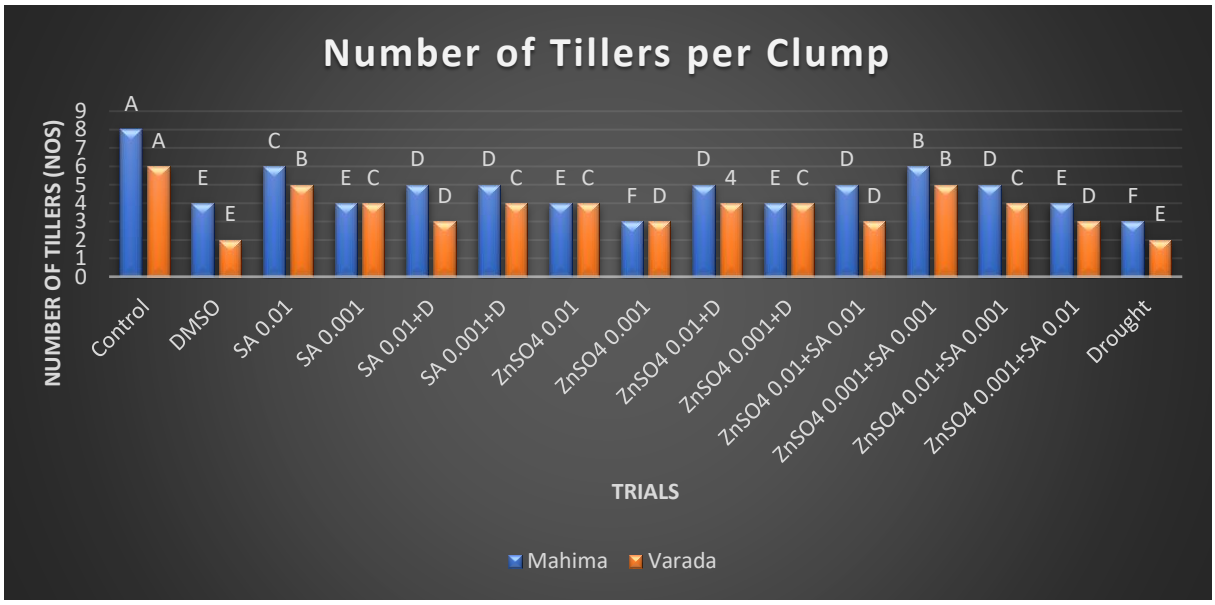

Figure 4.2 Effect of various stress signals on number of tillers per clump in *Z. officinale* cv- Mahima and *Z. officinale* cv-Varada.

[Number of tillers is expressed in nos. Bars with the same letters are not statistically different ($p \leq 0.05$). Capital letters is used for *Z. officinale* cv-Mahima and small letters used for *Z. officinale* cv-Varada].

4.3 A Comparative analysis on number of leaves per tiller in response to stress signals in *Z. officinale* cv- Mahima and *Z. officinale* cv- Varada

Response of plants to different stress signals was monitored in terms of number of leaves present each tiller identified. Here also the results show that number of leaves were found to be higher in number in control plants than that of any other stress. This indicates that stress factors somehow interfered with the number of leaves or it can also be assumed that stress factors significantly resulted in the defoliation of leaves in plants under study. Reduction of leaf number and thickness in response to drought stress in rice was previously reported by Hamim *et al.* (2016).

Table 4.3 Number of leaves per tiller in response to different stress signals including control in *Z. officinale* cv- Mahima and *Z. officinale* cv-Varada.

Sl. No.	Trial	*Z. officinale* cv- Mahima (No± S.E)	*Z. officinale* cv- Varada (No± S.E)
1	Control	22±1.15	18±0.57

2	DMSO	11.33±0.88	11±0.57
3	SA 0.01	14.33±1.20	17.33±0.57
4	SA 0.001	12±0.57	15.33±0.33
5	SA 0.01+ D	12±0.57	13±0.33
6	SA 0.001+ D	12±0.33	14±0.57
7	$ZnSO_4$ 0.01	14.667±1.52	13.66±0.57
8	$ZnSO_4$ 0.001	15±1.15	14.66±
9	$ZnSO_4$ 0.01+D	13±0.57	14±0.33
10	$ZnSO_4$ 0.001+D	17±0.57	15±0.88
11	$ZnSO_4$ 0.01+ SA 0.01	16±0.88	12.33±0.88
12	$ZnSO_4$ 0.001+ SA 0.001	11±0.57	12.33±0.33
13	$ZnSO_4$ 0.01+ SA 0.001	12.33±0.57	11.66±0.12
14	$ZnSO_4$ 0.001+ SA 0.01	12± 0.57	12.66±0.12
15	Drought	8±0.33	7.6±0.33

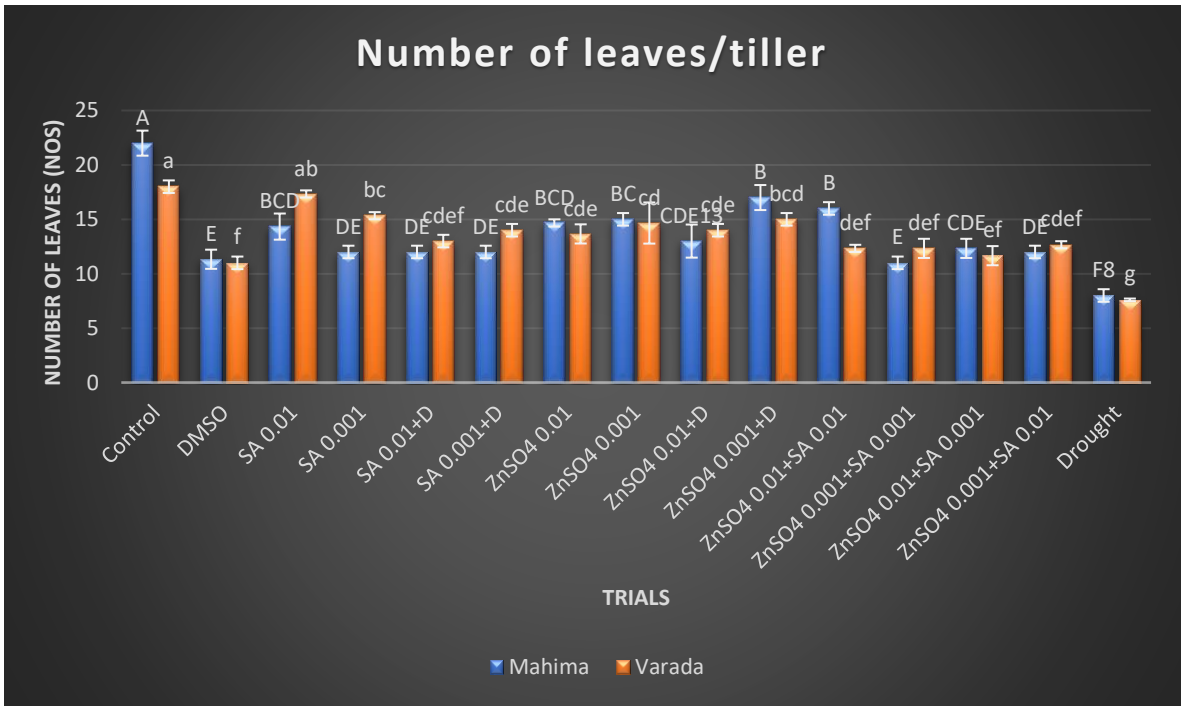

Figure 4.3 Effect of various stress signals on number of leaves per tiller in *Z. officinale* cv- Mahima and *Z. officinale* cv-Varada.

[Number of leaves is expressed in nos ± S.E. Bars with the same letters are not statistically different ($p \leq 0.05$). Capital letters are used for *Z. officinale* cv-Mahima and small letters used for *Z. officinale* cv-Varada].

Stress considerably decrease the vegetative growth of the plant. In the present study every stress considerably reduced vegetative growth of the plant in terms of height of the plant (**Table 4.1**), number of tillers per clump (**Table 4.2**) and leaves per tiller (**Table 4.3**). Bar diagrams were prepared based on the results obtained (**Fig.4.1,4.2,4.3**). There are so many eminent works by researchers around the world who studied and analyzed the effect of stress on vegetative and growth parameters of plant. Effects of Water Stress on Vegetative Growth and yield of Grapevine was studied by Chacón-Vozmediano *et al.* (2020). They found that the water stress considerably reduced the grapevine yield in the semiarid regions and adversely affected its production and market. A comparative study on influence of water stress on grain yield of two cultivars of bean *Phaseolus vulgaris* L. was given by Boutraa & Sanders, (2001). They found that water stress considerably reduced the grain yield by mainly affecting the flowering and pod filling stages. A study by Mauney, in 1986 on cotton plants showed that vegetative growth and development of fruiting sites were considerably affected by drought. At the same time there are studies which proved that exogenous application of foliar sprays like Salicylic acid and Sodium nitroprusside has improved the vegetative growth of Safflower under drought condition (Chavoushi *et al.*, 2019). Increasing the concentration of SA considerably increased the plant height and leaf number in *Zea mays* (Hussein *et al.*, 2007). So, it can be inferred that stress factors can crosstalk and result in a response which sometimes beneficial to the plant itself.

Brief Summary

Ensuring food security for the growing population is one of the major challenges that human population are going to face in the future decades. Plants which are under its natural conditions are prone to a variety of stress factors. In this study, ginger, which occupies a great role as a spice both in culinary and medical purposes are affected by stress factors. In the present study it was found that in response to stress factors, its vegetative growth has altered, especially plant height, number of tillers and number of leaves. Plants adopt themselves by changes its physiological mechanisms to adapt to its new environment.

This is may be because of these physiological parameters that it has reduced the number of leaves, plant height and number of tillers among them.

5.1Preliminary phytochemical analysis-Assessment of phytochemical components of selected ginger varieties (*Z. officinale* Rosc. cv- Varada and *Z. officinale* Rosc. cv- Mahima)

The methanolic extracts of ginger (both varieties) were taken for preliminary phytochemical analysis, which includes alkaloids, coumarins, flavonoids, glycosides, quinines, saponines, phenols, tannins, terpenoids, steroids and reducing sugars were done using standard protocols for different phytochemicals.

Table 5.1 Preliminary phytochemical analysis of Ginger.

Sl.no	Test	Name of the Test	Procedure	Observation	Inference
1	Alkaloids	Mayer's test	2 ml extract+Few drops of HCl+Mayer's reagent.	Cream Precipitation	+++
		Hager's test	2 ml extract+Few drops of HCl+Hager's reagent.	Yellow Precipitation	+++
2	Coumarins	Coumarins test	2 ml of sample extract+ three ml 10% Sodium Hydroxide solution is added along the sides of the test tube	Appearance of Yellow colored solution	+++

3	Flavonoids	Alkaline reagent test	2 ml of 2% NaOH solution + crude extract	intensive yellow colour was formed, which turned colourless on addition of dilute acid	+++
		Lead acetate test	2 ml extract+Few drops of Lead Acetate solution	Yellow Precipitation	+++
4	Glycosides	Keller Killiani's test	2 ml extract +Glacial Acetic Acid +one drop of 5% Ferric Chloride solution	No color junction	---
5	Quinines	Quinones test	2-3 ml of sample+3 ml HCl	Appearance of yellow colour	+++
6	Saponines	Foam test	2 ml sample+ 4 ml distilled water. mix well and vigorously	No foam formation	---

7	Phenols	Ferric chloride test	To 2-3 ml of extract a few drops of 5% Ferric Chloride	Presence of Deep blue black color	+++
8	Tannins	Braymer's test	2-3 ml extract is diluted by adding 2 ml of distilled water. To which 2-3 drops of 5% Ferric Chloride is added	Appearance of black green or bluish colour	+++
9	Terpenoids	Copper acetate test	2 ml extract is dissolved in distilled water, to which 3-4 drops Copper Acetate solution is added	Formation of emerald green	+++
10	Steroids	Salkowski test	To 2 ml of extract+ 2ml Chloroform. 2 ml concentrated	Chloroform layer appears red colour and acid layer shows	+++

			Sulphuric Acid is added along the sides of the test tube	greenish yellow fluorescence	
11	Carbohydrates	Molish Test	Molish Test	2 ml extract+2 drops of Molish reagent+Few drops of conc. H_2SO_4	+++
12	Proteins	Millons Test	3 ml of extract +5 ml of millon's reagent	White precipitate which turns brick red on warming	+++

• (+++) Present, (---) Absent

The analysis of phytochemicals from methanolic extracts of ginger shows that it is a rich source of secondary metabolites. Out of the twelve tests conducted for the preliminary phytochemical analysis, Keller Killiani's test for glycosides and foam tests for saponins showed a negative result. Alkaloids, coumarins, flavonoids, quinines, phenols, tannins, terpenoids, steroids, carbohydrates and proteins itself indicated its presence in the methanolic extract **(Table 5.1)**.

Bashir *et al*. (2015) published the results of a preliminary phytochemical analysis of *Z. officinale* hot water, methanol and chloroform extract. Various extracts were found to contain steroids, alkaloids, tannins, flavonoids, reducing sugars, and saponins. There are tannins, reducing sugars, saponins, and steroids in the hot water extract of ginger. Similar to this, Bashir *et al.* (2013) discovered that the aqueous extract of dried ginger rhizomes contained the same class of chemicals as well as flavonoids. Numerous phytoconstituents,

including alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, tannins, terpenoids, flavonoids and phytosterols were detected in the ethanolic and petroleum ether extracts of *Z. officinale* (Setty *et al.*, 2011). The hydro alcoholic extract of the dry rhizomes of *Z. officinale* revealed the presence of flavonoids, alkaloids, phenolic compounds, tannins and saponins (Babu *et al.*,2018). The fresh rhizomes of *Z. officinale* included steroids, phenols, alkaloids, glycosides, tannins, flavonoids, phlobatannins, saponins, cardenolides terpenes, quinones, steroids, phenols, alkaloids, glycosides and glycoside analogues in higher concentrations than the dried rhizomes of ginger (Taoheed *et al*., 2017).

Brief Summary

Plants produce a wide range of phytochemicals. Analyzing and understanding the class of phytochemicals and its properties is of great significance. The preliminary phytochemical analysis helped to understand different class of phytochemicals that were present in the selected cultivars of ginger. This analysis also gave a preliminary knowledge about the different kinds of phytochemicals belonging to different class of phyto compounds.

The total phytochemical components of rhizomes harvested from different stress trails including control was evaluated by means of quantitative estimation of phytochemicals. Estimation of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidant activities were done to have a clear idea about the influence of different stress trials in the formation of phytochemicals and to compare the impact of different stress signals in the bioaccumulation of phytochemicals. The average of percentage of content in five replications for each trail with standard error (M±SE) was evaluated and recorded.

6.1 Estimation of Total Phenolic Content (TPC)

The majority of a plant's secondary metabolites are phenolics, which are widely diversified throughout the plant kingdom. Plant polyphenols have gained growing attention as a result of their powerful antioxidant abilities and their notable contributions to the prevention of several oxidative stress-related illnesses, including cancer. An important field of health- and medical-related research in recent years has been the identification and development of phenolic chemicals or extracts from various plants (Dai & Mumper., 2010). Phenolic acids can be further split into two classes: Benzoic Acid derivatives and Cinnamic Acid derivatives. The most prevalent Phenolic Acid in many fruits and vegetables is Caffeine, which is most frequently esterified with Quinic Acid to form Chlorogenic Acid, the main phenolic ingredient in coffee. Ferulic acid, another typical Phenolic Acid that is found in cereals and is esterified to hemicelluloses in the cell wall (Archivio *et al.*,2007). According to Ainsworth & Gillespie, (2007) the assessment of total phenolic content often relies on the interaction between phenolic compounds and a particular colorimetric reagent. Based on the Folin-Ciocalteu test method, the Total Phenolic Content (TPC) was calculated in the current study.

Folin-Ciocalteu (F-C) colorimetry is based on the chemical reduction of the F-C reagent, which is a combination of tungsten and molybdenum oxides (Singleton & Rossi, 1965). The products of metal oxide reduction have a blue colour and a wide range of light absorption, having a peak at 765 nm. The phenolic chemicals in the extract reduced the F-C reagent, causing the blue complex to form as a result. The concentration of phenolic compounds present in the extract directly relates to how much light at that wavelength is absorbed (Waterhouse, 2002).

Table 6.1 Absorbance of different concentration of GA at 650nm

Concentration of Gallic Acid (µg/ml)	Absorbance at 650nm
50	0.228
100	0.685
200	1.099
300	1.498
400	2.10
500	2.531

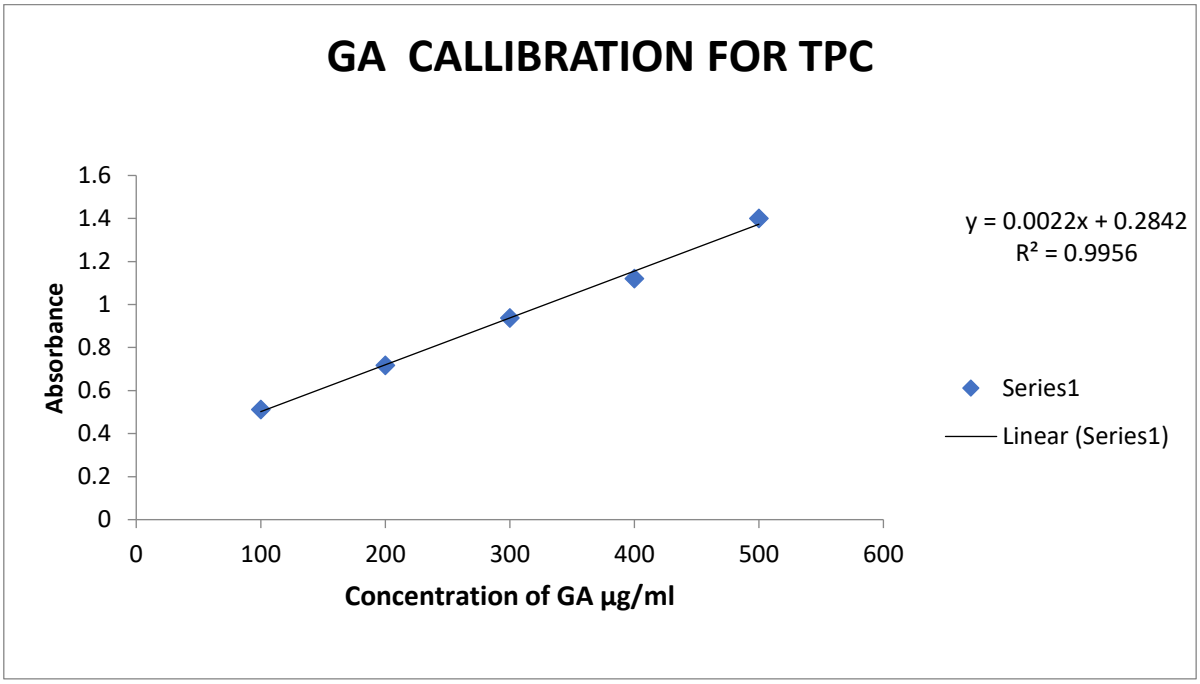

Figure 6.1 Standard calibration curve of Gallic Acid for TPC

Table 6.2 Total Phenolic Content (TPC) estimated from the standard curve.

Treatments	GA equivalent (mg GA/g) *Z. officinale* cv-Mahima (Mean±SE)	GA equivalent (mg GA/g) *Z. officinale* cv-Varada (Mean± SE)
Control	13.9±0.368	17.36±0.323
DMSO	21.79±0.436	23.40±0.651
SA 0.01 M	43.86±0.441	59±0.598

SA 0.001M	20.27±0.48	30.62±0.236
SA 0.01M +D	32.87±0.435	44.5±0.958
SA 0.001M+D	19.62±0.192	20.3±0.522
$ZnSO_4$ 0.01M	26.67±0.164	34.65±0.122
$ZnSO_4$ 0.001M	23.79±0.348	30.52±0.36
$ZnSO_4$ 0.01M+D	16.69±0.112	24.97±0.288
$ZnSO_4$ 0.001 M+D	13.87±0.103	19.44±0.16
$ZnSO_4$ 0.01M+ SA 0.01M	40.12±0.348	51.23±0.214
$ZnSO_4$ 0.001M+ SA 0.001M	35.13±0.435	46.98±0.263
$ZnSO_4$ 0.01M+ SA 0.001M	33.53±0.582	41.55±0.398
$ZnSO_4$ 0.001M+ SA 0.0.1M	39.78±0.433	47.36±0.151
Drought	21.12±0.96	22.98±0.102

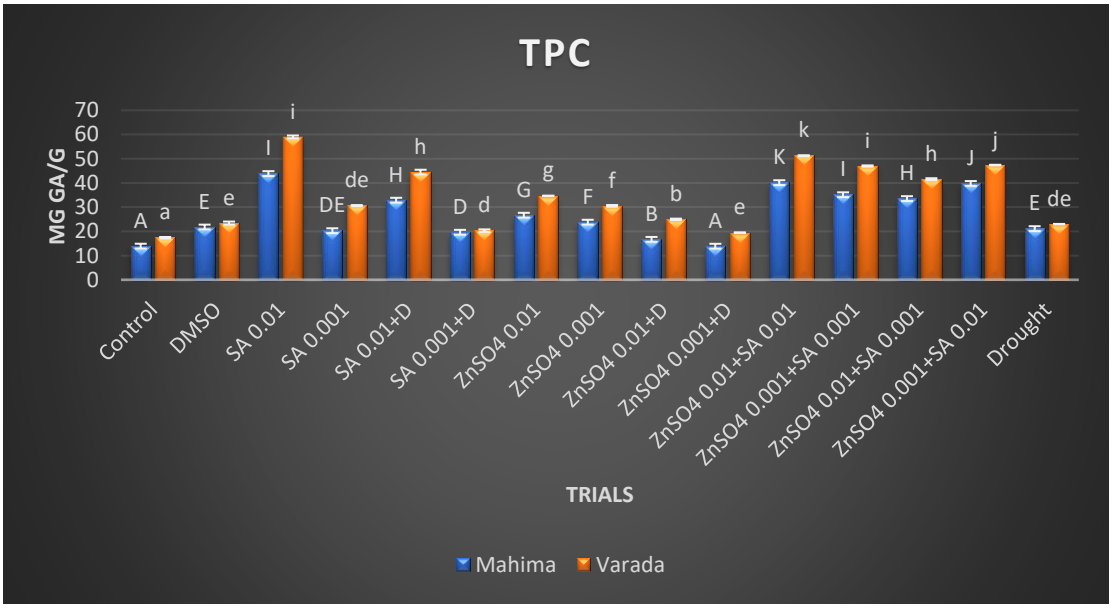

Figure 6.2 Effect of different stress trials on Total Phenolic Compounds (TPC) expressed as mg GA/g± Standard Error (S.E) of dry plant material.

From the results it can be inferred that increasing concentration of foliar spray of salicylic acid had a positive impact on the total phenolic content when comparing with the foliar application zinc sulphate of same concentration (**Figure 6.2**), and a common trend

observed in both the cultivars. Biological replicates sprayed with 0.01 M Salicylic Acid (SA) showed higher Total Phenolic Content in both cv-Varada and cv-Mahima (43.86±0.441mg GAE/g and 59±0.598441mg GAE/g each) (**Table 6.2**). Increasing concentration of both SA and $ZnSO_4.7H_2O$ in Mahima and Varada showed an increase in the concentration of total phenolic content in response to an increase in the concentration of respective foliar sprays. While in combination with drought, both the foliar sprays showed a negative cross tolerance. Salicylic acid and Zinc Sulphate in higher concentration (0.01 each) had a positive cross tolerance in terms of Total phenolic content, in comparison with solitary foliar applications it is higher than that of solitary application of Zinc Sulphate but is less than that of Solitary foliar application of SA. (**Table 6.2**).

Study by Bistgani *et al.* (2017) on *T. daenensis* reported that mild drought stress caused an increase in the concentration of phenolic compounds. In addition, a study by Manukyan, (2011) indicated that drought stress affected positively polyphenolic content in *Melissa officinalis* L. Similarly, exogenous application of SA increased Total Phenolic Content in table grapes (Blanch *et al.*, 2020). In contrary to the present investigation, Total Phenolic Content of *Z. officinale* cv- Varada was found to be higher than that of *Z. officinale* cv-Mahima (Sanwal *et al.*, 2010).

Estimation of Total Flavonoid Content (TFC)

Flavonoids are another type of naturally occurring antioxidant chemical that can scavenge free superoxide radicals, exhibiting anti-aging effects and lowering the risk of cancer (Feng *et al.*, 2012). They are a group of secondary plant metabolites with a polyphenolic structure that are prevalent in fruits, vegetables and some drinks. They have a variety of beneficial biochemical and antioxidant properties linked to a number of disorders, including cancer, Alzheimer's disease (AD), atherosclerosis etc. (Burak & Imen, 1999). Depending on the carbon atom of the C ring that the B ring is linked to as well as the level of unsaturation and oxidation of the C ring, flavonoids can be split into many subgroups. Isoflavones are flavonoids with a B ring attached to the third position of the C ring. Neoflavonoids are those in which the B ring is joined in position 4; those in which the B ring is linked in position 2 can be further split into a number of subgroups based on the structural characteristics of the C ring. These subgroups include chalcones, anthocyanins, flavones, flavonols, flavanones and flavanonols (Panche *et al.*, 2016).

Table 6.3 Absorbance of different concentration of Quercetin at 510 nm

Concentration of Quercetin (µg/ml)	Absorbance at 510nm
100	0.161
200	0.366
300	0.489
400	0.599
500	0.82

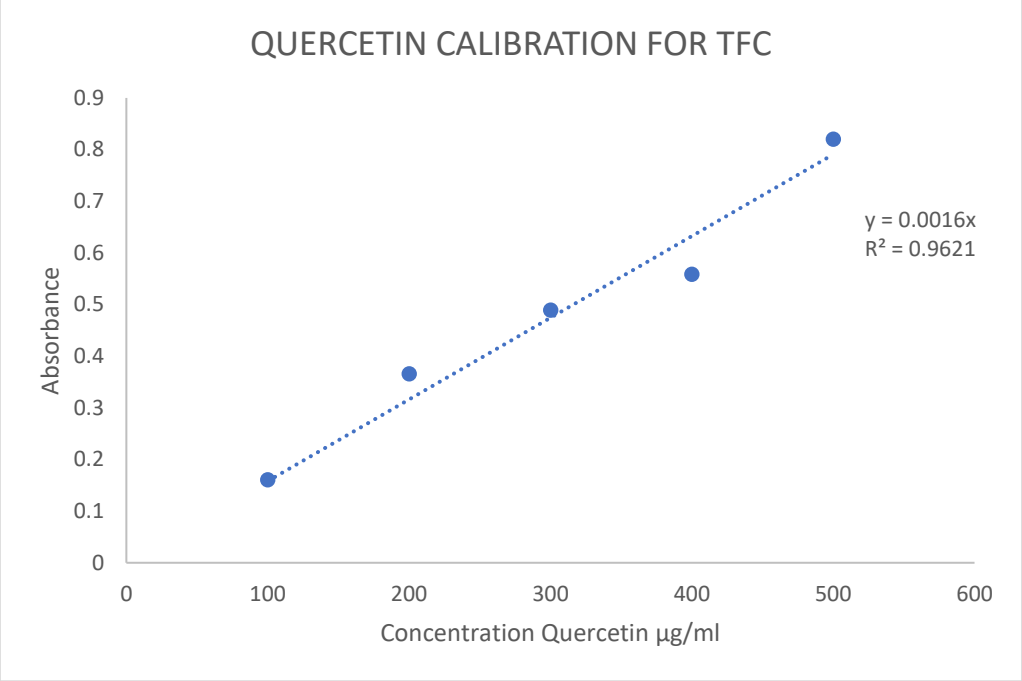

Figure 6.3 A standard calibration curve of Quercetin for TFC

Table 6.4 Total Flavonoid Content (TFC) estimated from the standard curve.

Treatments	**Quercetin equivalent (mg Q/g)** ***Z. officinale*** **cv-Mahima (Mean±SE)**	**Quercetin equivalent (mg Q/g)** ***Z. officinale*** **cv-Varada (Mean± SE)**
Control	6.72±0.05	6.86±0.86
DMSO	9.23±0.25	9.73±0.63
SA 0.01 M	11.23±0.12	11.56±0.89
SA 0.001M	10.04±0.36	10.75±0.57
SA 0.01M +D	11.39±0.4	11.65±0.64
SA 0.001M+D	11.02±0.192	11±0.12
$ZnSO_4$ 0.01M	10.08±0.369	9.89±0.023
$ZnSO_4$ 0.001M	9.65±0.25	9.95±0.58
$ZnSO_4$ 0.01M+D	10.36±0.012	10.52±0.69
$ZnSO_4$ 0.001 M+D	10.39±0.05	10.75±0.45
$ZnSO_4$ 0.01M+ SA 0.01M	12.83±0.23	13.02±0.25
$ZnSO_4$ 0.001M+ SA 0.001M	10.89±0.8	11.06±0.59
$ZnSO_4$ 0.01M+ SA 0.001M	10.69±0.65	11±0.78
$ZnSO_4$ 0.001M+ SA 0.0.1M	10.03±0.4	11.01±0.15
Drought	11±0.76	12.03±0.98

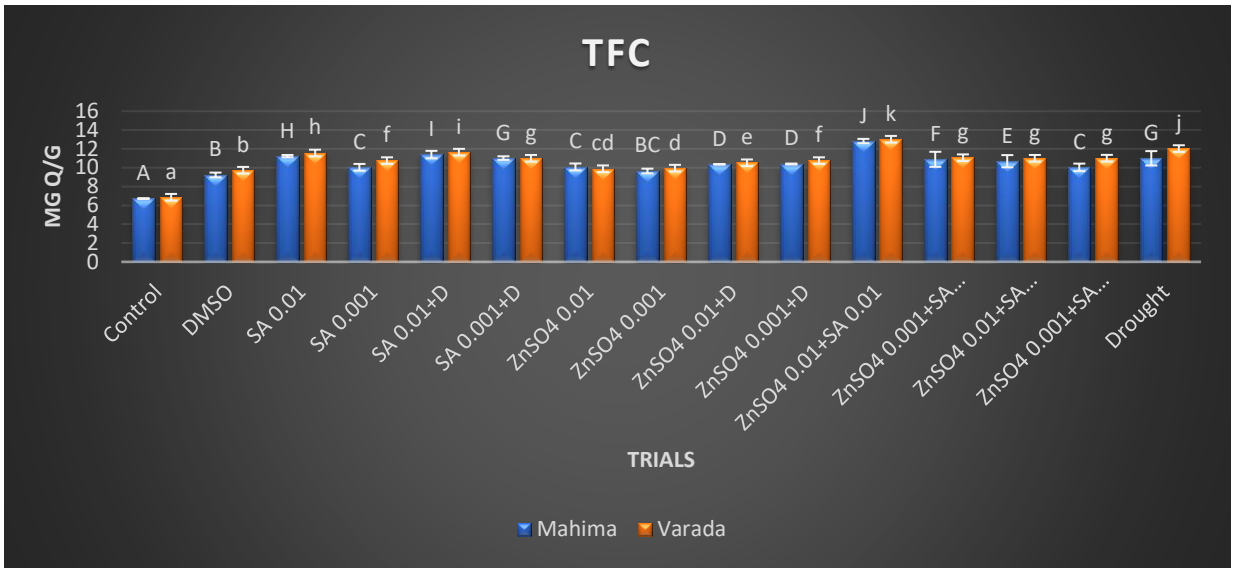

Figure 6.4 Effect of different stress trials on Total Flavonoid Compounds (TFC) expressed as mg Quercetin/g± Standard Error (S.E) of dry plant material.

It is clear from the experiment that Total flavonoid was found to be increasing while increasing the concentration of both the foliar sprays (**Table 6.4).** But the highest total flavonoid content was observed in the trials which are sprayed with both the foliar sprays in its highest concentration 12.83±0.23 mg QE/g and 13.02±0.25 mg QE/g for *Z. officinale* cv-Mahima and *Z. officinale* cv-Varada respectively. In combination with drought, the Total Flavonoid Content (TFC) found to be higher in comparison with solitary application of both the sprays.

Combined abiotic stress treatments altered the total flavonoid content in two *Capsicum* cultivars was reported by Genzel *et al.* (2021). Studies by Ellenberger *et al.* (2020) stated that combined abiotic stress treatments increased the total flavonoid content in a larger extend than solitary stress treatments in bell pepper and chilli. The combined effect of SA and CO_2 treatments in two varieties of ginger (Halia Bentong and Halia Bara) was studied by Ghasemzadeh & Jaafar (2012), and found that the combination of stress treatments enhanced anthocyanin and flavonoid production compared with single treatment effect.

Antioxidant activity by DPPH

Every significant phytochemical has pharmacological effects. The comparative study of antioxidant activity of ginger under various stress treatments reveal the effect of stress treatments in the production of phytochemicals and eventually its antioxidant

activity. Here, a comparative analysis of antioxidant activity of two cultivars of ginger were evaluated by Free radical scavenging activity by DPPH.

Table 6.5 Percentage of DPPH inhibition activity estimated.

Treatments	***Z. officinale* cv-Mahima (Mean±SE) (%)**	***Z. officinale* cv-Varada (Mean± SE) (%)**
Control	33.33±0.57	37±0.01
DMSO	33.66±0.33	38±0.02
SA 0.01 M	66.66±0.33	70.667±0.05
SA 0.001M	47.33±0.33	60.33±0.33
SA 0.01M +D	51.833±0.166	58.33±0.36
SA 0.001M+D	41±0.5	53.5±0.5
$ZnSO_4$ 0.01M	51.667±0.4	57.33±0.23
$ZnSO_4$ 0.001M	48.66±0.3	53.667±0.21
$ZnSO_4$ 0.01M+D	49±0.1	53.01±1.5
$ZnSO_4$ 0.001 M+D	45±0.25	49.667±1.2
$ZnSO_4$ 0.01M+ SA 0.01M	53.50±0.45	55.16±0.4
$ZnSO_4$ 0.001M+ SA 0.001M	51.66±0.37	56.33±0.14
$ZnSO_4$ 0.01M+ SA 0.001M	45.89±0.65	55.7±0.13
$ZnSO_4$ 0.001M+ SA 0.0.1M	46.78±0.8	50.67±0.65
Drought	41.26±0.81	48.33±0.21

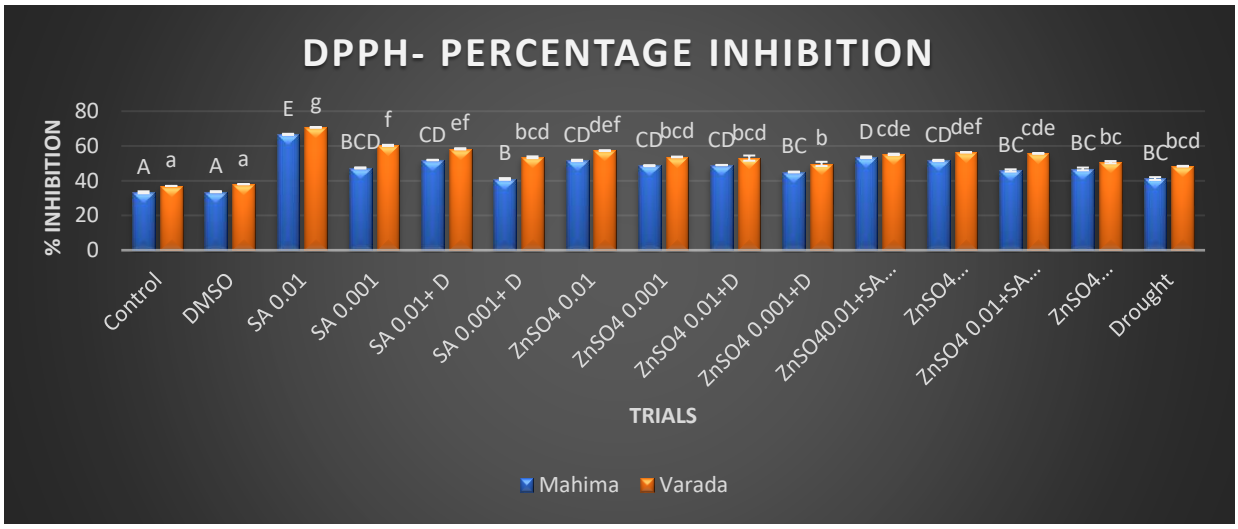

Figure 6.5 Antioxidant activity by DPPH expressed as percentage± Standard Error (S.E) of dry plant material.

Antioxidant activity estimated by the radical scavenging activity by DPPH was found to be higher in the case of *Z. officinale* cv-Varada than that of *Z. officinale* cv-Mahima. Trials which are sprayed with SA in its higher concentration was found to have higher antioxidant activity than other trials (70.667±0.05 for *Z. officinale* cv- Mahima and 66.66±0.33 for *Z. officinale* cv-Varada) **(Table 6.5**). Increasing concentration of both the foliar sprays had a positive impact on the antioxidant activity. The trend was similar to that of Total Phenolic Content estimated for different stress trials. From this it can be inferred that Total Phenolic Content and antioxidant activity is directly proportional to each other. In combination with drought, both the foliar spray stress treatment showed a decrease in the antioxidant activity. In foliar spray combination trials (Zn+SA) both the foliar sprays in its higher concentration showed high antioxidant activity. Here, a positive relationship between antioxidant activities and total phenolic contents was also observed.

The results are in harmony with various studies reported by different authors. Total Phenolic Content and antioxidant activity was found to be related in ginger rhizomes was also studied under various drying conditions. This good correlation was observed by Ghafoor *et al.* (2020). Previous investigations on the phenolic content and antioxidant properties of ginger rhizomes was reported by various scientists (Kuo *et al.*, 2005; Chrubasik *et al.*, 2005). Tohma *et al.* (2017) also measured the Total Phenolic Content and antioxidant activity of the aqueous extract of the ginger rhizome. The antioxidant activity

was discovered to be rising with the concentration of polyphenols in the extract, and the phenolic content was determined to be 52.8 g/mg Gallic acid equivalents. According to Prakash, (2010) the aqueous extracts of the *Z. officinale* rhizomes were compared to the various solvent extracts in terms of their phenolic composition and antioxidant activity. Studies by (Shan *et al.,* 2005; Wu *et al.,* 2006; Wong *et al.,* 2006) have shown that the antioxidant activities of spices and herbs are substantially attributed to the presence of phenolic compounds in it.

Brief Summary

Quantitative estimation of total phenolics, total flavonoids and a comparative analysis of antioxidant activity of ginger rhizomes under different stress condition gave clear picture on how the stress factors has altered/ influenced the production of secondary metabolites in the selected ginger varieties, especially phytoconstituents which belonging to the category of phenolics and flavonoids. The results indicated that stress signals considerably increased the production of secondary metabolites in trials. This can be considered as a result of its defense mechanism and its adaptation to grow under abiotic stress conditions. The present results also showed how combination of stress results were different from that of solitary stress responses. Which gives a clear picture of how the one stress alters the effect of another. A strong correlation has been noted between quantitative estimation of phytochemicals and antioxidant activity of rhizomes under stress trials.

7.1 HPLC Analysis of 6- gingerol

The analytical instrument HPLC can identify, isolate and measure compound, as well as any contaminants and drug-related degradation products that may develop during manufacturing or storage. It requires knowledge of the chemistry of the chemical ingredient and aids in the creation of analytical techniques. To improve the procedure, a number of chromatographic parameters were assessed. Finding the right mobile phase, column, column temperature, wavelength and gradient is essential for ensuring the compound's compatibility, stability, and resistance to degradation products and contaminants (Gupta *et al.*, 2012). The examination of ginger extracts has been conducted using a number of High-Performance Liquid chromatographic (HPLC) techniques. These techniques were mostly utilized to examine the gingerols in ginger that was cultivated in the field (Pawar *et al.*, 2011). There are no studies focused on comparative analysis of concentration of gingerol in response to different stress treatments. In the present study, a comparative approach to evaluate the effect of different stress treatments in the concentration of gingerol in two cultivars of ginger is evaluated.

HPLC analysis of methanolic extract of dried ginger powder showed difference in concentration of 6-gingerol (in percentage) in different trials including control. The concentration of 6-gingerol was found to be varying with stress trials. Comparing two cultivars, *Z. officinale* cv-Mahima was found to have comparatively greater percentage of gingerol than that of the cultivar *Z. officinale* cv-Varada. When coming on to the quantity of gingerol in each treatment, plants which are sprayed with SA in two different concentrations SA 0.01M and 0.001M along with its control, that is 0.02% DMSO, trials which are sprayed with DMSO showed comparatively a higher concentration of gingerol than SA 0.01M and 0.001 M as well as plants which are not irrigated or trials which are completely withdrawn water supply (Drought Control).

For all the analysis a standard HPLC chromatogram was prepared for 6-gingerol to identify the retention time of 6-Gingerol.

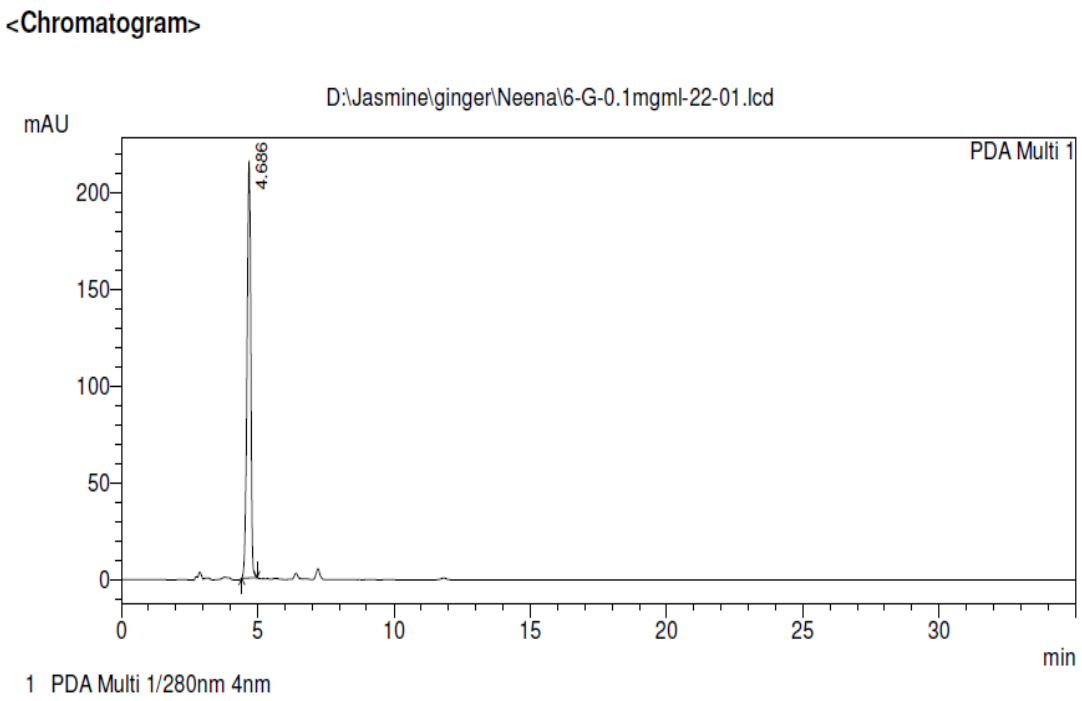

Figure 7.1 Chromatogram for 6-gingerol

A standard curve was prepared using Capsaicin (NVA 8-methyl-N-Vanillyl-trans-6-nonenamide) which is a structural analogue of 6-gingerol to estimate the percentage of gingerol in all the trials.

Table 7.1 Peak area of NVA at different concentration

Sl.No	Concentartion (µg/ml)	Retention time (min.)	Area of the Peak
1	0.2	4.68	1189501.1
2	0.4	4.69	2565117.5
3	0.6	4.68	4314675.1
4	0.8	4.7	5332346.7
5	1	4.68	7089047.2

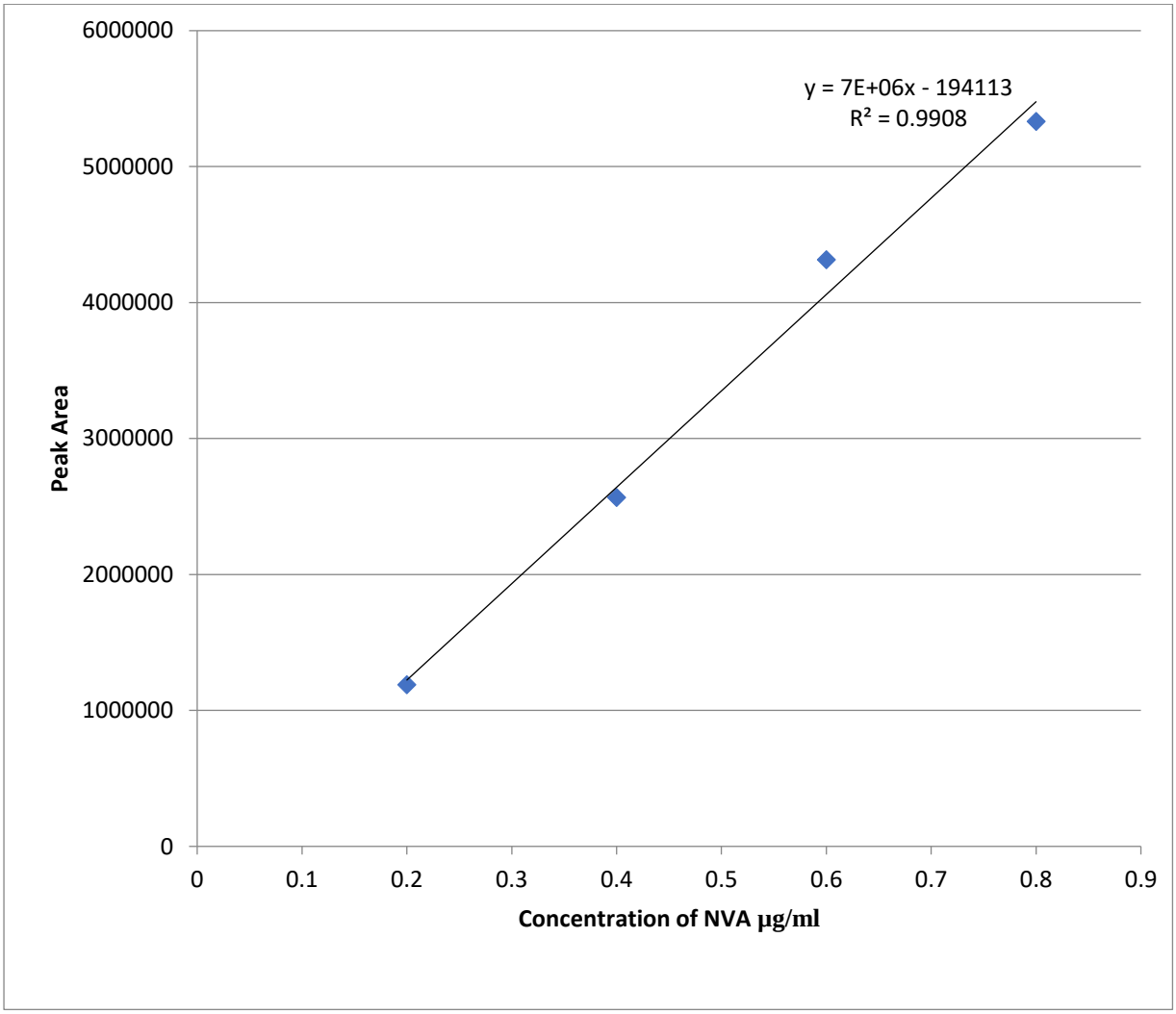

Figure 7.2 Standard Curve for NVA

Plant trials which are sprayed with DMSO had considerable quantity of gingerol (2.45% for *Z. officinale* cv-Mahima and 1.56% for *Z. officinale* cv-Varada (at a retention time of 4.702 for *Z. officinale* cv-Varada and 4.693min for *Z. officinale* cv-Mahima) (**Fig 7.3 & 7.4**) From this it can be inferred that DMSO which is an organosulphate itself has crucial role in the plant response.

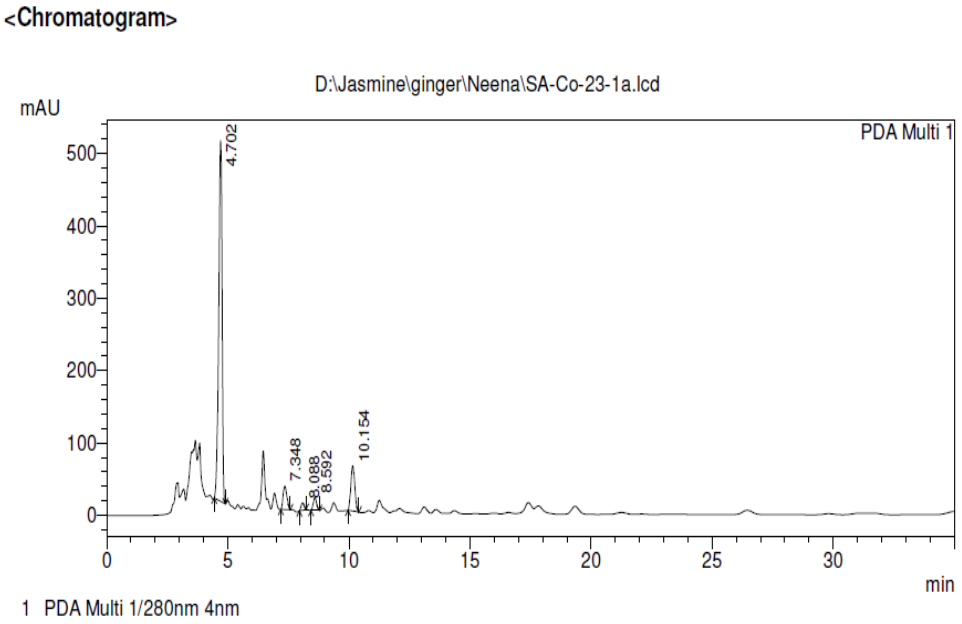

Figure 7.3 Chromatogram for *Zingiber officinale* cv-Varada under the foliar spray of DMSO (Control for SA).

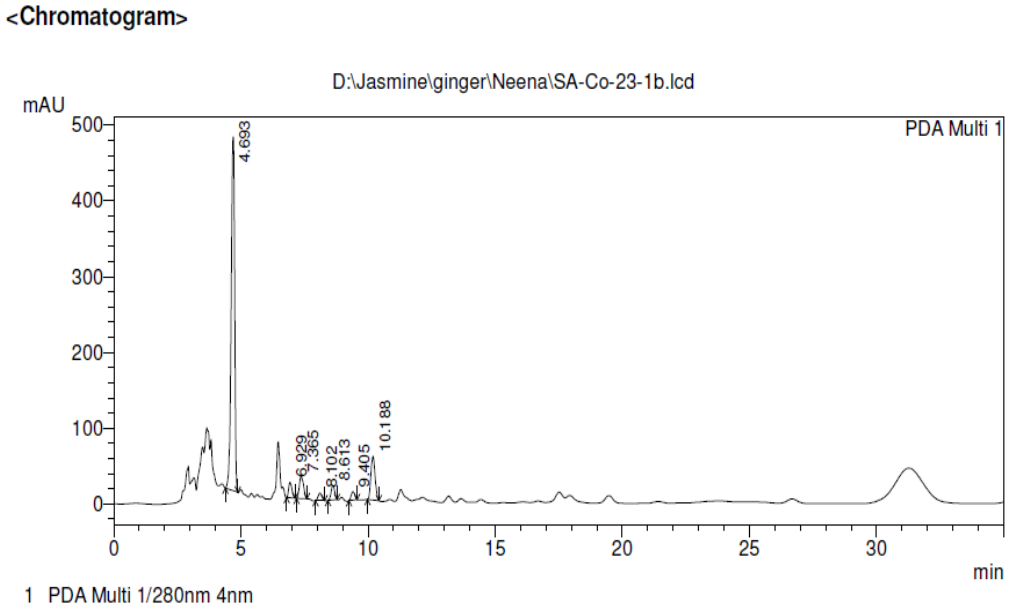

Figure 7.4 Chromatogram for *Zingiber officinale* cv-Mahima under the foliar spray of DMSO (Control for SA).

The percentage of gingerol was found to be higher in the case of *Z. officinale* cv-Mahima comparing with *Z. officinale* cv-Varada.

Increasing concentration of Salicylic acid showed a positive effect on the production of gingerol. SA 0.01 M has 1.8% of gingerol in *Z. officinale* cv- Mahima (R.T. 4.719min.) and 1.21% (R.T.4.722min.) in the case of *Z. officinale* cv-Varada. **(Fig 7.5& 7.6).**

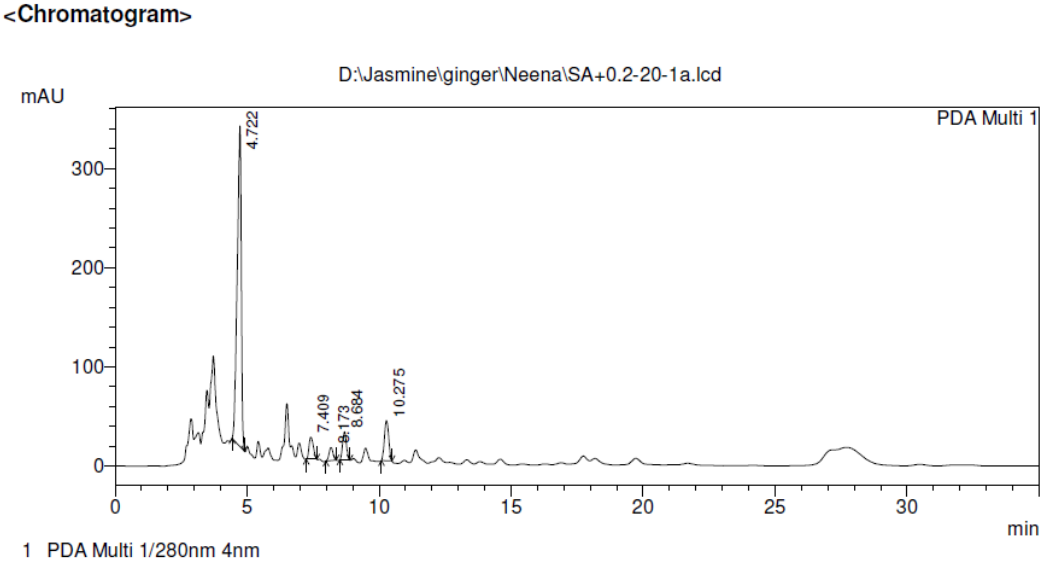

Figure 7.5 Chromatogram for *Zingiber officinale* cv-Varada under the foliar spray of SA 0.01M.

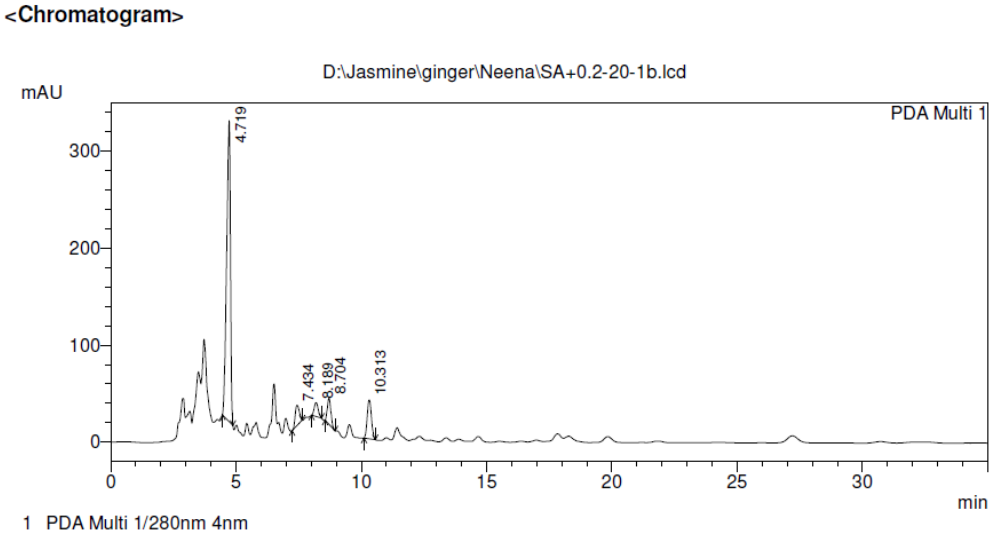

Figure 7.6 Chromatogram for *Zingiber officinale* cv-Mahima under the foliar spray of SA 0.01M.

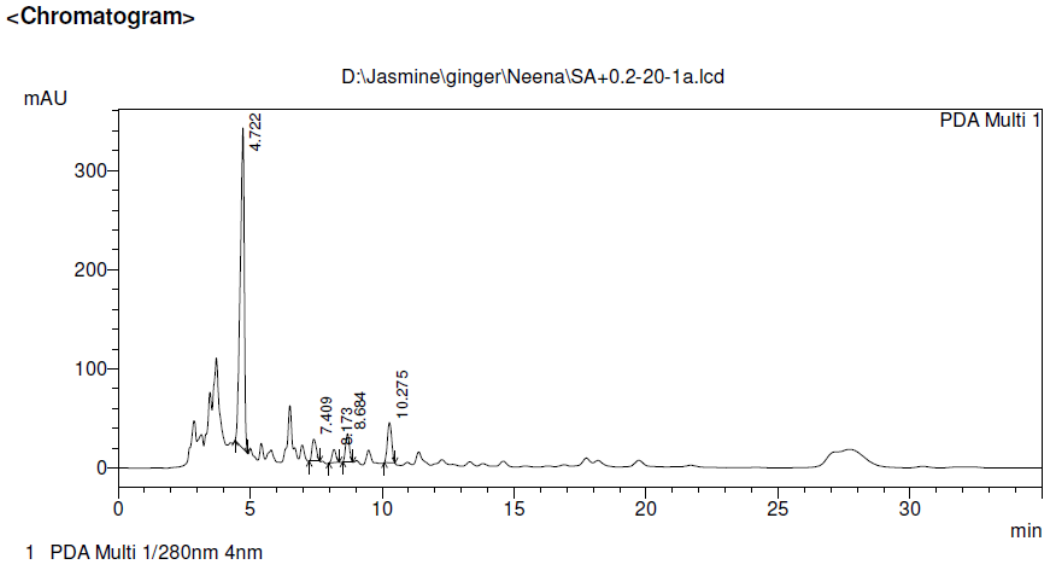

Figure 7.7 Chromatogram for *Zingiber officinale* cv-Varada under the foliar spray of SA 0.001M.

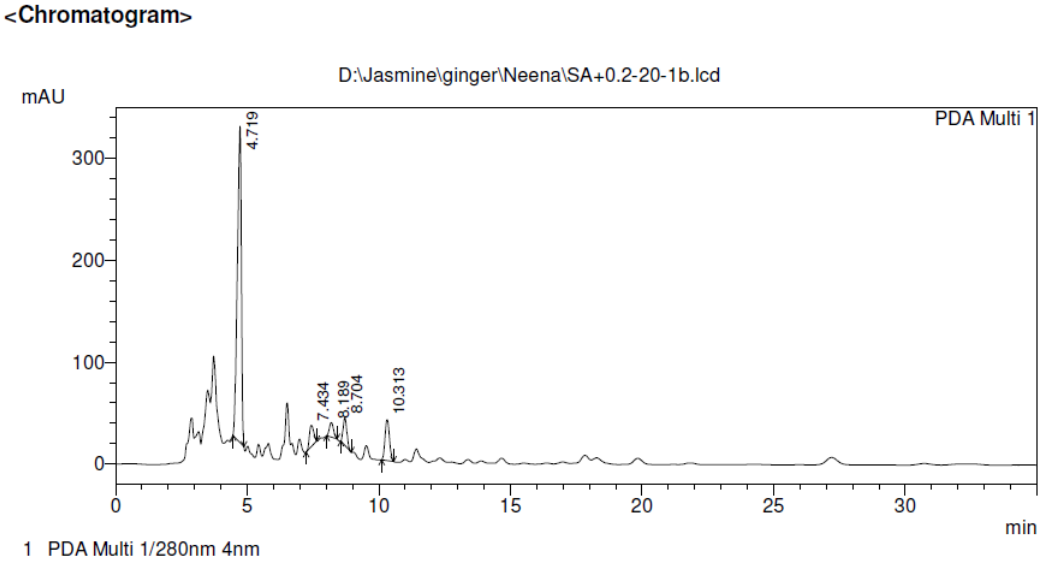

Figure 7.8 Chromatogram for *Zingiber officinale* cv-Mahima under the foliar spray of SA 0.001M.

And SA 0.001 M has 1.2% for *Z. officinale* cv-Mahima at retention time of 4.719min. and 0.89% for *Z. officinale* cv-Varada at a retention time of 4.722 **(Fig 7.7&7.8)**.

Drought control showed 0.98 % of gingerol at a retention time of 4.682 min for *Z. officinale* cv-Mahima and 0.9% of gingerol at a retention time of 4.706 min. for *Z. officinale* cv-Varada **(Fig 7.9& 7.10).**

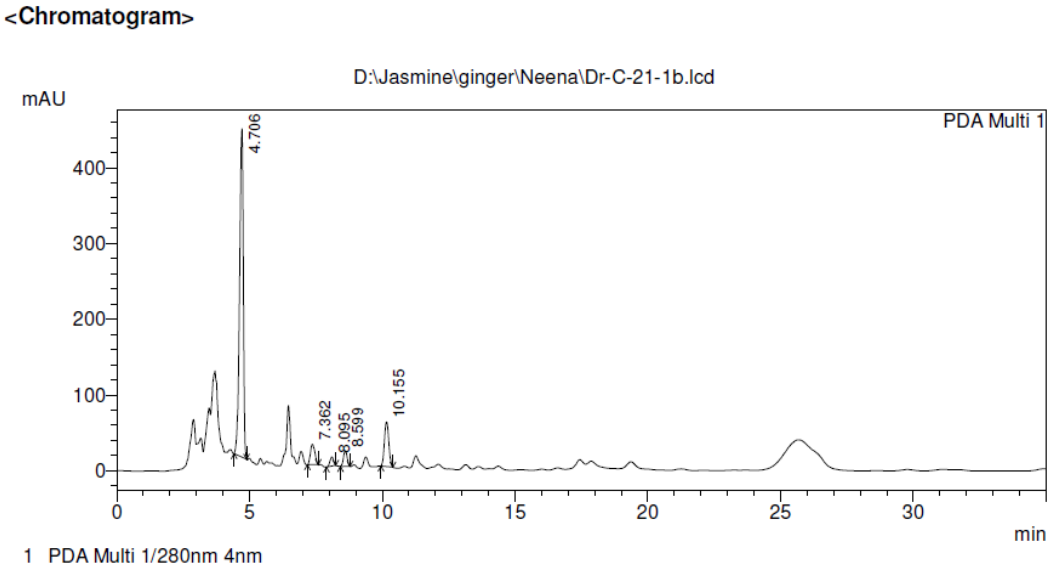

Figure 7.9 Chromatogram for *Zingiber* officinale cv-Varada under drought control.

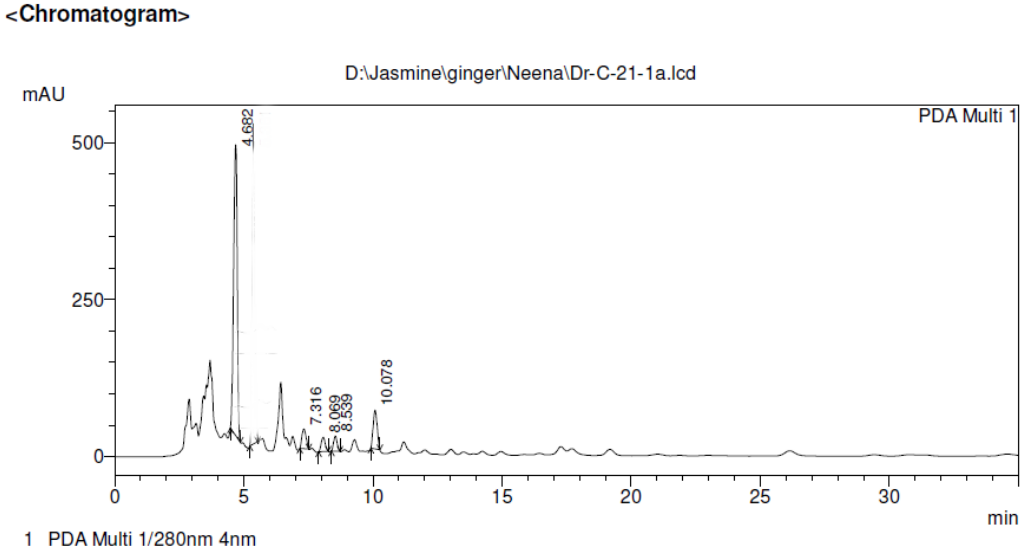

Figure 7.10 Chromatogram for *Zingiber officinale* cv-Mahima under drought control.

In combination with drought SA 0.01 + D and SA 0.001+ D showed greater percentage of gingerol than its solitary stress signals. It was higher than that of the percentage obtained from solitary application of Salicylic acid or drought control. In drought and SA 0.01 M combination had 1.99% of gingerol in *Z. officinale* cv- Mahima (R.T. 4.70 min.) and 1.27% of gingerol in *Z. officinale* cv-Varada (R.T.4.697 min) which was greater than that of its solitary applications **(Fig 7.11 & 7.12)**. The same was observed in the case of SA 0.001M and drought combination. In that case 1.46% of gingerol (R.T.

4.688 min) for Z. *officinale* cv- Mahima and 1.03% for *Z. officinale* cv- Varada (R.T.4.689 min). **(Fig 7.13& 7.14).**

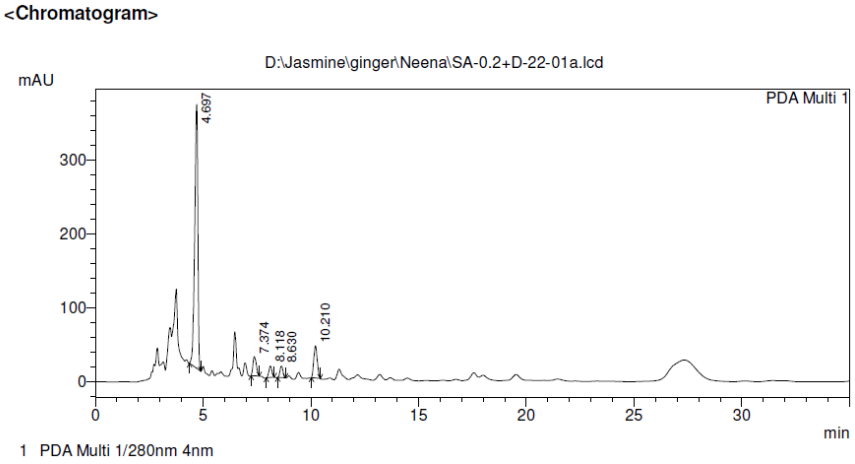

Figure 7.11 Chromatogram for *Zingiber officinale* cv-Varada under the combination of SA 0.01M+D

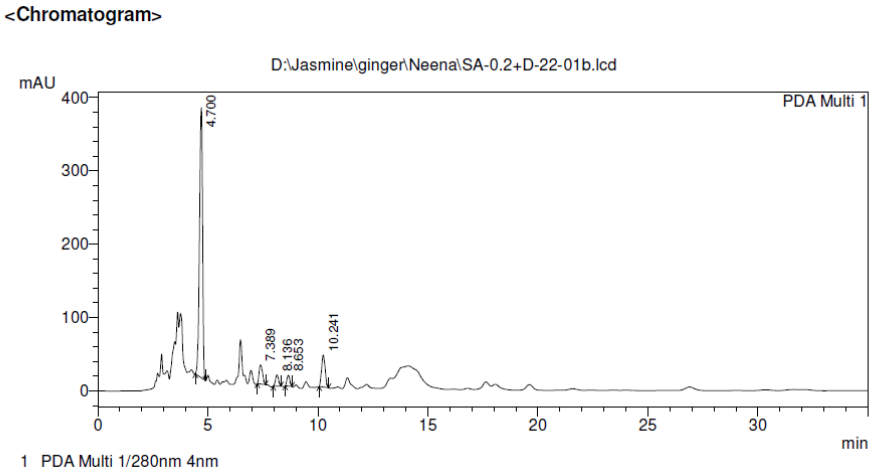

Figure 7.12 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of SA 0.01M+D

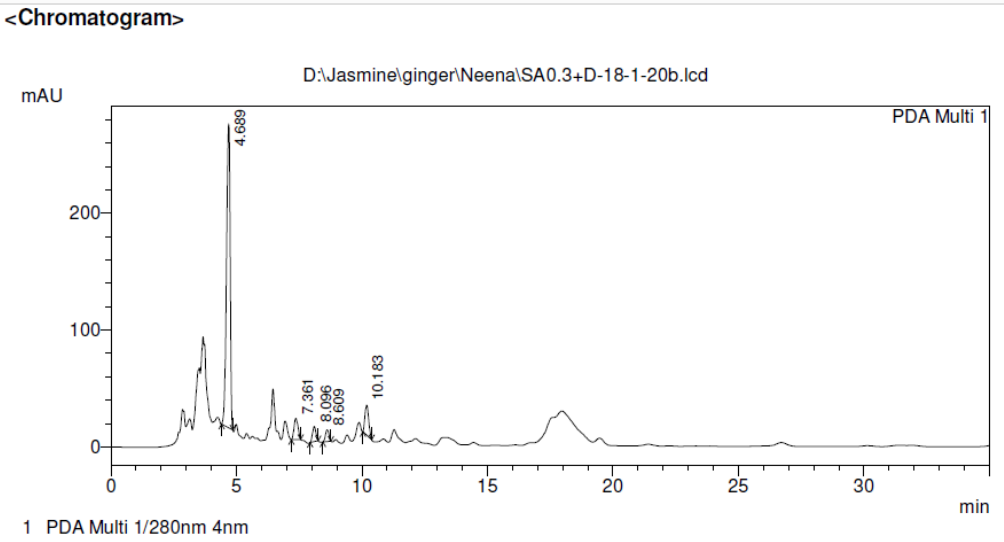

Figure 7.13 Chromatogram for *Zingiber officinale* cv-Varada under the combination of SA 0.001M+D

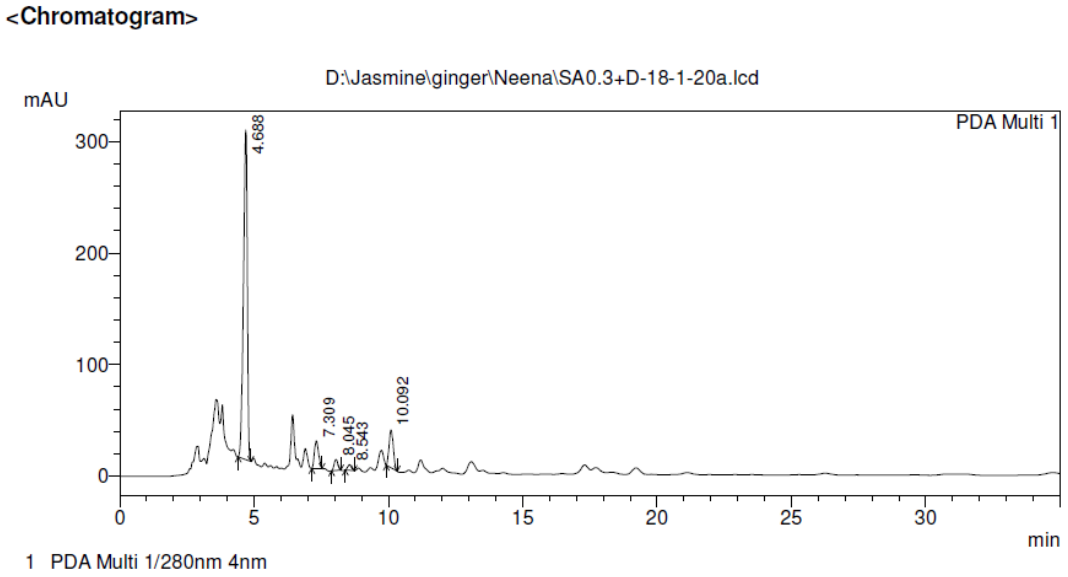

Figure 7.14 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of SA 0.001M+D

Even though the concentration was found to be varying but the trend observed was the same in both *Zingiber officinale* cv-Mahima and *Zingiber officinale* cv-Varada. Percentage of gingerol was found to be in the order DMSO> SA 0.01 M+D> SA 0.001

M+D> SA 0.01 M> SA 0.001 M > Drought. This is the first report on *Z. officinale* cultivars that increasing concentration of Salicylic Acid has a positive impact on the production of gingerol content as well as in combination with drought, aerial spraying of SA has compensated to some degree for the negative impacts of reduced irrigation on production of this important secondary metabolite Gingerol.

When coming in to the foliar application of Zinc Sulphate increasing concentration of Zinc Sulphate had a negative impact on gingerol content. $ZnSO_4$ $7H_2O$ at 0.001 M showed a greater quantity of gingerol than that of $ZnSO_4$ $7H_2O$ at 0.01 M concentration. In the case of *Zingiber officinale* cv-Mahima $ZnSO_4$ $7H_2O$ at 0.001 M has a percentage of 1.59 of gingerol at a retention time of 4.707min. and 0.01M has 1.28% of gingerol (R.T.4.692min.) **(Fig 7.15, Fig 7.16).** In the case of *Zingiber officinale* cv-Varada it is 1.44% for $ZnSO_4$ $7H_2O$ at 0.001Mconcentration and for $ZnSO_4$ $7H_2O$ at 0.001M it is 1.12% (R. T. 4.707 min. and 4.695 min. respectively) **(Fig 7.17, Fig 7.18).**

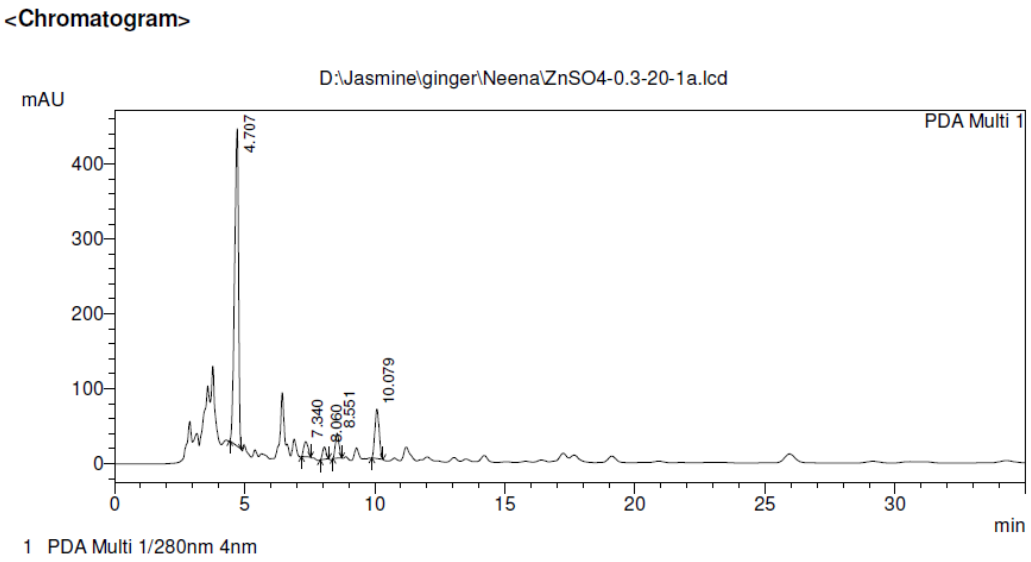

Figure 7.15 Chromatogram for *Zingiber officinale* cv-Varada under the foliar spray of $ZnSO_4$ $7.H_2O$ 0.01M

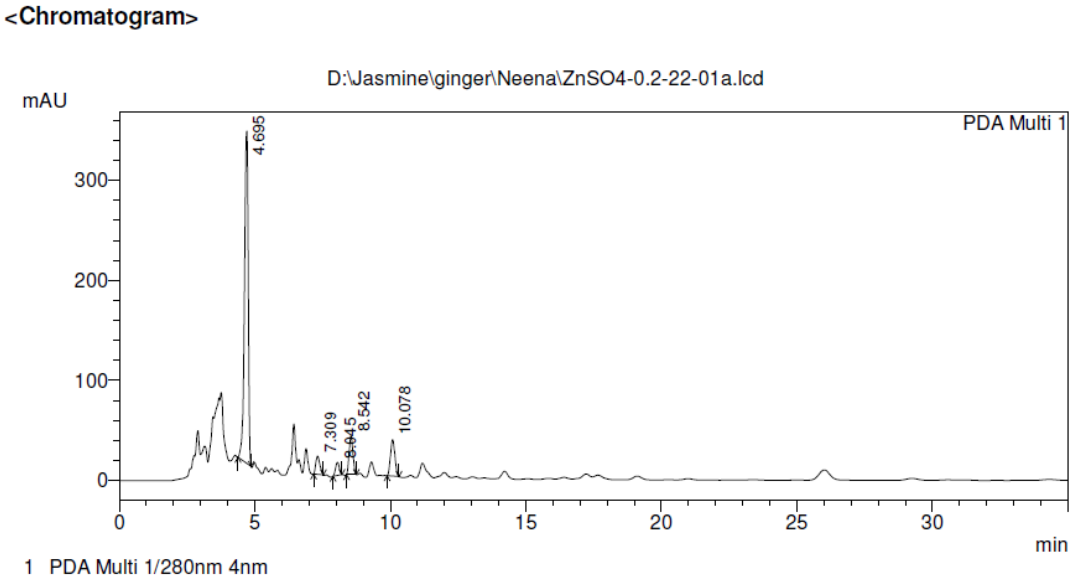

Figure 7.16 Chromatogram for *Zingiber officinale* cv-Mahima under the foliar spray of $ZnSO_4$ 7.H_2O 0.01M

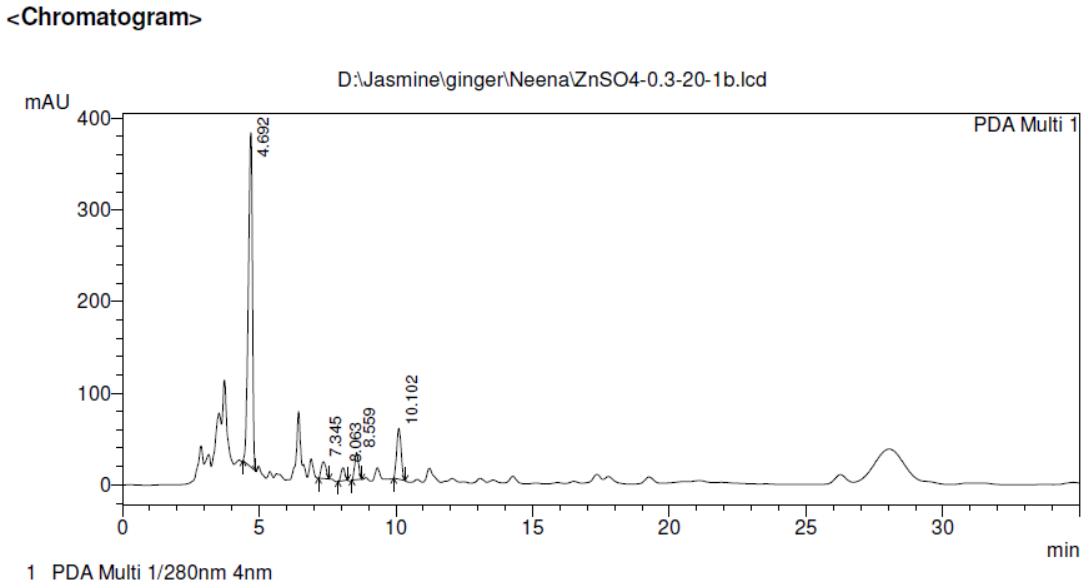

Figure 7.17 Chromatogram for *Zingiber officinale* cv-Varada under the foliar spray of $ZnSO_4$ 7.H_2O 0.001M

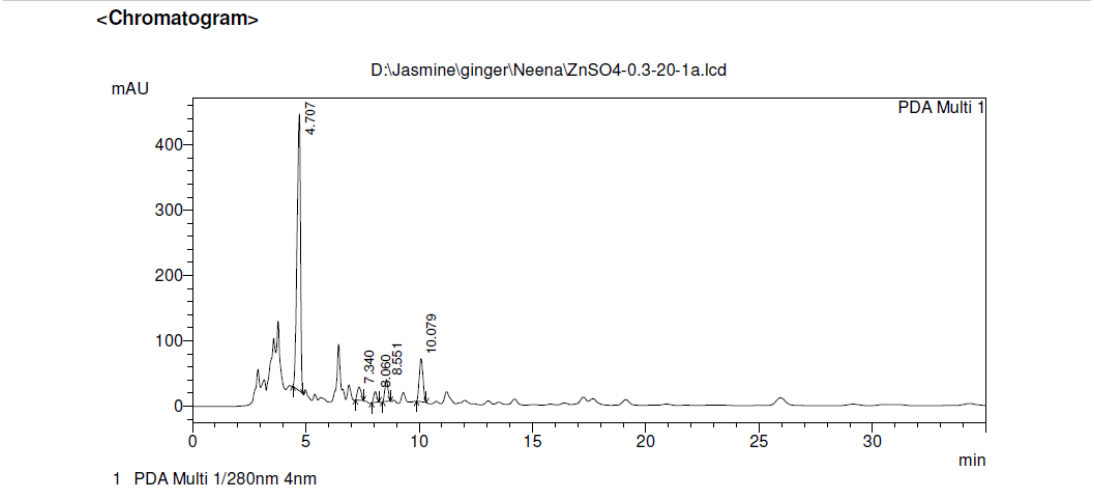

Figure 7.18 Chromatogram for *Zingiber officinale* cv-Mahima under the foliar spray of $ZnSO_4$ 7.H_2O 0.001M

In combination with drought they showed a negative cross relation between the foliar spray and drought. In the case of *Zingiber officinale* cv-Mahima it has reduced from 1.59% to 1.32% (R.T.4.716 min.) **(Fig 7.19)** in the case of $ZnSO_4$ 7.H_2O 0.001M+D, and 1.28% to 1.01% in the case of $ZnSO_4$ 7.H_2O 0.01M+D (R.T. 4.703 min) **(Fig 7.21)**. In the case of variety *Zingiber officinale* cv-Varada it has reduced from 1.44% to 1.20% for $ZnSO_4$ 7.H_2O 0.001M+D (R.T.4.716) **(Fig 7.20)** and 1.12% to 1.10% in $ZnSO_4$ 7.H_2O 0.01M+D (R.T.4.714) **(Fig 7.22)**.

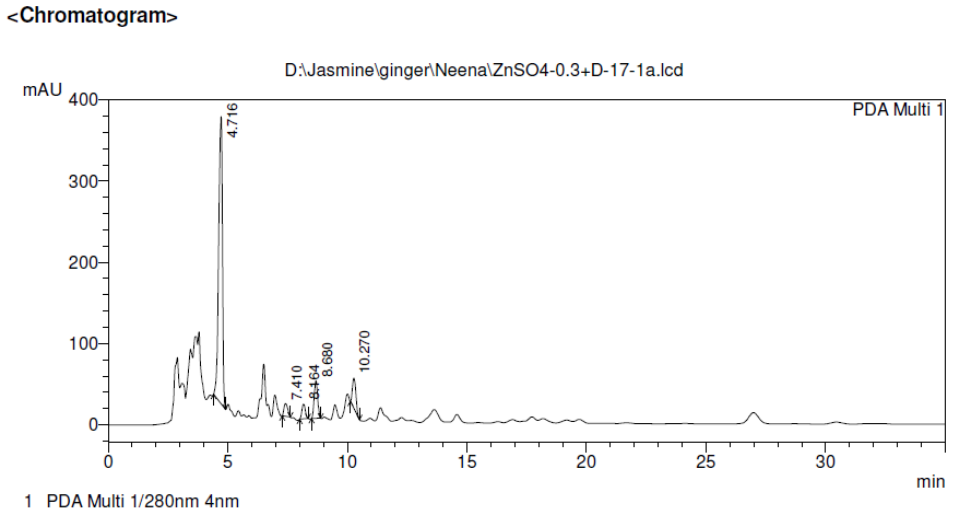

Figure 7.19 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of $ZnSO_4$ 7.H_2O 0.001M+D

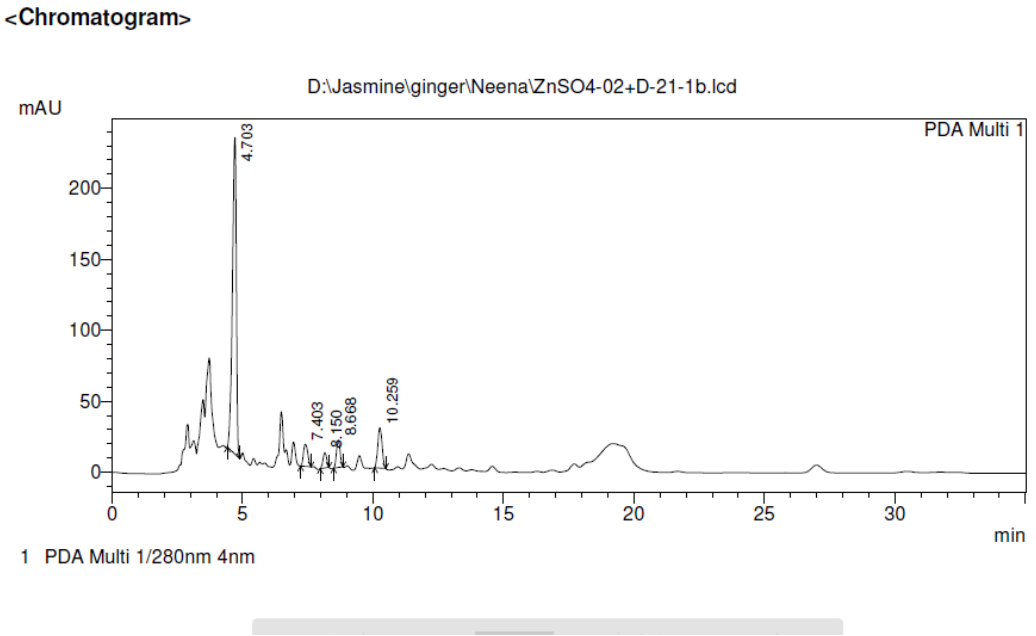

Figure 7.20 Chromatogram for *Zingiber officinale* cv-Varada under the combination of $ZnSO_4$ 7.H_2O 0.001M+D

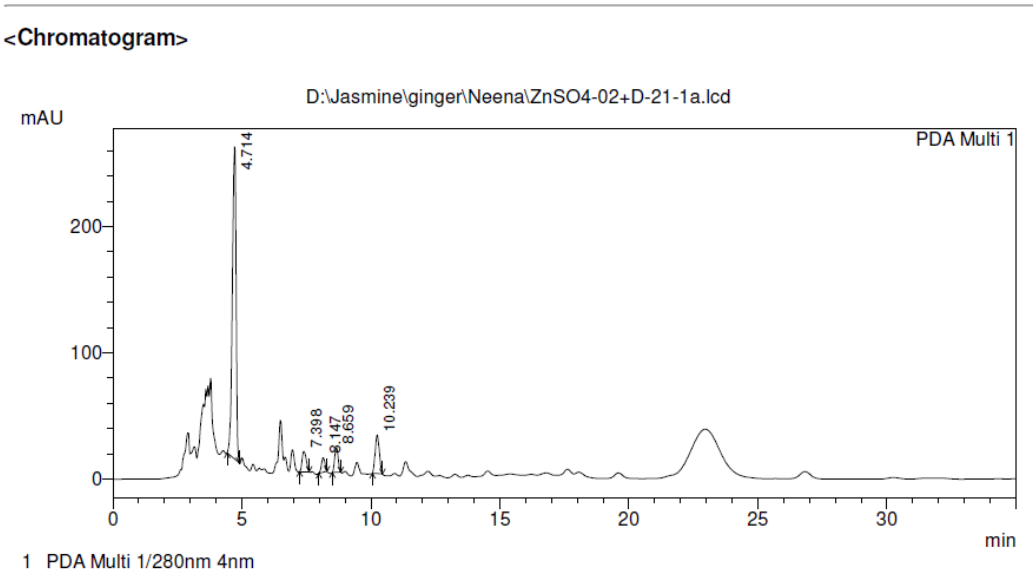

Figure 7.21 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of $ZnSO_4$ 7.H_2O 0.01M+D

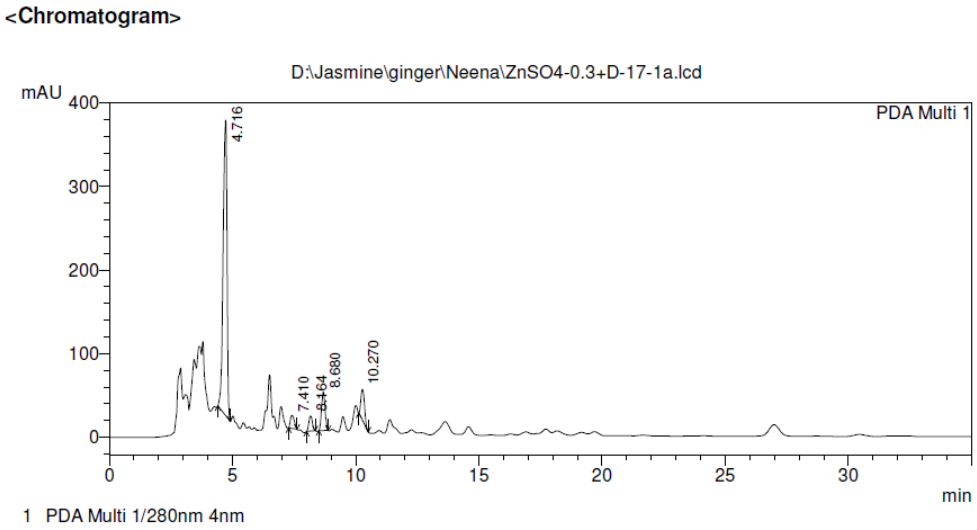

Figure 7.22 Chromatogram for *Zingiber officinale* cv-Varada under the combination of $ZnSO_4$ 7.H_2O 0.01M+D

when the combination studies are compared with the solitary applications of Zinc Sulphate Heptahydrate in the two different combinations. Still these trials show gingerol percentage higher than that of drought stress. Here the control, that is the plants sprayed with distilled water has a gingerol percentage of 1.2% for Mahima and 1.01% for *Zingiber officinale* cv-Varada. **(Fig 7.23, 7.24).**

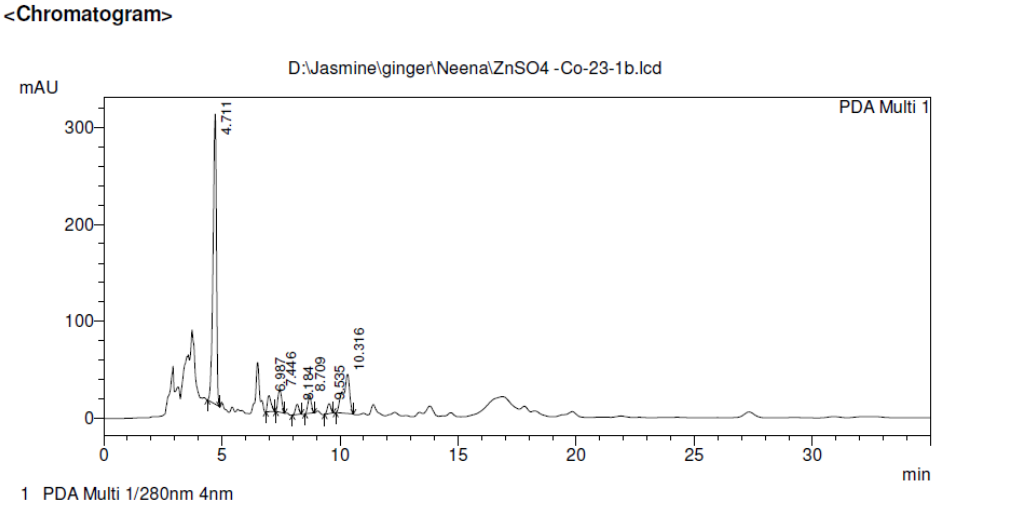

Figure 7.23 Chromatogram for *Zingiber officinale* cv-Mahima under the foliar spray of distilled water (control for $ZnSO_4$ 7 H_2O).

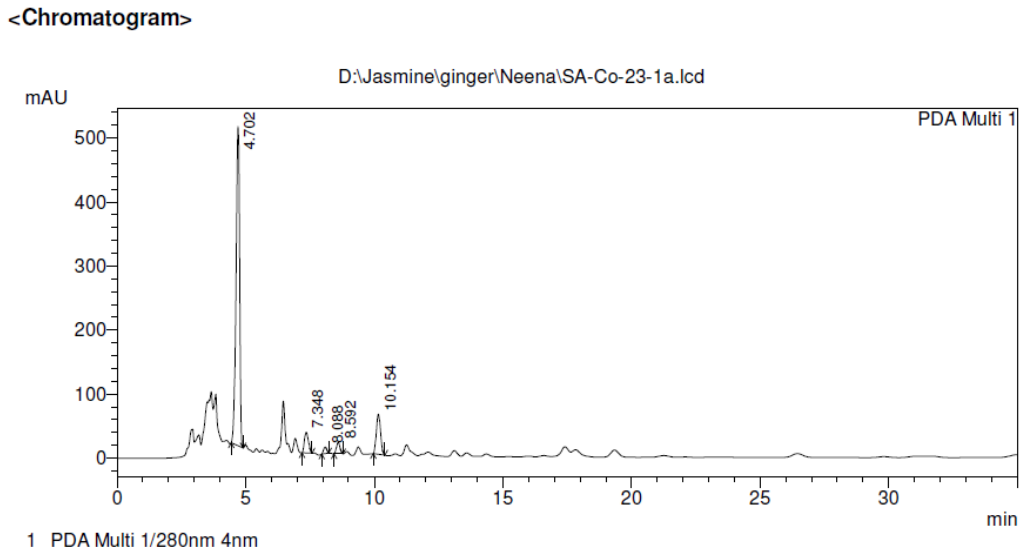

Figure 7.24 Chromatogram for *Zingiber officinale* cv-Varada under the foliar spray of distilled water (control for $ZnSO_4$ 7.H_2O).

In combination of Foliar sprays both the foliar sprays in its lower concentration showed comparatively higher percentage of gingerol (2.02% for *Zingiber officinale* cv-Mahima and 1.53% *Zingiber officinale* cv-Varada) **(Fig 7.25, Fig 7.26)**. Both foliar sprays in its higher concentration considerably reduced the percentage quantity of gingerol in it. For *Zingiber officinale* cv-Varada it is 0.95% and for *Zingiber officinale* cv-Mahima it is 1.1% **(Fig 7.27, Fig 7.28)**. Salicylic acid in lower concentration with Zinc Sulphate in higher concentration has 1.9% of gingerol in *Zingiber officinale* cv-Mahima and 1.44% in *Zingiber officinale* cv-Varada **(Fig 7.29, Fig 7.30)**. Zinc Sulphate lower concentration along with Salicylic acid higher concentration have a gingerol percentage of 1.25% for *Zingiber officinale* cv- Mahima and 1.07% for V *Zingiber officinale* cv-Varada **(Fig 7.31 Fig 7.32)**.

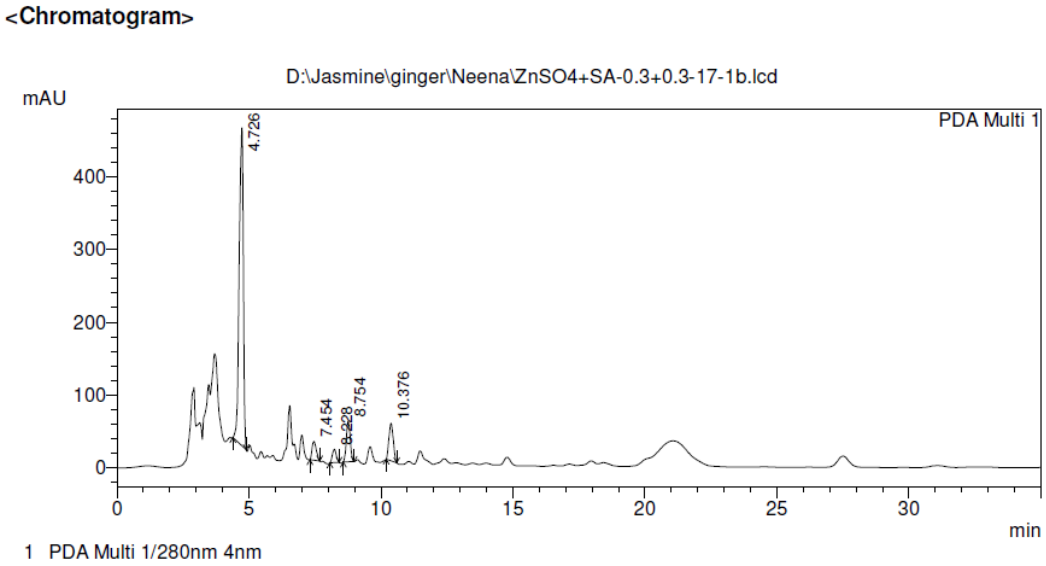

Figure 7.25 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of foliar sprays ($ZnSO_4$ 7 H_2o+ SA, 0.001M each).

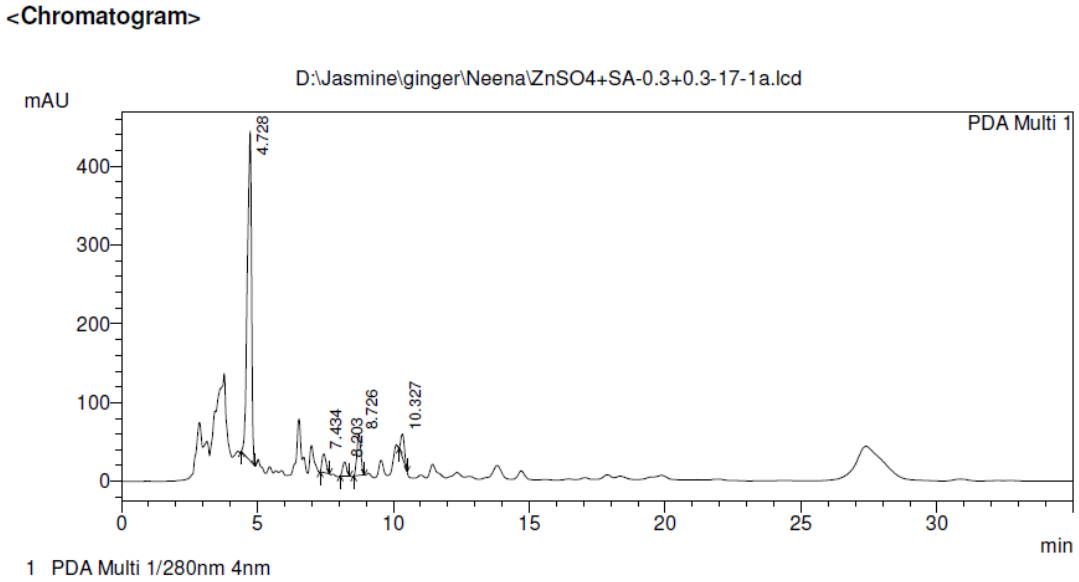

Figure 7.26 Chromatogram for *Zingiber officinale* cv-Varada under the combination of foliar sprays ($ZnSO_4$ 7 H_2O+ SA, 0.001M each).

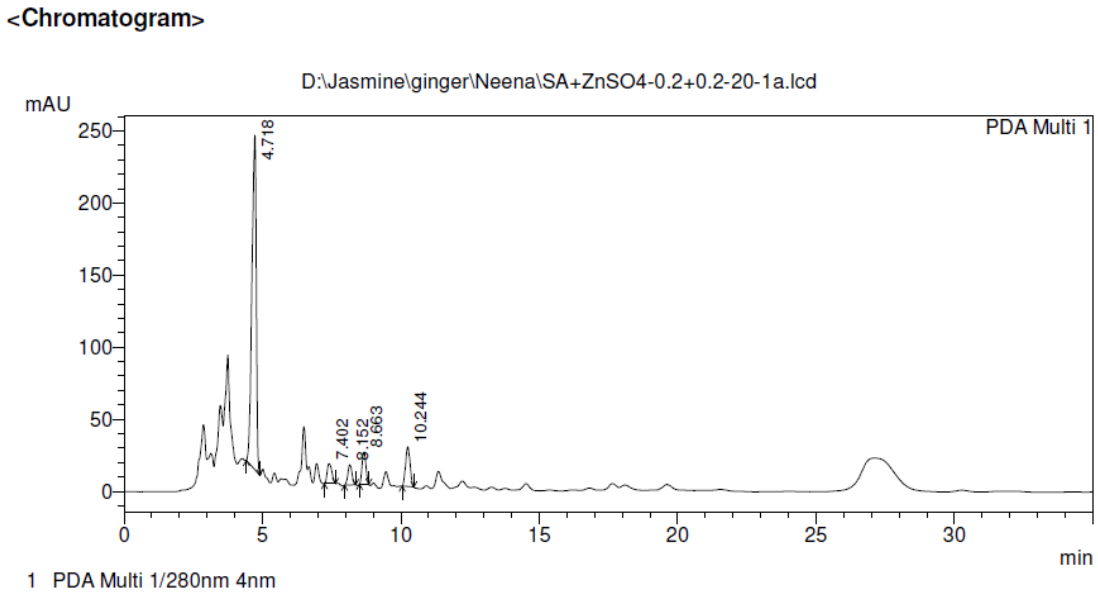

Figure 7.27 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of foliar sprays ($ZnSO_4$ 7 H_2O+SA, 0.01M each).

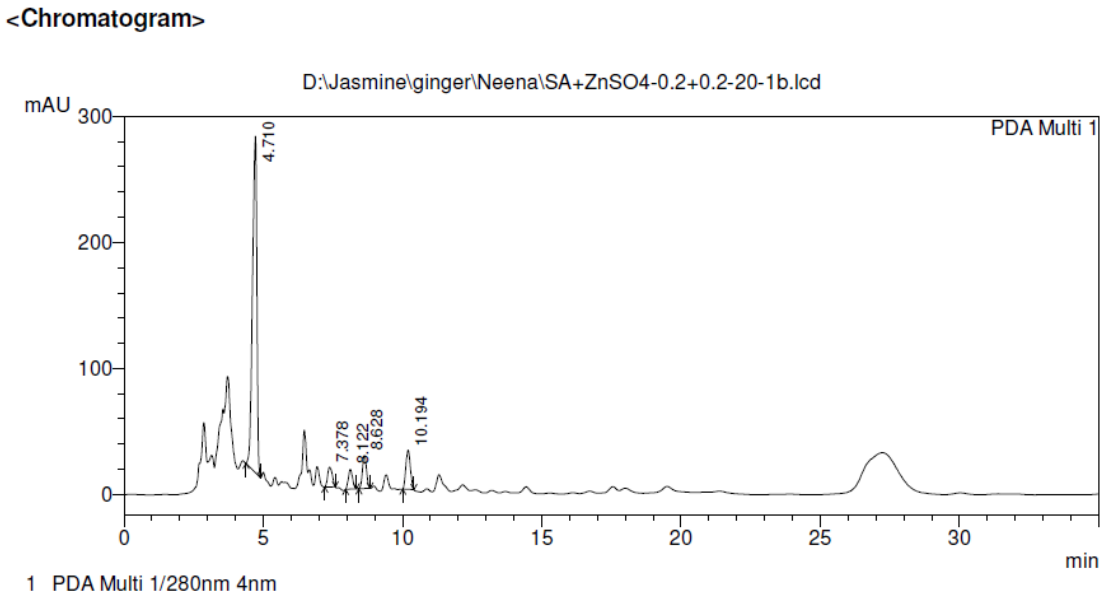

Figure 7.28 Chromatogram for *Zingiber officinale* cv-Varada under the combination of foliar sprays ($ZnSO_4$ 7 H_2O+SA, 0.01M each).

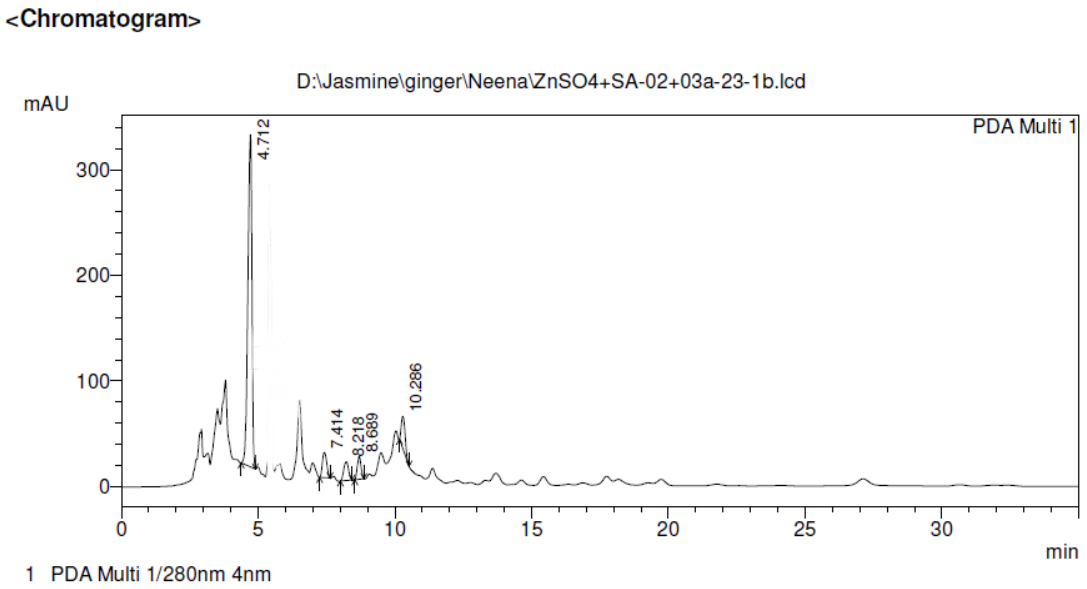

Figure 7.29 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of foliar sprays ($ZnSO_4$ 7 H_2O 0.01M+SA 0.001M).

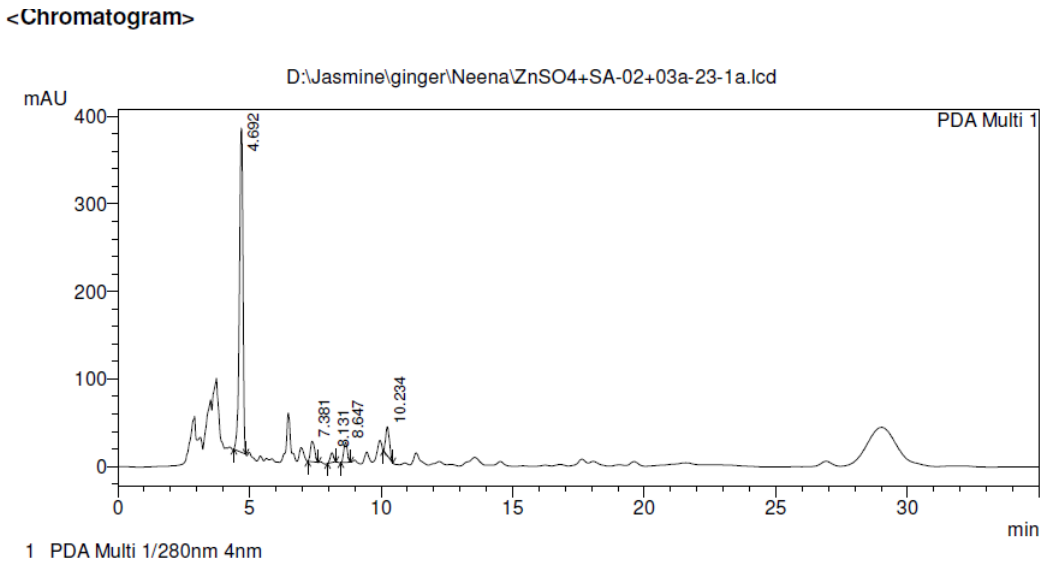

Figure 7.30 Chromatogram for *Zingiber officinale* cv-Varada under the combination of foliar sprays ($ZnSO_4$ 7 H_2O 0.01M+SA 0.001M).

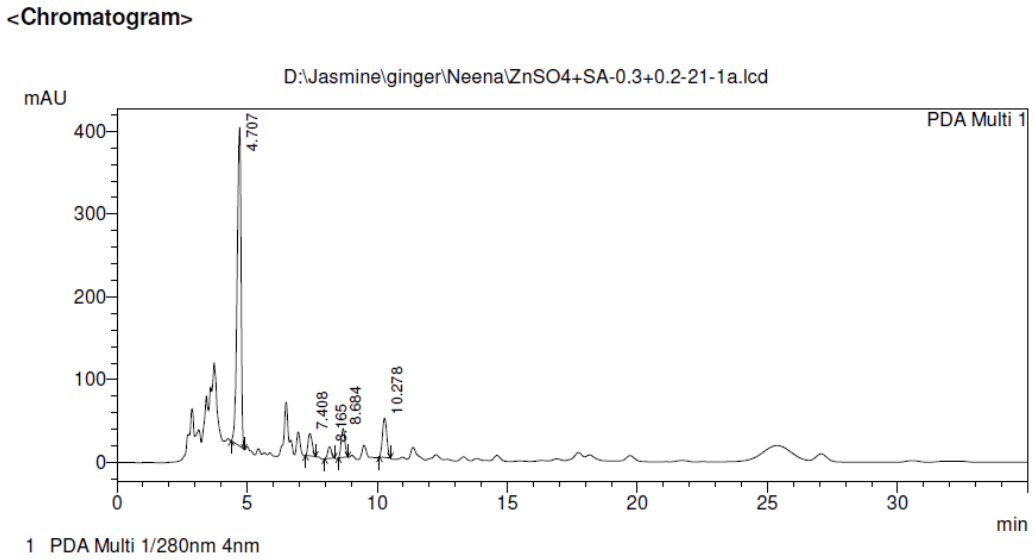

Figure 7.31 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of foliar sprays ($ZnSO_4$ 7 H_2O 0.001M+SA 0.01M).

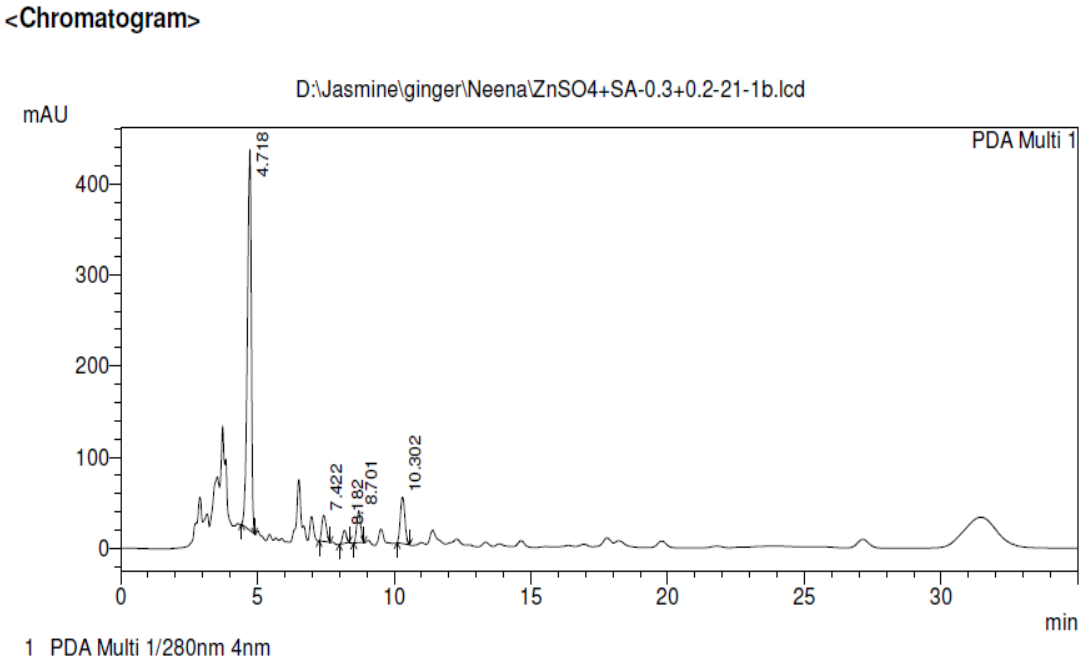

Figure 7.32 Chromatogram for *Zingiber officinale* cv-Mahima under the combination of foliar sprays ($ZnSO_4$ 7 H_2O 0.001M+SA 0.01M).

Table 7.2 Analysis data of chromatogram produced for *Zingiber officinale* cv-Mahima.

Sl.No.	Trial	R.T. (min.)	Percentage of Gingerol (%)
1	Control	4.711	1.2
2	DMSO	4.693	2.45
3	SA 0.01 M	4.719	1.8
4	SA 0.001M	4.719	1.2
5	SA 0.01M +D	4.70	1.99
6	SA 0.001M+D	4.68	1.46
7	$ZnSO_4$ 0.01M	4.707	1.28
8	$ZnSO_4$ 0.001M	4.695	1.59
9	$ZnSO_4$ 0.01M+D	4.703	1.01
10	$ZnSO_4$ 0.001 M+D	4.716	1.32
11	$ZnSO_4$ 0.01M+ SA 0.01M	4.718	1.1
12	$ZnSO_4$ 0.001M+ SA 0.001M	4.726	2.02
13	$ZnSO_4$ 0.01M+ SA 0.001M	4.712	1.9
14	$ZnSO_4$ 0.001M+ SA 0.0.1M	4.718	1.25
15	Drought	4.682	0.98

Table 7.3 Analysis data of chromatogram produced for *Zingiber officinale* cv-Varada

Sl.No.	Trial	R.T.(min.)	Percentage of Gingerol (%)
1	Control	4.702	1.01
2	DMSO	4.702	1.56
3	SA 0.01 M	4.722	1.21

4	SA 0.001M	4.722	0.89
5	SA 0.01M +D	4.697	1.27
6	SA 0.001M+D	4.698	1.06
7	$ZnSO_4$ 0.01M	4.707	1.12
8	$ZnSO_4$ 0.001M	4.692	1.44
9	$ZnSO_4$ 0.01M+D	4.716	1.10
10	$ZnSO_4$ 0.001 M+D	4.714	1.20
11	$ZnSO_4$ 0.01M+ SA 0.01M	4.710	0.95
12	$ZnSO_4$ 0.001M+ SA 0.001M	4.728	1.53
13	$ZnSO_4$ 0.01M+ SA 0.001M	4.692	1.44
14	$ZnSO_4$ 0.001M+ SA 0.0.1M	4.718	1.07
15	Drought	4.706	0.9

7.2 Comparative analysis of concentration of 6-gingerol in *Z. officinale* cv- Mahima and *Z. officinale* cv-Varada

A bar diagram is prepared to compare the concentration of 6-gingerol in *Z. officinale* cv-Varada and *Z. officinale* cv-Mahima.

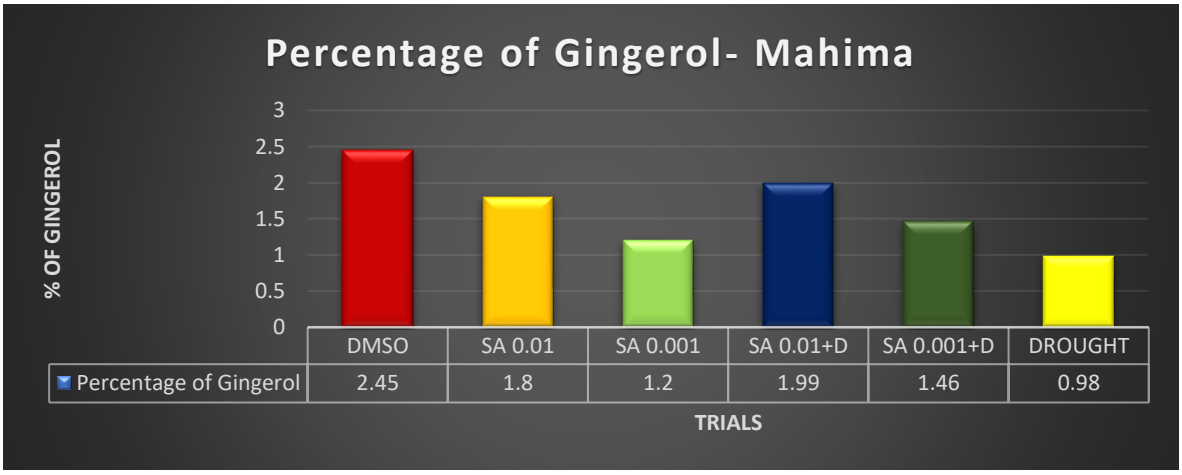

Figure 7.33 Comparative analysis of percentage of 6-gingerol in *Z. Officinale* cv-Mahima (SA trials)

From the figure **(fig 7.33)** it is clear that increasing concentration of Salicylic acid positively regulated the production of 6-gingerol in it. The control for SA, i.e., plants sprayed with 0.02% of DMSO the highest percentage of 6-gingerol in it. Also, HPLC analysis of *Z. officianle* cv-Mahima under the SA trials indicate that in combination with drought and SA there occurred a cross talk between the stress signals which resulted in the reduction of concentration of 6-gingerol in it.

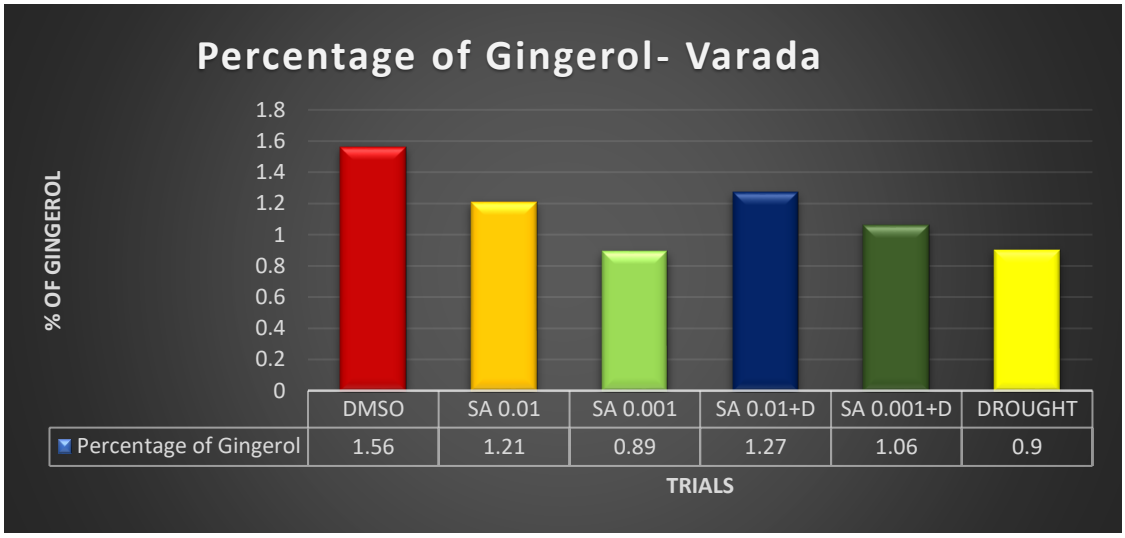

Figure 7.34 Comparative analysis of percentage of 6-gingerol in *Z. officinale* cv-Varada (SA trials)

A same trend has been noticed in the case of *Z. officinale* cv-Varada like *Z. officinale* cv-Mahima **(Fig.7.44)**. SA had a positive effect on the concentration of 6-gingerol in int. But the concentration of 6-gingerol in the cultivar variety *Z. officinale* cv- Varada was comparatively lesser than that of the cultivar variety *Z. officinale* cv- Mahima.

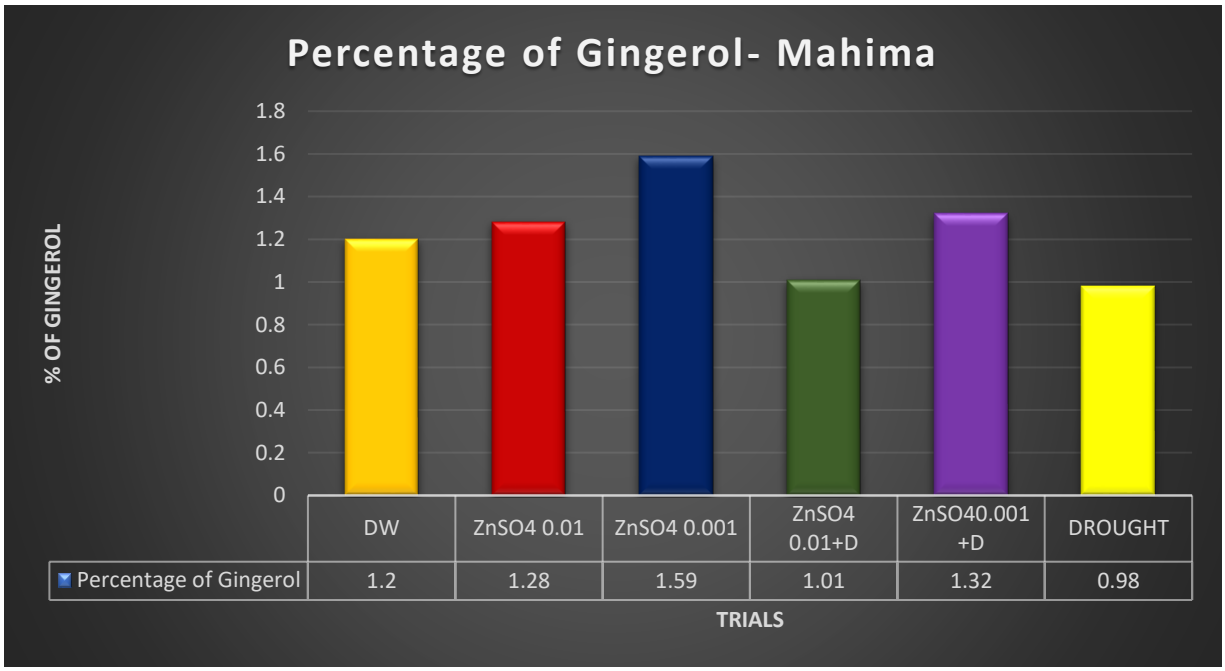

Figure 7.35 Comparative analysis of percentage of 6-gingerol in *Z. officinale* cv-Mahima (Zinc Sulphate Heptahydrate trials)

Increasing concentration of $ZnSO_4$ 7.H_2O reduced the percentage concentration of 6-gingerol in *Z. officinale* cv-Mahima **(Fig 7.35)**. At the same time in combination with drought considerably decreased the concentration of 6-gingerol in it. A same trend was noticed in the case of *Z. officinale* cv-Varada **(Fig 7.36).**

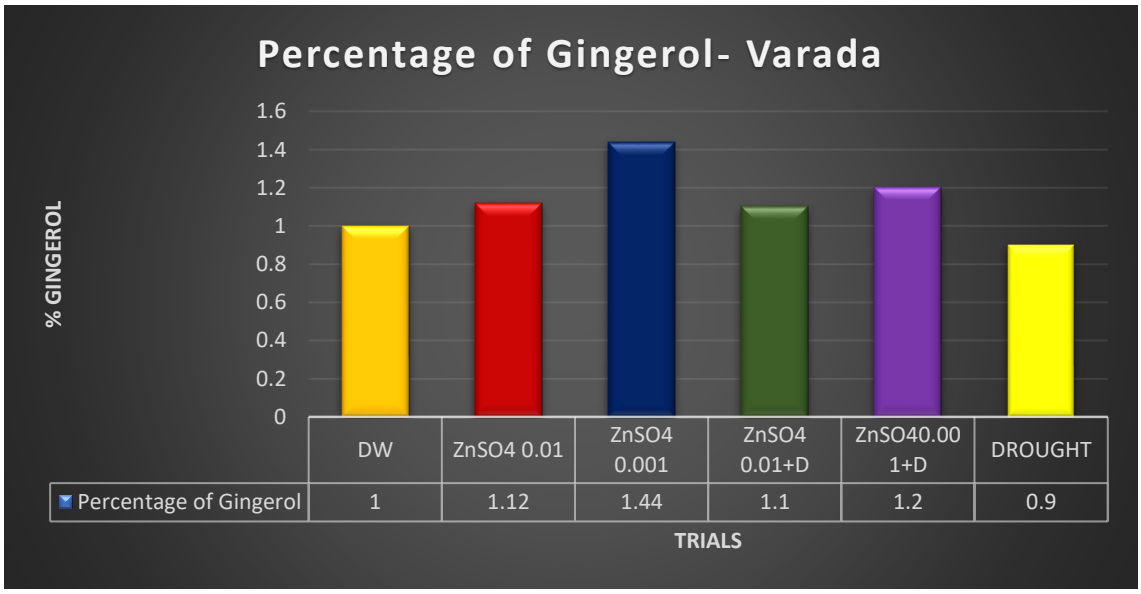

Figure 7.36 Comparative analysis of percentage of 6-gingerol in *Z. officinale* cv-Varada (Zinc Sulphate Heptahydrate trials)

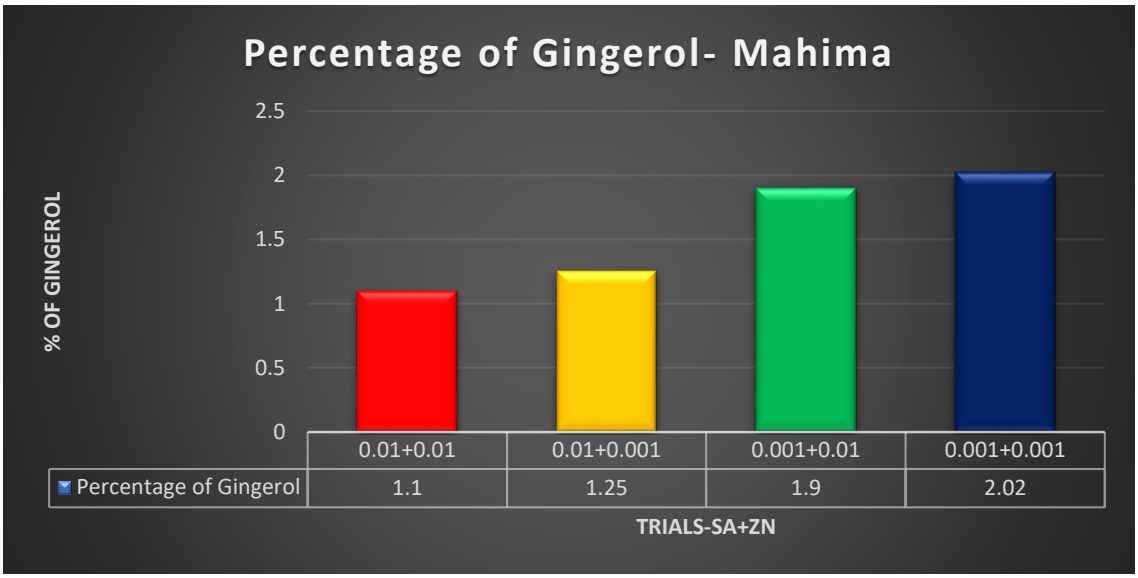

Figure 7.37 Comparative analysis of percentage of 6-gingerol in *Z. officinale* cv-Mahima (combination trials)

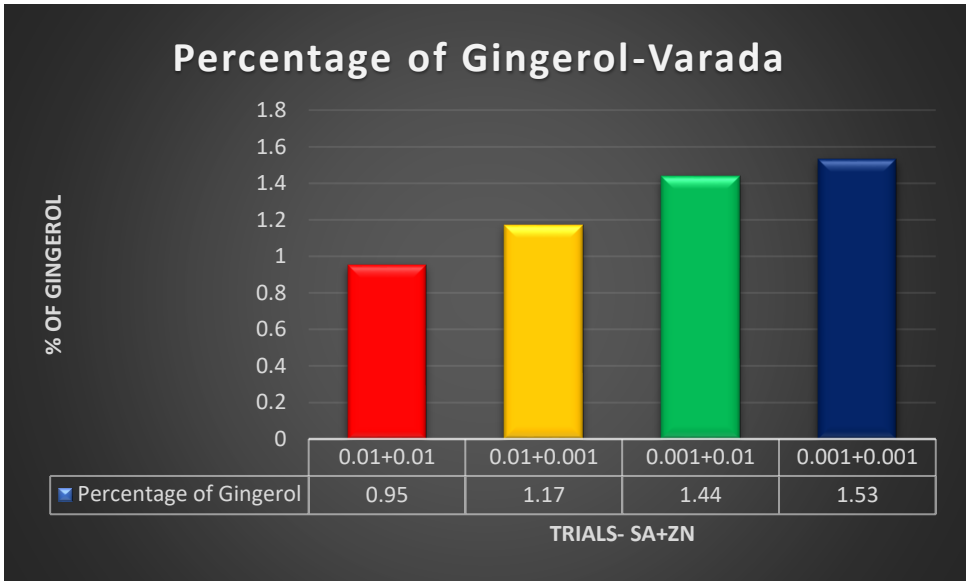

Figure 7.38 Comparative analysis of percentage of 6-gingerol in *Z. officinale* cv-Varada (combination trials)

In the foliar spray combination analysis, both the foliar sprays in its lower concentration (0.001M each) had a greater percentage of 6-gingerol. Both the foliar sprays in higher concentration considerably reduced the percentage quantity of 6-gingerol in both the varieties. At the same time $ZnSO_4$ 7. H_2O in higher concentration (0.01M) and Salicylic Acid in lower concentration (0.001M) had a higher amount of 6-gingerol, when comparing with $ZnSO_4$ 7. H_2O+ SA (0.001M+0.01M) **(Fig 7.37& 7.38)**. In all trials, the HPLC

analysis proved that the cultivar Mahima has higher amount of 6-gingerol than that of *Z. officinale* cv-Varada, both good cultivar varieties released by IISR. There are so many earlier studies which support the same.

Superiority of *Z. officinale* cv-Mahima in terms of gingerol content was reported by reported by Vedashree & Madhava (2023). Ginger has a wide range of chemical components that depend on the region of origin, the harvesting method, and the storage circumstances. It has been claimed that several HPLC techniques can be used to quantitatively analyse ginger components (Promdam & Panichayupakaranant, 2022).

The determination of bioactive components via comparisons to standards is a common use of HPLC methods. In the HPLC analysis, the sesquiterpene hydrocarbons sesquiphellandrene, zingiberene, farnesene and bisabolene, as well as gingerol, shogaol, and curcumene were identified as seven major compounds present in the crude extracts of *Z. officinale* (Hasan *et al.*, 2012). HPTLC and HPLC were used to characterise the Jamaican ginger (*Zingiber officinale* Roscoe) varieties. The ginger cultivars' HPTLC fingerprints revealed chemical homogeneity with only minor qualitative variations in the intensities of the gingerol and shogaol zones. Significant variations in overall pungency were found among the cultivars when these chemicals were quantified using High-Performance Liquid Chromatography (Salmon *et al.*, 2012). The dried powdered ginger of the various cultivars of Jamaican ginger was subjected to HPLC analysis, which revealed that 6-gingerol was four times more concentrated than the other pungent components in every variety. This is consistent with literature-based reports on ginger analysis. Hawaiian ginger concentrations ranged from 0.527±0.002% by mass to 0.811±0.011% for Yellow Tambric (Salmon *et al*., 2012). The quantity of gingerol in *Z. officinale* under different drying conditions was studied by An *et al.* (2016). They found that Fresh ginger contained 5.91 mg/g of 6-gingerol; however, following drying, especially with microwave drying, the amount of 6-gingerol drastically fell to only 2.12 mg/g. The highest amount was found in the FD samples (freeze drying) (3.54 mg/g), followed by the IR (Infrared Drying), IM&CD (intermittent microwave & convective drying, and AD (air drying) samples (3.44, 3.21, and 2.50 mg/g, respectively). It might be assumed that the 6-gingerol's degradation and conversion would be facilitated by the high temperature and lengthy drying period. With the exception of the FD procedure, the amount of 8-gingerol and 10-gingerol likewise showed a trend towards

decreasing after drying. The gingerol content of rhizome under biotic stress was elucidated by Sunil Kumar, (2016). Root Knot nematode infection considerably decreased the quantity of gingerol in it. He found that the percentage reduction in gingerol content in rhizome samples in plants inoculated with 1000 J2 was 19.95% over 31.01% for un inoculated plants. Choudhari & Kareppa, (2013) also found that the gingerol content considerably decreased under *Fusarium spp* infection in ginger. These results are in contrary with the present study, in that, stress altered the production of the well-known secondary metabolite gingerol, and under abiotic stress condition the gingerol production has been found to be increasing in comparison with untreated plants.

Brief Summary

Gingerol which is considered as one of the elixir compounds was identified through HPLC analysis. A marker compound for 6-gingerol was used to identify and quantify this compound in the present study. The study also emphasized on how the concentration of this compound changed with respect different stress treatments. It was found that some foliar sprays remarkably increased the concentration of 6- gingerol production. 0.2% DMSO foliar sprays which was considered as the control for SA has considerably increased the amount of 6-Gingerol in it. Which was followed by SA 0.01M foliar spray and then by other trials. The effect of drought on the production of 6-gingerol was compensated by its combination with foliar sprays. Also, a varietal level comparison was also done to evaluate the effect of stress signals on two stress varieties under study.

After completing the preliminary phytochemical analysis, Quantitative estimation of phenolics, flavonoids, antioxidant analysis and HPLC analysis of gingerol, the trials were narrow down on the basis of the best results and the superior trials were subjected to Chlorophyll estimation, PAL analysis and chromatographic studies by GCMS. Ten trials were selected from cultivars *Z. officinale* cv- Varada and *Z. officinale* cv- Mahima (5 trials from each, including control plants). And the trials include Varada/Mahima-SA 0.01M, Varada/Mahima- $ZnSO_4$ 7.H_2O 0.01M, Varada/Mahima- SA 0.01M+ $ZnSO_4$ 7.H_2O 0.01M, Varada/ Mahima- Dw, and Varada/ Mahima- DMSO.

8.1 Chlorophyll estimation

The leaves of plants under various solitary as well as combined stress treatments along with its control has been monitored for its photosynthetic pigments, namely chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. All the experiments were done in triplicate and One-way ANOVA was performed for all the trials and Homogeneity by Duncan test using SPSS software, the results are represented as Mean± Standard Error (S.E).

Table 8.1 Analysis of chlorophyll-a content in trials under study.

Trial	***Z. officinale* cv- Varada Chlorophyll a µg/g (Mean± S.E)**	***Z. officinale* cv- Mahima Chlorophyll a µg/g (Mean± S.E)**
Control	823±59	1097±65
DMSO	650±165	803±69
SA 0.01 M	790±168	952±75
$ZnSO_4$ 7.H_2O 0.01M	703±95	876±124
$ZnSO_4$ 0.01M + SA 0.01M	569±98	812±105

Chl-a was found to be higher in the control plants rather than all other trials. These are plants which are sprayed with Distilled Water. All the other trials, including the control for Salicylic Acid showed a decrease in the chlorophyll a. It is very clear from this analysis that all the stress treatments have interfered with the photosynthetic activity. The highest chlorophyll a was found in control plants and is 1097±65 µg/g for Mahima and 823±59 µg/g for Varada. Followed by plants which are sprayed with SA 0.01M (952±75 µg/g for Mahima and 790±168 µg/g for Varada). For Mahima lowest chlorophyll a was found in plants sprayed with DMSO 803±69 µg/g and for Varada it was found in the trials which are sprayed with the combination of both foliar sprays 569±98 µg/g.

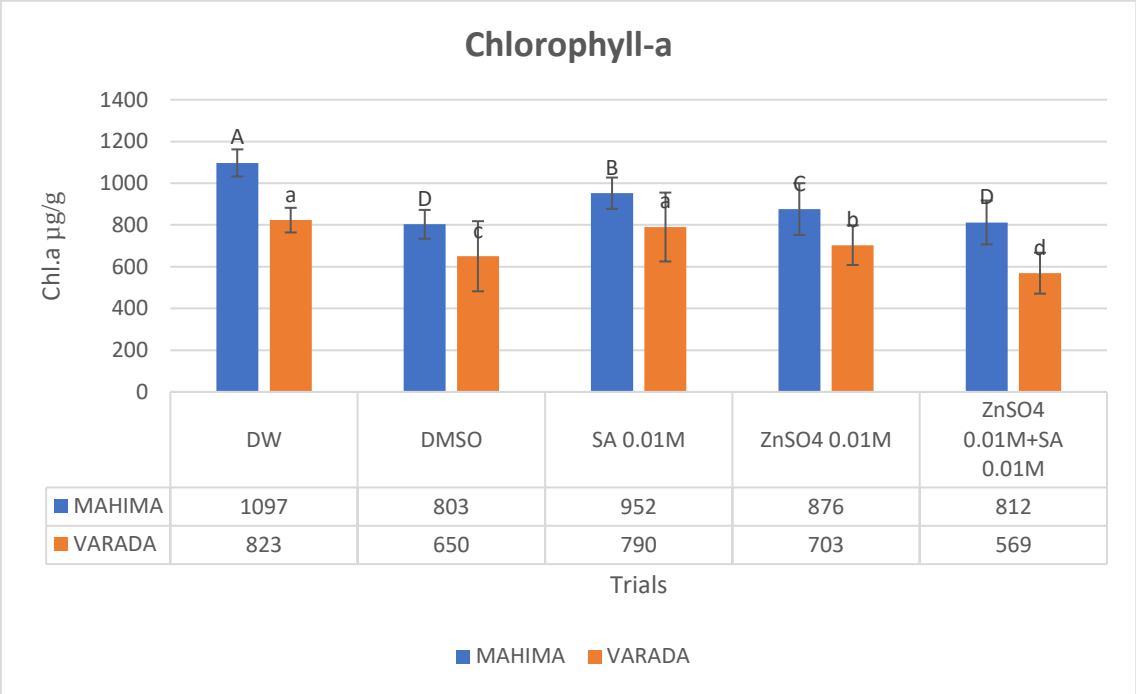

Figure 8.1 Chlorophyll a content in trials under study

Table 8.2 Analysis of Chlorophyll b content in trials under study.

Trial	***Z. officinale* cv- Varada** **Chlorophyll b** **µg/g (Mean± S.E)**	***Z. officinale* cv- Mahima** **Chlorophyll b** **µg/g (Mean± S.E)**
Control	798±59	896±88
DMSO	576±89	606±69
SA 0.01 M	623±129	705±89
$ZnSO_4$ 7.H_2O 0.01M	616±107	689±82
$ZnSO_4$ 0.01M + SA 0.01M	598±95	650±75

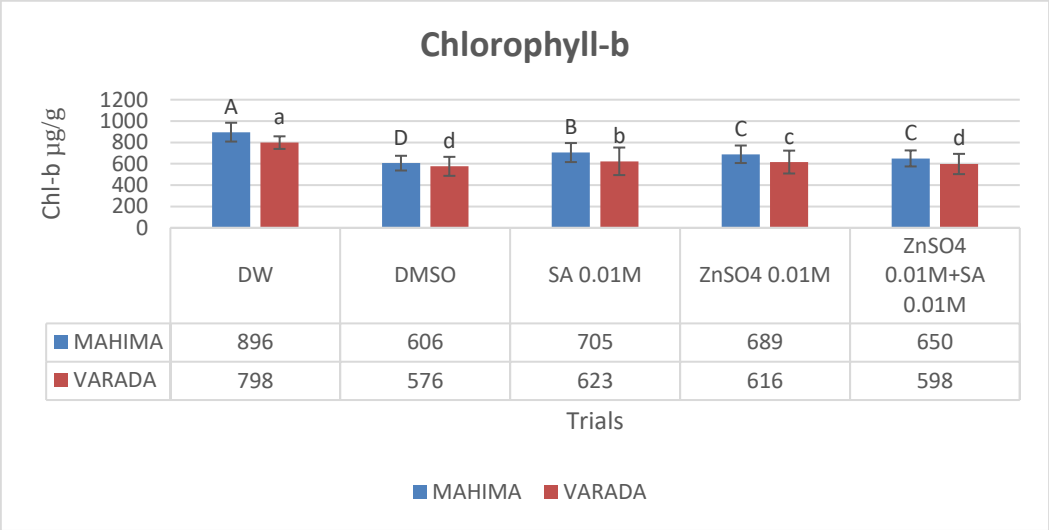

Figure 8.2 Chlorophyll b content in trials under study

A same trend was noted in the case of Chlorophyll-b also. Chlorophyll b also found to be higher in the control plants (896±88 µg/g for cv-Mahima and 798±59 µg/g for Varada) and was found decreasing in stress trials SA 0.01≥ $ZnSO_4$ $7H_2O$ 0.01M ≥ $ZnSO_4$ 0.01M + SA0.01M 0.01M≥DMSO. Chlorophyll- b content in plants which were sprayed with SA 0.01M was 623±129 µg/g for Varada and 705±89 µg/g for Mahima. For plants under the stress $ZnSO_4$ $7.H_2O$ 0.01M is 623±129 µg/g for the cultivar variety Varada and 705±89 µg/g Mahima. $ZnSO_4$ 0.01M + SA0.01M 0.01M had a chlorophyll-b content 598±95 µg/g for Varada and 650±75 µg/g for Mahima. Least quantity of chlorophyll-b was observed in plants sprayed with DMSO; 576±89 µg/g for Varada and 606± 69 µg/g for Mahima.

Table 8.3 Analysis of total chlorophyll content in trials under study

Trial	***Z. officinale* cv- Varada Total chlorophyll µg/g (Mean± S.E)**	***Z. officinale* cv- Mahima Total chlorophyll µg/g (Mean± S.E)**
Control	1300±85	1565±77
DMSO	676±89	882±69
SA 0.01 M	901±102	1103±89
$ZnSO_4$ $7.H_2O$ 0.01M	816±98	983±92

$ZnSO_4$ 0.01M + SA 0.01M	798±56	851±52

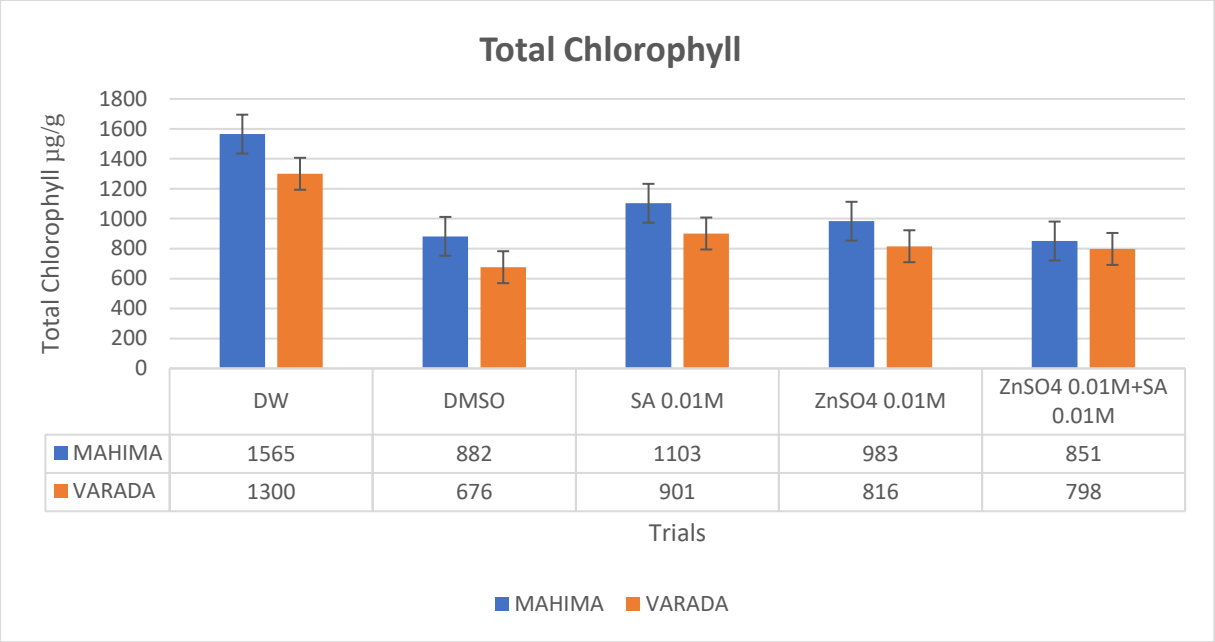

Figure 8.3 Total chlorophyll content in trials under study

In the case of total chlorophyll content, control plants found to have more total chlorophyll than that of all the trials. Control plants had a highest concentration of Total chlorophyll 1300±85 µg/g for Varada and1565±77 µg/g for Mahima. After control plants higher amount of total chlorophyll was observed in plants sprayed with SA in 0.01M concentration 901±102 µg/g for Varada and 1103±89 µg/g for Mahima. ZnSO4 7.H2O 0.01M trial had a total chlorophyll content of 816±98 µg/g for Varada and 983±92 µg/g for Mahima. Combination of foliar sprays had a total chlorophyll content of 798±56 µg/g for Varada and 851±52 µg/g for Mahima. Plants which were sprayed with DMSO had the lowest total chlorophyll content among all the trials 676±89 µg/g for Varada 882±69 µg/g for Mahima.

Table 8.4 Analysis of Carotenoids content in trials under study

Trial	***Z. officinale* cv- Varada Carotenoids µg/g (Mean± S.E)**	***Z. officinale* cv- Mahima Carotenoids µg/g (Mean± S.E)**
Control	782±95	758±84

DMSO	878±67	880±52
SA 0.01 M	800±124	823±89
$ZnSO_4$ 7.H_2O 0.01M	862±84	815±102
$ZnSO_4$ 0.01M + SA 0.01M	762±51	789±95

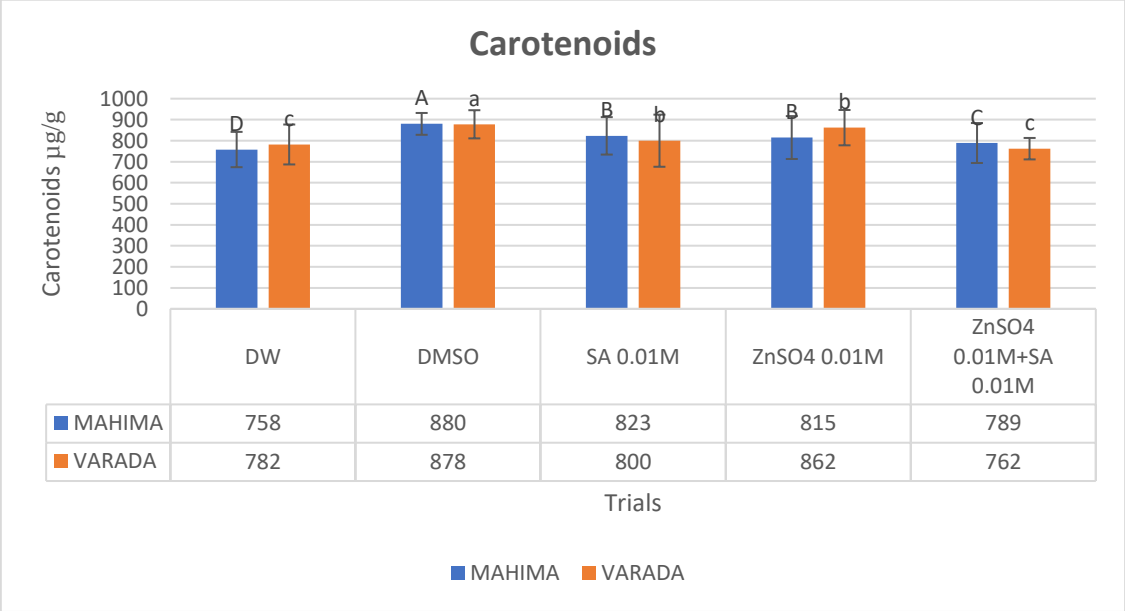

Figure 8.4 Carotenoids content in trials under study

The estimation of carotenoid content under different trails were done. Comparing Carotenoids with chlorophyll content in the sample, Carotenoids was found to be less in control plants under study. And at the same time Carotenoids was found to be high in plants under the foliar spray of DMSO, which was taken as a control for salicylic acid stress 878±67 µg/g for Varada and 880±52 µg/g for Mahima, followed by SA 0.01M 823±89 µg/g for Varada and 800±124 µg/g for Mahima which is almost similar to that of plants which are sprayed with $ZnSO_4$ 7. H_2O. which is 862±84 µg/g for Varada and 815±102 µg/g for Mahima. Least carotenoid content was observed in combination analysis 762±51 µg/g for ±Varada and 789±95 µg/g for Mahima.

When comparing two varieties photosynthetic pigments were found to be higher in the cultivar variety Mahima than that of Varada. Water stress on chlorophyll content in *Olea europaea* cv. Dezful was studied by Khaleghi *et al*. 2012, they found that water stress

significantly reduced the chlorophyll content. The results of current study agree with previous studies conducted on three accessions on *Vicia faba* L. Total chlorophyll was found to be decreasing in heat stress trials when comparing with the control plants under study (Enneb *et al.,* 2020). Influence of Silicon supply on chlorophyll content in tomato under salt stress was studied by Al-aghabary *et al.* (2005), and found that the chlorophyll content found to related to salt stress.

Brief Summary

Chlorophyll estimation by Arnon's Method was performed on selected trials to monitor the effect of stress treatments in chlorophyll content. It was noticed that chlorophyll pigments except carotenoids were found to be decreasing when compared to control plants. Plants reduce the rate of photosynthesis in order to cope up with the environmental stress. This may be the reason for the observed results. In order to minimize the energy loss plants have adapted to minimize the rate of photosynthesis by reducing the concentration of chlorophyll in the leaves.

9.1 Estimation of Phenylalanine Ammonia Lyase (PAL) activity

The shikimate-phenylpropanoid-flavonoid pathway is used to produce phenolics, which have been linked to resistance and incompatibility factors (Osbourn,1996; Harborne, 1999). One of the key regulating enzymes in the phenylpropanoid pathway include Phenylalanine Ammonia-lyase (PAL; EC 4.3.1.5).

The first enzyme of the phenylpropanoid pathway, Phenylalanine Ammonia Lyase (PAL), serves as a gateway into the potential pathways that can result in the creation of gingerols. Trans-cinnamic acid and ammonium ion are produced when this enzyme catalyses the non-oxidative deamination of L-Phenylalanine. In the present study. PAL converts L-Phenylalanine to trans-Cinnamic Acid (Koukol & Conn, 1961). Subsequent metabolism results in generation of a wide variety of phenolic metabolites that include simple phenolics or salicylates, coumarins, lignins, tannins, flavonoids and anthocyanins (Jones, 1984; Morrison & Buxton, 1993; Dixon & Paiva, 1995; Dixon *et al*., 2002).

In response to numerous plant diseases, systemic induction and accumulation of plant polyphenolics have been documented (Picinelli *et al.,* 1995; Matern & Kneusel, 1988 Wallis *et al*., 2008; Petkovšek *et al.,* 2008). In addition , studies showing that phenolics are crucial markers for pathogen resistance (Witzell & Martin, 2008), significant changes in PAL activity have also been noted in plant tissues in response to a variety of physical and chemical stimuli (Jones, 1984; Ju *et al.,* 1995; Schmidt *et al.,* 2004). In the present study PAL activity was analyzed for fresh leaves and Rhizomes of selected ginger cultivars (*Z. officinale* cv- Varada and *Z. officinale* cv- Mahima).

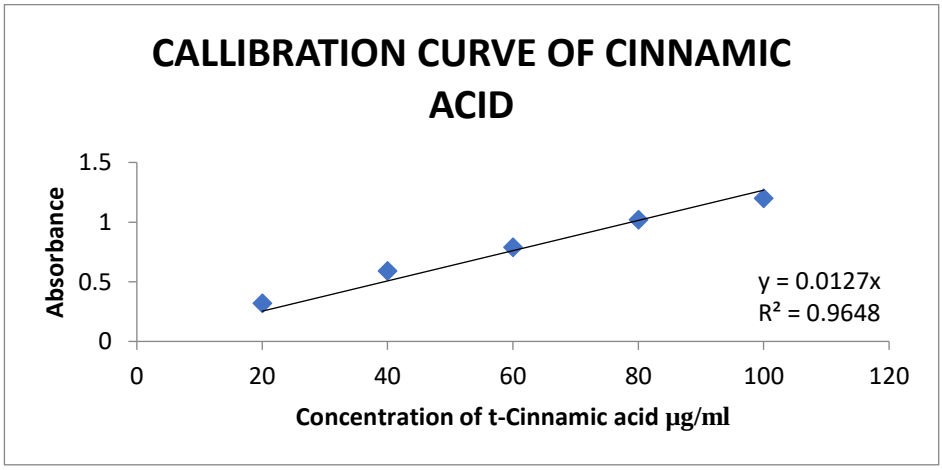

Figure 9.1 Standard calibration curve of Cinnamic Acid for Phenylalanine Ammonia Lyase

Table 9.1 PAL activity (Quantity of Cinnamic Acid produced) in ginger leaves under different stress trials (Day 1)

Trial **Day 1**	***Z. officinale* cv- Mahima** **U/mg (Mean± S.E)**	***Z. officinale* cv- Varada** **U/mg (Mean± S.E)**
Control	47.824±0.6	74.496±0.03
DMSO	35.266±0.02	64.29±0.05
SA 0.01 M	27.804±0.06	59.964±0.01
$ZnSO_4$ 7.H_2O 0.01M	25.184±0.5	56.596±0.3
$ZnSO_4$ 0.01M + SA 0.01M	24.092±0.2	50.763±0.09

Table 9.2 PAL activity (Quantity of Cinnamic Acid produced) in ginger leaves under different stress trials (Day 2)

Trial **Day 2**	**Mahima U/mg (Mean± S.E)**	**Varada U/mg (Mean± S.E)**
Control	39.21±0.04	65.05±0.05
DMSO	37.86±0.12	66.29±0.05
SA 0.01 M	31.82±0.16	62.364±0.31
$ZnSO_4$ 7.H_2O 0.01M	26.96±0.15	59.5±0.3
$ZnSO_4$ 0.01M + SA 0.01M	25.9±0.2	52.3±0.23

Table 9.3 PAL activity (Quantity of Cinnamic Acid produced) in ginger leaves under different stress trials (Day 3)

Trial **Day 3**	**Mahima U/mg (Mean± S.E)**	**Varada U/mg (Mean± S.E)**
Control	35.4±0.7	60.4±0.13
DMSO	39.66±0.33	67.29±0.65
SA 0.01 M	36.9±0.06	69.4±0.02
$ZnSO_4$ 7.H_2O 0.01M	29.1±0.25	61.5±0.13
$ZnSO_4$ 0.01M + SA 0.01M	29.01±0.2	54.3±0.09

Table 9.4 PAL activity (Quantity of Cinnamic Acid produced) in ginger leaves under different stress trials (Day 4)

Trial Day 4	**Mahima U/mg (Mean± S.E)**	**Varada U/mg (Mean± S.E)**
Control	32.4±0.5	59.4±0.13
DMSO	40.1±0.52	71.2±0.34
SA 0.01 M	41.23±0.06	73.69±0.21
$ZnSO_4$ 7.H_2O 0.01M	31.05±0.5	63.5±0.5
$ZnSO_4$ 0.01M + SA 0.01M	30.92±0.21	57.7±0.09

Table 9.5 PAL activity (Quantity of Cinnamic Acid produced) in ginger leaves under different stress trials (Day 5)

Trial Day 5	**Mahima U/mg (Mean± S.E)**	**Varada U/mg (Mean± S.E)**
Control	31.05±0.16	57.03±0.03
DMSO	44.6±0.32	74.19±0.15
SA 0.01 M	45.4±0.16	75.9±0.03
$ZnSO_4$ 7.H_2O 0.01M	33.1±0.2	65.6±0.33
$ZnSO_4$ 0.01M + SA 0.01M	31.02±0.05	58.1±0.19

Table 9.6 PAL activity (Quantity of Cinnamic Acid produced) in ginger rhizomes under different stress trials (Day 1)

Trial	**Mahima U/mg (Mean± S.E)**	**Varada U/mg (Mean± S.E)**
Control	51.24±0.16	52.544±0.13
DMSO	46.925±0.02	40.006±0.01
SA 0.01 M	47±0.06	43.672±0.05
$ZnSO_4$ 7.H_2O 0.01M	49.53±0.15	49.566±0.12
$ZnSO_4$ 0.01M + SA 0.01M	49.54±0.2	40.06±0.5

As expected all crude extracts of control as well as the stress trials showed PAL activity. The results of these assays demonstrated that the PAL activity was found to be higher in leaves rather than in rhizome. In addition, the highest activity was found in leaves which was kept as control. That is the one which was not undergone any stress treatment (47.824±0.6 U/mg and 74.496±0.03 U/mg respectively). In the consecutive days the PAL activity was found to be increasing in stress trials at the same time it was found decresing in control plants. Among the two ginger varieties the PAL activity was higher in Varada as compared to Mahima. This high level of PAL activity in developing ginger leaves was unexpected because the shoots and rhizomes should have significant PAL activity because lignin is produced in the growing xylem, whereas ginger leaves are not known to contain high levels of flavonoids, lignins, or other commonly found phenylpropanoid pathway derived compounds. The high level of PAL activity in the growing leaves can be due to the production of a certain group of metabolites derived from the phenylpropanoid pathway was increased in the leaves. The highest activity of PAL in ginger leaves may be due to the high production of ginger phenolics and flavonoids in ginger leaves than that of ginger flower, shoot or rhizome (Tanweer *et al.*, 2020). The present study was in harmony with the findings of numerous scientists who agreed that the flavonoids exist in different concentrations in all the parts of ginger crop. The highest flavonoid contents were observed in leaves as compared to flowers and rhizome (Prakash, 2010; Panwar *et al.*, 2011). A group of scientists, Stoilova *et al.* 2007 worked on the different parts of ginger and concluded that different parts have different amounts of total phenolic contents. The maximum phenolic contents were found in green part of ginger. Although a low basal activity of PAL is present in the plant tissues, in response to stress the activity of PAL increases many folds. Thus, perception of stress induces the expression of PAL activity. Similar conditions are mimicked by certain biotic and abiotic compounds like SA. The application of SA induces PAL activity in plants (Șimonați, 2009).

At the same time reverse trend in the case PAL activity in ginger rhizomes were observed. All stress signals considerably reduced the quantity of PAL. Whereas control rhizome possessed high concentration of PAL. This can be explained by the negative/feedback regulation of PAL. PAL which is the first enzyme in Phenyl propanoid pathway sometimes regulated by feedback inhibition by its own products or intermediates.

Here we can say that the secondary metabolites produced as a result of stress signals might have inhibited the production of PAL enzyme in rhizome at the time of harvest there is no need for the production of secondary metabolites which is itself an expenditure of energy for the plant (Zhang & Liu, 2015).

Brief Summary

The enzyme Phenylalanine Ammonia Lyase which is the first enzyme of phenylpropanoid pathway is found to be regulated in leaves and rhizomes under different stress treatments. During the initial time control plants(leaves) had high concentration of PAL and after day by day the leaves which are sprayed with Salicylic Acid and Zinc Sulphate Heptahydrate found to be increasing the concentration of PAL. After the harvest rhizomes were analyzed for PAL activity and found that the enzyme was found to be lower in concentration in rhizomes than in leaves.

Mass Spectrometric analysis of selected trials of two varieties of ginger including control were done by using Gas Chromatography-Mass Spectrometry (GC-MS) to estimate the quantitative differences in the phytochemicals, which is produced as a result of different stress treatments. This chromatographic analysis revealed the quantitative difference among the phytochemicals present in different samples. The retention time is the length of time that a chemical typically stays in the column following sample introduction. If all other parameters, including mobile phase and flow rate, remain fixed and the detector is tuned to the same wavelength, the retention period for a particular molecule won't change (Nagy & Vekey, 2008). Thus, the retention time will be distinct for each component.

10.1 GC-MS Analysis of hexane fraction of rhizomes under different stress condition

The GC-MS analysis of hexane fraction of samples was studied and the identification of compounds present in different samples and its quantitative variation among samples were analyzed and recorded. According to Sermakkani & Thangapandian, (2012) and Kumar *et al.* (2014). GC-MS analysis is a useful technique for identifying non-polar components, volatile substances, acids, esters and alkaloids. There were many studies on identification and quantification of phytoconstituents in ginger using GC-MS analysis (Bartley & Foley,1994; Sasidharan & Menon, 2010; Kamaliroosta *et al.*,2013; Shareef *et al.*, 2016; Munda *et al.*, 2018; Yu *et al.*, 2022;). The peak produced at different retention time and the mass fragmentation pattern of different phytoconstituents was compared with library available at CMPR kottakkal and NIST-14 library and the spectral interpretation revealed the identity of different compounds present in the hexane fraction of ginger sample.

10.2 GC- MS analysis of dried rhizome of *Zingiber officinale* cv-Varada and *Zingiber officinale* cv- Mahima (Control)

GC-MS analysis of dried rhizome samples of *Zingiber officinale* cv-Varada and cv-Mahima (Control) plants were studied. A total of 16 compounds for cv-Varada and 9 compounds for cv-Mahima were identified. A Total Ion Chromatogram (TIC) of the hexane fraction of both the varieties were prepared. Numerous phytochemicals were found in the Total Ion Chromatogram at different retention durations. The results of the TIC analysis were shown, including the retention indices, area and height of several chemical constituents.

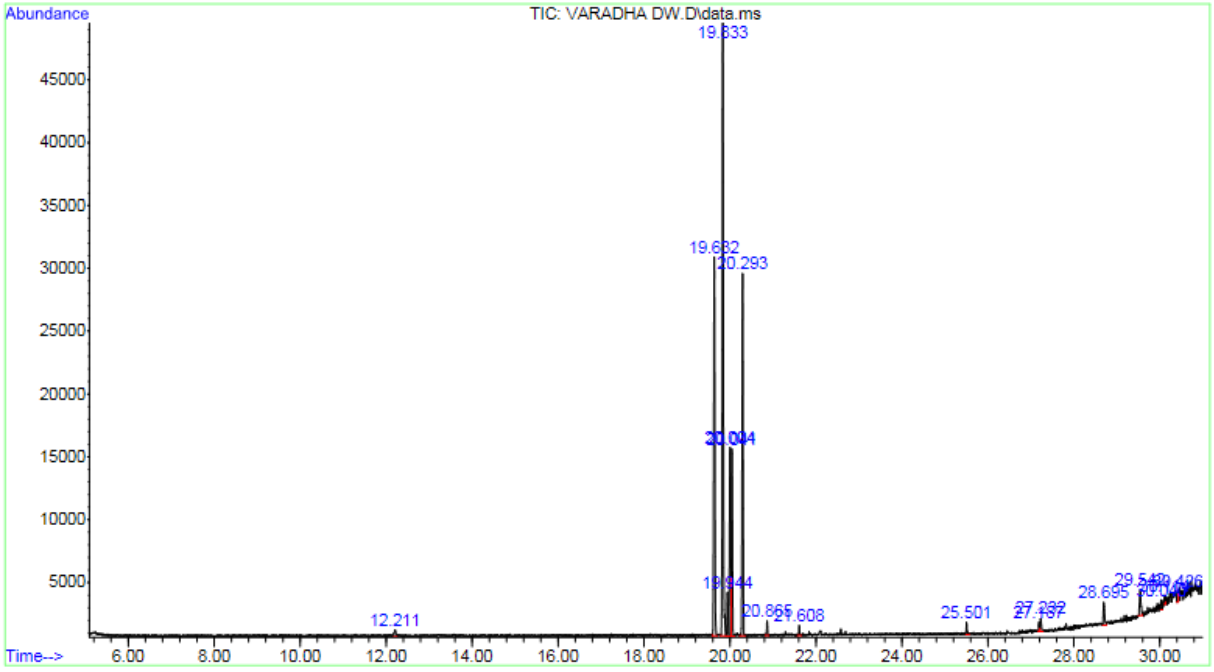

Figure 10.1 TIC of Hexane Fraction of *Z. officinale* cv-Varada (Control)

Table 10.1 The TIC analysis data for the ginger rhizome (*Z. officinale* cv-Varada-Control)

Peak No.	R.T. (min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	12.204	954	959	964	rBV	554	1390	1.66	0.529
2	19.632	1952	1958	1968	rVB	30139	54723	65.38	20.828
3	19.8328	1978	1985	1995	rVB	48748	83694	100.00	31.855
4	19.9443	1995	2000	2004	rVV	3491	5532	6.61	2.106
5	20.037	2004	2008	2011	rVV	15018	27122	32.41	10.323
6	20.041	2011	2013	2022	rVB	14921	23073	27.57	8.782
7	20.293	2038	2047	2055	rVB	28824	47887	57.22	18.226
8	20.865	2118	2124	2128	rVB	1151	1472	1.76	0.560
9	21.608	2219	2224	2231	rBB	798	1042	1.25	0.397
10	25.501	2746	2748	2756	rBV3	973	1386	1.66	0.528
11	27.2301	2972	2975	2978	rBV2	711	931	1.11	1.1098
12	28.695	3175	3178	3186	rBV2	1828	4001	4.78	1.523

13	29.542	3287	3292	3299	rBV4	2069	4637	5.54	1.765
14	30.039	33356	3359	3362	rBV2	781	1202	1.44	0.457
15	30.114	3367	3369	3372	rBV	723	942	1.13	0.359
16	30.426	3407	3411	3412	rBV2	920	1718	2.05	0.654

Table 10.2 Phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Varada under foliar spray of DW(Control).

Peak No.	R.T.(min)	Area (%)	Name of the compound	Ref. No	CAS No	Quality index
1	12.204	0.529	Acetonitrile2,2'-iminobis-	2686	000628-87-5	2
2	19.632	20.8281	.alpha.- Curcumene	66865	000644-30-4	99
3	19.833	31.8547	Nortricyclyl bromide	41891	1000342-32-2	2
4	19.944	2.1055	Xylopropamine	34152	075659-60-8	9
5	20.037	10.3229	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	68740	020307-83-9	87
6	20.0408	8.7818	.beta.-Bisabolene	68571	000495-61-4	96
7	20.2934	18.2262	.alpha.- Farnesene	68740	020307-83-9	87
8	20.8654	0.5603	N-Benzyl-N-ethyl-p-isopropylbenzamide	141055	015089-22-2	43
9	21.6083	0.3966	3-Methyl-3-hexene	3364	003404-65-7	9
10	25.5009	0.5275	Propanamide	735	000079-05-0	4

12	27.2317	1.1098	Cyclopentadecanone, 2-hydroxy-	102369	004727-18-8	27
12	28.6952	1.5228	6-Shogaol	136506	000555-66-8	9
13	29.5421	1.7649	4-Hydroxyphenyl pyrrolidinyl thione	71384	084783-02-8	7
14	30.0398	0.4575	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	206983	101869-40-3	25
15	30.114	0.3585	2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino)pyridine	71190	339352-50-0	47
16	30.4261	0.6539	Silicic acid, diethyl bis(trimethylsilyl) ester	154747	003555-45-1	28

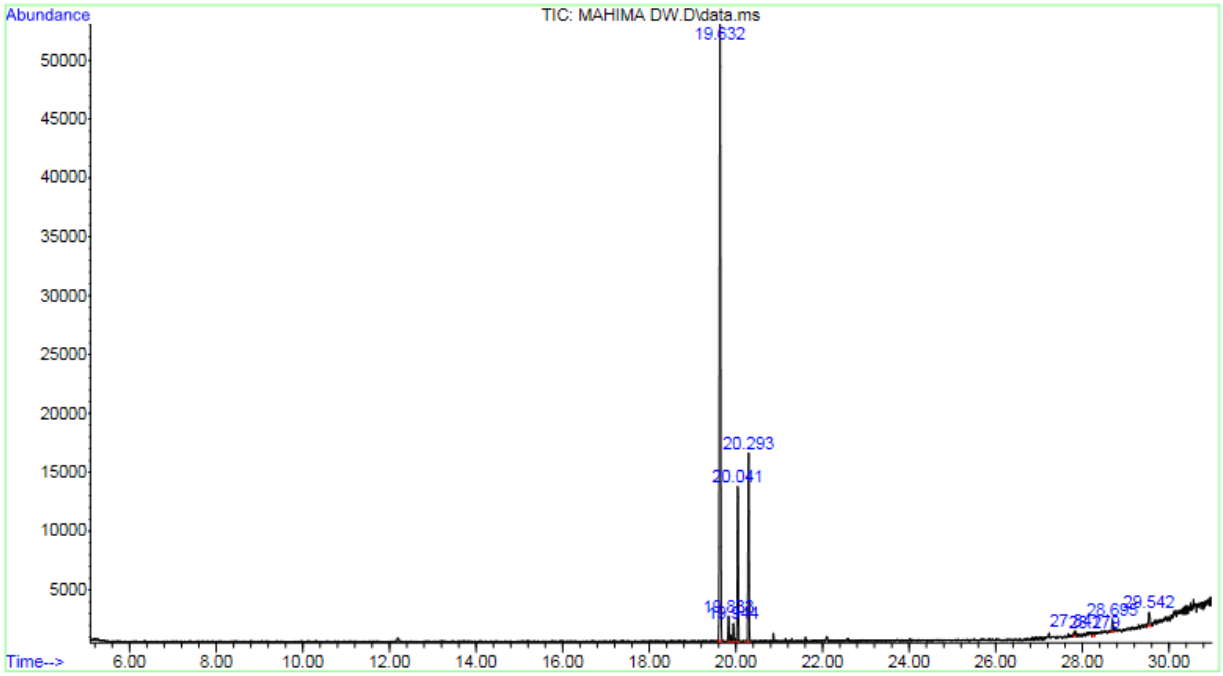

Figure 10.2 TIC of Hexane Fraction of *Z. officinale* cv-Mahima (Control)

Table 10.3 The TIC analysis data for the ginger rhizome *Z. officinale* cv-Mahima (Control)

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	19.632	1952	1958	1965	rBB	52379	89522	100.00	44.873
2	19.833	1980	1985	1989	rBV	2232	3742	4.18	2.503
3	19.944	1994	2000	2004	rVV	1569	2457	2.74	11.643
4	20.041	2004	2013	2019	rVV	13204	21477	23.99	19.367
5	20.293	2037	2047	2052	rVB	16078	25624	28.62	8.3537
6	27.841	3056	3063	3066	rVB	430	956	1.07	5.639
7	28.279	3114	3122	3123	rBV	334	1045	1.17	5.1289
8	28.695	3174	3178	3183	rBV	1007	1930	2.16	1.291
9	29.542	3288	3292	3299	rVB2	1177	2767	3.09	1.851

Table 10.4 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Mahima under foliar spray of DW(Control)

Peak No.	R.T. (min)	Area (%)	Name of the compound	Ref. No	CAS No	Quality index
1	19.632	44.873	.alpha.-Curcumene	66865	000644-30-4	99
2	19.833	2.5027	Nortricyclyl bromide	41891	1000342-32-2	2
3	19.944	11.6433	Xylopropamine	34152	075659-60-8	9
4	20.041	19.367	.beta.-Bisabolene	68571	000495-61-4	96
5	20.293	8.3537	.alpha.-Farnesene	68740	020307-83-9	87
6	27.841	5.6394	Hexadecanoic acid, methyl Ester	130817	000112-39-0	46
7	28.279	5.1289	1- (2 Adamantyliden) semicarbazide	71395	065814-27-9	4
8	28.695	1.2908	6-Shogaol	136506	000555-66-8	9
9	29.542	1.8506	4 Hydroxyphenyl pyrrolidinyl thione	71384	084783-02-8	7

.alpha.-Curcumene, Nortricyclyl bromide, Xylopropamine, .alpha.-Farnesene, .beta.-Bisabolene, 6-Shogaol, 4-Hydroxyphenyl pyrrolidinyl thione were the common compounds were present in both the cultivars of ginger. .alpha.-Curcumene was the major compound present in *Z. officinale* cv-Mahima at a retention time of 19.632 (44.573% area),

and for *Z. officinale* cv-Varada major percentage area is occupied by Nortricyclyl bromide at a retention time of 19.833 (31.8547%).

10.5 Table showing the area percentage of common compounds present in the trial (DW), in both the cultivars.

Sl.No.	Name of the Compound	Area% of Compound in *Z. officinale* cv- Varada (Control)	Area% of Compound in *Z. officinale* cv- Mahima (Control)
1	.alpha.- Curcumene	20.8281%	44.573%
2	Nortricyclyl bromide	31.8547%	2.5027%
3	Xylopropamine	2.1055%	11.6433%
4	.beta.-Bisabolene	8.7818%	19.367%
5	.alpha.-Farnesene	10.3229%	8.3537%
6	6-Shogaol	1.5228%	1.2908%
7	4-Hydroxyphenyl pyrrolidinyl thione	1.4649%	1.8506%

In the case of plant replicates which are sprayed with DW (Control plants), a total of 7 common compounds identified through GC-MS analysis in both the varieties. The compound Nortricyclyl bromide was found in greater area percentage in *Z. officinale* cv-Varada (31.8547%). In the case of *Z. officinale* cv-Mahima it is 2.5027% which is comparatively low when compared to *Z. officinale* cv-Varada. .alpha.- Curcumene was present in both *Z. officinale* cv-Varada and *Z. officinale* cv- Mahima (20.821% for Varada and 44.573% for Mahima). Xylopropamine with an area percentage of 2.1055% in Varada and 11.6433% in Mahima. The next compound .alpha.-Farnesene was found to have an area percentage of 10.3229% in Varada and 8.3537% in Mahima. 6-Shogaol was found to have an area percentage of 1.5228% in Varada and 1.2908% in Mahima. 4-Hydroxyphenyl pyrrolidinyl thione was the next compound with an area percentage of 1.4649% in *Z. officinale* cv- Varada and 1.8506% in *Z. officinale* cv-Mahima.

10.3 GC- MS analysis of dried rhizome of *Zingiber officinale* cv-Varada and cv-Mahima (DMSO).

Since Salicylic doesn't dissolve in water, it is dissolved in an organosulfur that is DMSO (Dimethyl sulfoxide). So, for a comparative analysis of ginger rhizomes under stress condition, a control is given for Salicylic Acid also. Control for Salicylic Acid were plant replicates which are sprayed with DMSO (0.02%, i.e., 0.2ml of Dimethyl sulfoxide in 100ml of distilled water). GC-MS analysis of dried rhizome of ginger *Z. officinale* cv-Varada and *Z. officinale* cv-Mahima under the foliar spray of DMSO were done. A total of 24 compounds were identified for the cultivar Varada and a total of 7 compounds identified for Mahima. A Total Ion Chromatogram for both Varada and Mahima under DMSO stress were prepared. The Total Ion Chromatogram demonstrated the presence of several phytochemicals at various retention times. The retention indices, area and height of several compounds were displayed TIC analysis data. The proportion amount of these phytochemicals in the current sample under study is compared to the percentage quantity of the same phytochemicals in the ginger, which has been observed through library research.

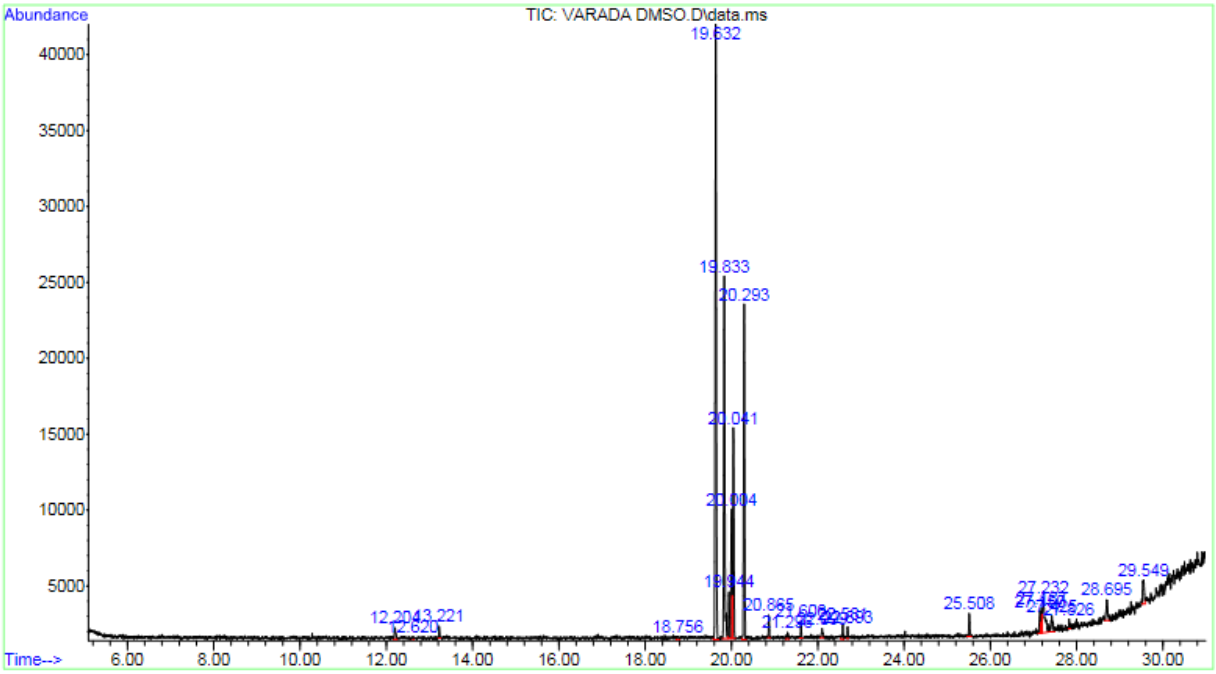

Figure 10.3 TIC of Hexane Fraction of *Z. officinale* cv-Varada (DMSO).

Table 10.6 The TIC analysis data for the ginger rhizome (*Z. officinale* cv-Varada under foliar spray of DMSO (Control))

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	12.204	954	958	964	rBV	863	1567	2.13	0.678
2	12.620	1007	1014	1016	rBV	284	776	1.06	0.336
3	13.221	1093	1095	1100	rBV2	791	929	1.26	0.402
4	18.756	1836	1840	1846	rVB	244	757	1.03	0.328
5	19.632	1951	1958	1968	rVB	40522	73442	100.00	31.782
6	19.833	1979	1985	1996	rBV2	23877	41854	56.99	18.112
7	19.944	1996	2000	2004	rVV	3071	4744	6.46	2.053
8	19.966	2004	2008	2010	rVV	8524	13657	18.60	5.910
9	20.041	2010	2013	2019	rVB	13881	22197	30.22	9.606
10	20.293	2040	2047	2053	rVB	22112	37280	50.76	16.133
11	20.865	2121	2124	2128	rVB	1480	1716	2.34	0.743
12	21.296	2177	2182	2186	rBB2	430	736	1.00	0.319
13	21.608	2219	2224	2229	rBV2	1120	1174	1.60	0.508
14	22.099	2284	2290	2295	rBV2	600	1047	1.43	0.453
15	22.581	2349	2355	2358	rVB2	937	1287	1.75	0.557
16	22.693	2367	2370	2373	rVB	787	1024	1.39	0.443
17	25.508	2742	2749	2755	rBV2	1530	2523	3.44	1.092
18	27.150	2965	2970	2972	rBV3	1487	2610	3.55	1.129
19	27.187	2972	2975	2976	rVV2	1641	2338	3.18	1.012
20	27.232	2977	2981	2995	rVV2	2305	8171	11.13	3.536
21	27.425	3000	3007	3015	rVV3	1059	2964	4.04	1.283
22	27.826	3059	3061	3066	rVB	629	771	1.05	0.334
23	28.695	3173	3178	3186	rVB2	1347	3477	4.73	1.505
24	29.549	3289	3293	3298	rBV3	1595	4040	5.50	1.748

Table 10.7 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Varada under foliar spray of DMSO 0.02% (Control for SA)

Peak No.	R.T. (min)	Area (%)	Name of the compound	Ref. No	CAS No	Quality index
1	12.203	0.6781	Acetonitrile 2,2'-iminobis-	2686	000628-87-5	2
2	12.619	0.3358	Naphthalene	12196	000091-20-3	78
3	13.221	0.402	.alpha.-Phellandrene	29129	000112-31-2	50
4	18.756	0.3276	Cyclohexanol, 3-	4	18.756	0.3276
5	19.632	31.781	.alpha.-Curcumene	66865	000644-30-4	99
6	19.832	18.112	Nortricyclyl bromide	41891	1000342-32-2	2
7	19.944	2.053	Xylopropamine	7	19.9442	2.053
8	19.966	5.91	trans-.beta.-Ocimene	16098	003779-61-1	27
9	20.040	9.6057	.beta.-Bisabolene	9	20.0408	9.6057
10	20.293	16.13	.alpha.- Farnesene	68740	020307-83-9	87
11	20.865	0.742	N-Benzyl-N-ethyl-p-isopropylbenzamide	141055	015089-22-2	43
12	21.296	0.318	2,6-Octadienal,3,7-dimethyl-,(Z)	19651	000124-18-5	72
13	21.608	0.508	3-Methyl-3-hexene	3364	003404-65-7	9
14	22.098	0.453	Diethyl Phthalate	85001	000084-66-2	97
15	22.571	0.556	Acetamide, 2-chloro-	2508	000079-07-2	4
16	22.692	0.443	p-Toluic acid, 2-octyl ester	109790	1000293-34-0	35
17	25.508	1.091	N-(3-Methylbutyl)acetamide	17	25.5083	1.0918
18	27.15	1.129	Benzaldehyde, 2-nitro diaminomethyl idenhydrazone	71767	18	27.15
19	27.18	1.011	Cyclododecanol, 1-aminomethyl-	77240	19	27.1871

20	27.23	3.536	Cyclopentadecanone, 2-hydroxy-	102369	004727-18-8	27
21	27.42	1.282	3,4- dihydroxyphenylglycol, 4TMS	258664	056114-62-6	25
22	27.82	0.333	2-Myristynoyl-glycinamide	139899	1000111-57-7	38
23	28.69	1.504	6-Shogaol	136506	000555-66-8	9
24	29.54	1.748	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	24	29.5494	1.7483

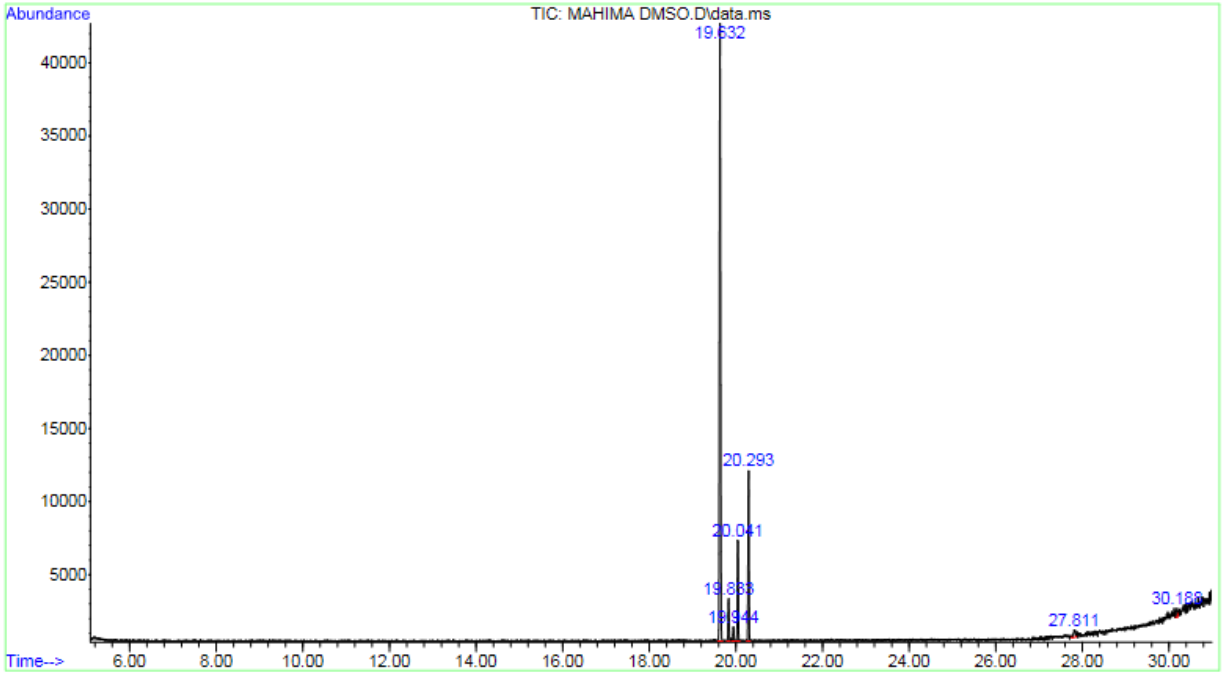

Figure 10.4 TIC of Hexane Fraction of *Z. officinale* cv-Mahima (DMSO).

Table 10.8 The TIC analysis data for the ginger rhizome (*Z. officinale* cv-Mahimaa under foliar spray of DMSO (Control))

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	19.632	1951	1958	1966	rVB	42256	73028	100.00	66.579
2	19.833	1979	1985	1989	rBV	2899	4531	6.20	4.131

3	19.944	1995	2000	2006	rBB	977	1508	2.06	1.375
4	20.041	2009	2013	2019	rBV	6902	10478	14.35	9.553
5	20.293	2042	2047	2052	rVB	11646	17540	24.02	15.991
6	27.811	3052	3059	3066	rVV	505	1619	2.22	1.476
7	30.188	3375	3379	3381	rBV3	600	983	1.35	0.896

Table 10.9 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Mahima under foliar spray of DMSO 0.02%(Control for SA)

Peak No.	R.T.(min)	Area (%)	Name of the compound	Ref. No	CAS No	Quality index
1	19.6322	66.5785	.alpha.-Curcumene	1	19.6322	66.5785
2	19.8328	4.1308	Nortricyclyl bromide	2	19.8328	4.1308
3	19.9442	1.3748	Xylopropamine	3	19.9442	1.3748
4	20.0408	9.5526	.beta. -Bisabolene	4	20.0408	9.5526
5	20.2934	15.991	.alpha.-Farnesene	68740	020307-83-9	87
6	27.821	1.476	2-Myristynoyl-glycinamide	139899	1000111-57-7	38
7	30.1883	0.8962	2-Methyl-6-(5-methyl-2-	7	30.1883	0.8962

Table 10.10 Area percentage of common compounds present in the trial (DMSO), in both the cultivars.

Sl.No.	Name of the Compound	Area% of Compound in *Z. officinale* cv- Varada (DMSO)	Area% of Compound in *Z. officinale* cv- Mahima (DMSO)

1	.alpha.- Curcumene	31.7819%	66.5785%
2	Nortricyclyl bromide	18.1123%	4.1308%
3	Xylopropamine	2.0583%	1.3748%
4	.beta.-Bisabolene	9.6057%	9.5526%
5	.alpha.- Farnesene	16.1329%	15.991%
6	2-Myristynoyl-glycinamide	0.3336%	1.476%

There are 6 common compounds common to both *Z. officinale* cv-Varada and *Z. officinale* cv-Mahima under the stress trial of DMSO. Out of the 7 compounds. .alpha-Curcumene was the compound found to have a greater area percentage when comparing with other compounds. In cultivar Mahima the percentage of .alpha.-Curcumene was 66.5785% and in Varada it is 31.7819%. The next highest concentration was seen in the peak identified for Norticyclyl bromide (18.1123%, in Mahima the area percentage for Norticyclyl bromide is 2.5027%) in Varada. In the case of Mahima after .alpha.-Curcumene .beta.-Bisabolene was the most important compound with respect to area percentage (19.367%) .alpha. -Farnesene which has an area percentage of 16.1329% in Varada and 8.5357% in Mahima was the next important compound which was commonly found in both the varieties under DMSO trial. 2-Myristynoyl-glycinamide is the next two common compounds identified. The area percentage of 2-Myristynoyl-glycinamide in Varada is 0.3336% and in Mahima it is 1.4706%.

10.4 GC- MS analysis of dried rhizome of *Zingiber officinale* cv-Varada and cv-Mahima under the foliar spray Salicylic Acid (0.01M).

GC-MS analysis dried ginger rhizomes of replicates which are sprayed Salicylic acid (0.01M) were analyzed for the identification phytocompunds in it. A total of 16 compounds identified for the cultivar Varada and a total of 5 compounds for cultivar Mahima. A Total Ion Chromatogram of (TIC) of hexane fraction of *Z. officinale* cv-Varada and *Z. officinale* cv-Mahima under the stress condition $ZnSO_4$ 7. H_2O and Salicylic Acid at 0.01M concentration were developed. At different retention times different phytochemicals has been identified for two cultivars, and from the area percentage, the quantity of several phytochemicals identified has been recorded in percentage.

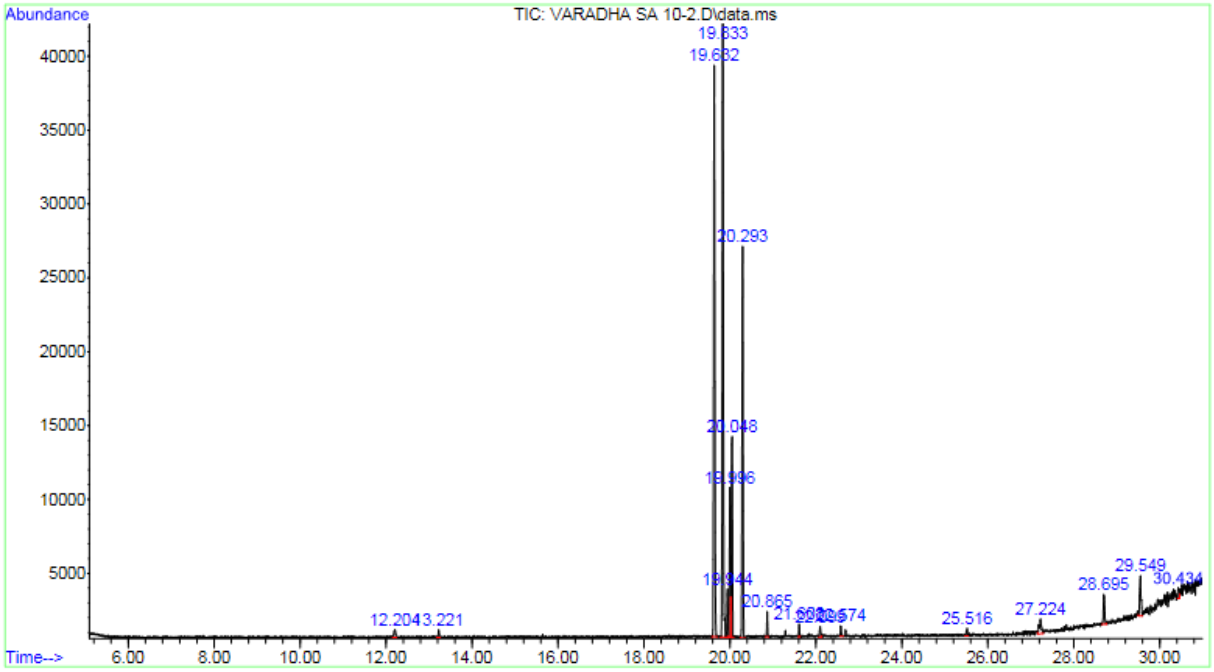

Figure 10.5 TIC of Hexane Fraction of *Z. officinale* cv-Varada (SA 0.01M).

Table 10.11 The TIC analysis data for the ginger rhizome (*Z. officinale* cv-Varada under foliar spray of Salicylic Acid (0.01M))

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	12.204	951	958	963	rVB	518	1376	1.19	0.550
2	13.221	1089	1095	1100	rVB	503	743	1.03	0.297
3	19.632	1951	1958	1968	rVB	34681	68219	94.60	47.263
4	19.944	1978	1985	1995	rVB	41520	72112	100.00	8.819
5	19.944	1995	2000	2003	rBV	3262	4875	6.76	1.948
6	19.966	2003	2007	2010	rVV	10140	15647	21.70	6.253
7	20.004	2010	2014	2019	rVB	13566	22290	30.91	8.908
8	20.293	2040	2047	2054	rVB	26505	43970	60.97	17.573
9	21.608	2119	2124	2128	rBV	1700	1811	2.51	0.724
10	22.099	2221	2224	2227	rBV	860	847	1.17	0.339
11	22.574	2286	2290	2295	rBV	692	1250	1.73	0.500
12	25.501	2352	2354	2362	rVB	713	942	1.31	0.376

13	27.224	2971	2980	2989	rVB3	979	3372	4.68	1.348
14	28.695	3173	3178	3186	rBV	2023	4281	5.94	1.711
15	29.549	3287	3299	3299	rVB3	2715	5874	8.15	2.348
16	30.434	3409	3412	3416	rBV3	715	1709	2.37	0.683

Table 10.12 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Varada under foliar spray of Salicylic Acid 0.01M

Peak No.	R.T.(min)	Area (%)	Name of the compound	Ref. No	CAS No	Quality index
1	12.204	0.5499	Acetonitrile 2,2'-iminobis-	2686	000628-87-5	2
2	13.221	0.2969	.alpha.-Phellandrene	29129	000112-31-2	50
3	19.632	47.2636	.alpha.- Curcumene	3	19.632	47.2636
4	19.944	8.8194	Nortricyclyl bromide	41891	1000342-32-2	2
5	19.944	1.9483	Xylopropamine	5	19.944	1.9483
6	19.966	6.2533	trans-.beta.-Ocimene	6	19.966	6.2533
7	20.04	8.9082	.beta.-Bisabolene	7	20.04	8.9082
8	20.293	17.5725	.alpha.-Farnesene	68740	020307-83-9	87
9	20.865	0.7238	N-Benzyl-N-ethyl-p-isopropylbenzamide	141055	015089-22-2	43
10	22.099	0.3385	Diethyl Phthalate	85001	000084-66-2	97
11	22.574	0.4996	Acetamide, 2-chloro-	2508	000079-07-2	4
12	25.501	0.737	Propanamide	735	000079-05-0	4
13	27.224	1.3476	Cyclopentadecanone, 2-hydroxy-	13	27.224	1.3476
14	28.695	1.7109	6-Shogaol	136506	000555-66-8	9
15	29.549	2.3475	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	15	29.549	2.3475
16	30.434	0.683	Arsenous acid, tris(trimethylsilyl) ester	199618	055429-29-3	16

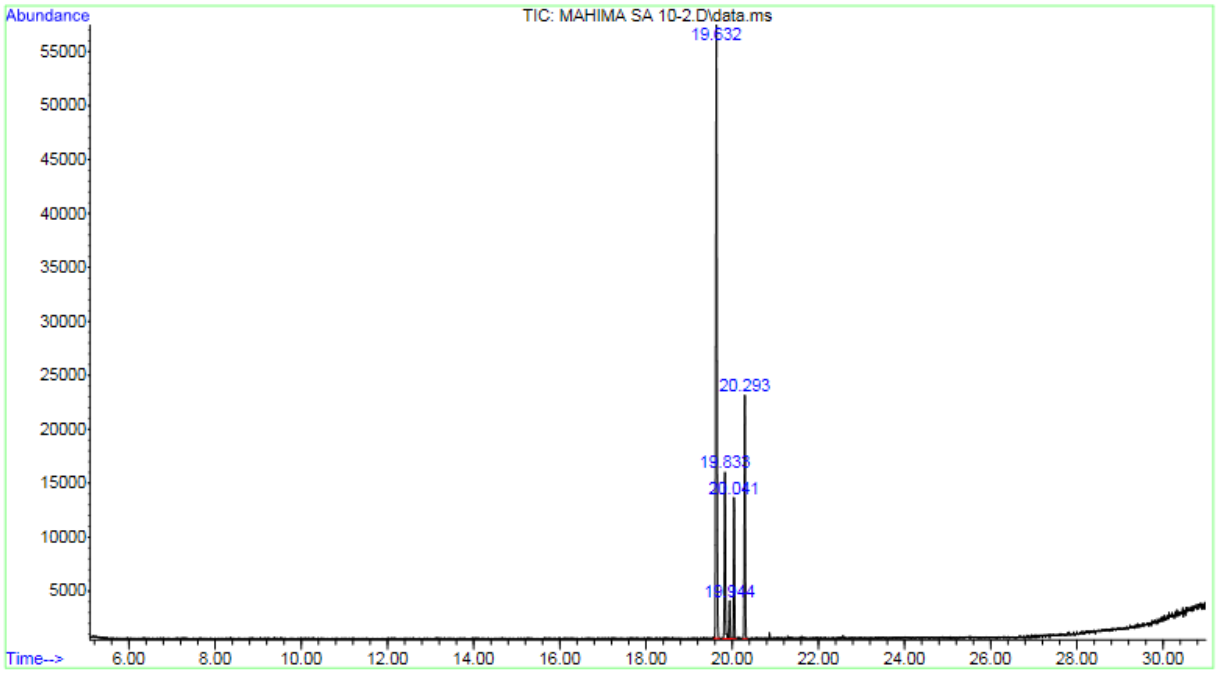

Figure 10.6 TIC of Hexane Fraction of *Z. officinale* cv-Mahima (SA 0.01M).

Table 10.13 The TIC analysis data for the ginger rhizome (*Z. officinale* cv-Mahima under foliar spray of Salicylic Acid (0.01M))

Peak No.	R.T. (min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	19.6332	1951	1958	1967	rVB	56856	101345	100.00	53.446
2	19.833	1979	1985	1985	rVB	15424	26812	26.46	14.140
3	19.944	1995	2000	2007	rVV	3536	5222	5.15	2.754
4	20.041	2007	2013	2019	rVB	13111	19345	19.09	10.202
5	20.293	2041	2047	2053	rBv	22614	22614	36.41	19.458

Table 10.14 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Mahima under foliar spray of Salicylic Acid 0.01M

Peak No.	R.T. (min)	Area (%)	Name of the compound	Ref. No	CAS No	Quality index
1	19.6321	53.4461	.alpha.- Curcumene	66865	000644-30-4	99
2	19.8327	14.1398	Nortricyclyl bromide	2	19.8327	14.1398
3	19.9442	2.7539	Xylopropamine	3	19.9442	2.7539
4	20.0407	10.2019	.beta.-Bisabolene	4	20.0407	10.2019
5	20.2933	19.4583	.alpha.-Farnesene	68740	020307-83-9	87

Table 10.15 The area percentage of common compounds present in the trial (sa 0.01m), in both the cultivars.

Sl.No.	**Name of the Compound**	**Area% of Compound in *Z. officinale* cv-Varada (SA 0.01M)**	**Area% of Compound in *Z. officinale* cv- Mahima (SA 0.01M)**
1	.alpha.-Curcumene	47.2636	53.4461
2	Nortricyclyl bromide	8.8194	14.1398
3	Xylopropamine	1.9483	2.7539
4	.beta.-Bisabolene	8.9082	10.2019
5	.alpha.-Farnesene	17.5725	19.4583

There are 5 common compounds identified for cultivars Varada and Mahima under the foliar spray of Salicylic Acid 0.01M. .alpha.-Curcumene was found to have highest area percentage in both (47.2636% in Varada and 53.4461% in Mahima). The area percentage of .alpha.-Farnesene for both the varieties were 17.5725% for Varada and 19.4583% for Mahima. The next common compound identified is .beta.-Bisobolene with an area percentage of 8.9082% in Varada and 10.2019% in Mahima. Nortricyclyl bromide has an area percentage of 8.8194% in Varada and 14.1398% in Mahima. Xylopropamine has an area percentage of 1.9483% in Varada and 2.7539% in Mahima.

10.5 GC- MS analysis of dried rhizome of *Zingiber officinale* cv-Varada and cv-Mahima under the foliar spray $ZnSO_4$.$7H_2O$ (0.01 M).

In case of plant replicates which are sprayed with Zinc Sulphate Heptahydrate at 0.01 M, a total of 14 compounds were isolated for *Z. officinale* cv- Varada and 13 compunds for *Z. officinale* cv-Mahima and its percentage quantity calculated. The Total Ion Chromatogram (TIC) of hexane fraction of *Z. officinale* cv-Varada and *Z. officinale* cv-Mahima under the stress condition $ZnSO_4$.$7H_2O$ was developed. Under the stress condition $ZnSO_4.7H_2O$, a Total Ion Chromatogram (TIC) of the hexane fraction of both varieties were created. The Total Ion Chromatogram demonstrated the presence of several phytochemicals at various retention times. The retention indices, area, and height of several compounds were displayed TIC analysis data. The proportion amount of these phytochemicals in the current sample under study is compared to the percentage quantity of the same phytochemicals in the ginger, which has been observed through library research. The TIC analysis of hexane fraction of ginger cultivar Varada and Mahima under the stress trial $ZnSO_4.7H_2O$ at 0.01M revealed the presence of characteristic phytochemicals. A total of 14 phytochemicals were identified at different retention time for Varada and 13 for the cultivar Mahima. The TIC showed the variation in the concentration of phytochemicals in percentage in comparing with the concentration of the same in control trials. And also showed the presence of some phytochemicals which is unique for the biological replicates which are sprayed with trial $ZnSO_4.7\ H_2O$ at 0.01M.

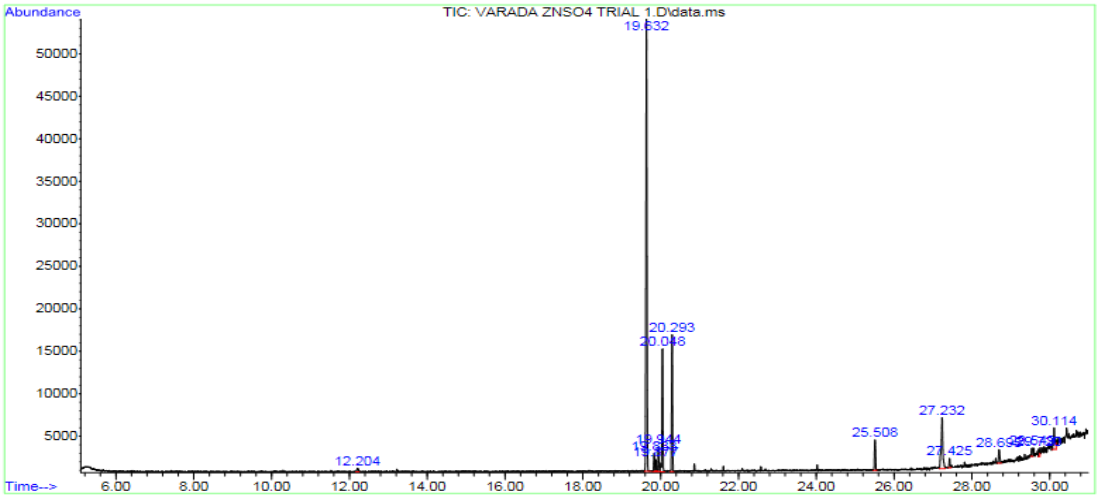

Figure 10.7 TIC of Hexane Fraction of *Z. officinale* cv-Varada under foliar spray of $ZnSO_4.7\ H_2O$ (0.01M)

Table 10.16 The TIC analysis data for the ginger rhizome (*Z. officinale* cv-Varada under foliar spray of $ZnSO_4.7\ H_2O$ (0.01M))

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	12.204	953	958	964	rBV	447	1084	1.16	0.553
2	19.632	1952	1958	1971	rBV	53193	93346	100.00	47.659
3	19.833	1979	1985	1989	rBV	2174	3607	3.86	1.842
4	19.877	1989	1991	1994	rVV	1410	1413	1.51	0.721
5	19.944	1994	2000	2005	rVV	2940	4538	4.86	2.317
6	20.048	2005	2014	2021	rVB	14450	24975	26.76	12.751
7	20.293	2042	2047	2052	rVB	16112	26340	28.22	13.448
8	25.508	2743	2749	2755	rBV2	3568	7084	7.59	3.617
9	27.232	2972	2981	2992	rBV4	5996	17541	18.79	8.956
10	27.425	3003	3007	3013	rBv	1091	1835	1.97	0.937
11	28.695	3174	3178	3185	rVV3	1591	3536	3.79	1.805
12	29.549	3288	3293	3296	rBV	972	2101	2.25	1.073
13	29.720	3314	3316	3320	rBV2	885	1681	1.80	0.858
14	30.114	3363	3369	3376	rBV3	2545	6780	7.26	3.462

Table 10.17 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Varada under foliar spray of $ZnSO_4.7\ H_2O$ (0.01M)

Peak No.	R.T.(min)	Area(%)	Name of the Compound	Ref no	CAS No	Quality index
1	12.204	0.553	Acetonitrile 2,2'-iminobis-	2686	000628-87-5	2
2	19.632	47.659	.alpha.- Curcumene	66865	000644-30-4	99

3	19.833	1.842	Nortricyclyl bromide	41891	100034 2-32-2	2
4	19.877	0.721	Propanedioicacid,(hydroxyimino)-, diethyl ester	55444	006829-41-0	5
5	19.944	2.317	Xylopropamine	34152	075659-60-8	9
6	20.048	12.751	.beta.-Bisabolene	68571	000495-61-4	96
7	20.293	13.448	.alpha.- farnesene	68740	020307-83-9	87
8	25.508	3.617	N-(3-Methylbutyl) acetamide	254928	038147-00-1	33
9	27.232	8.956	Cyclopentadecanone, 2-hydroxy-	102369	004727-18-8	27
10	27.425	0.937	3,4- dihydroxyphenylglycol, 4TMS	258664	056114-62-6	25
11	28.695	1.805	6-Shogaol	136506	000555-66-8	9
12	29.549	0.73	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	71757	100014 7-64-6	9
13	29.720	0.858	Copaene	262268	100030 9-06-1	53
14	30.114	3.462	2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino)pyridine	71190	339352-50-0	47

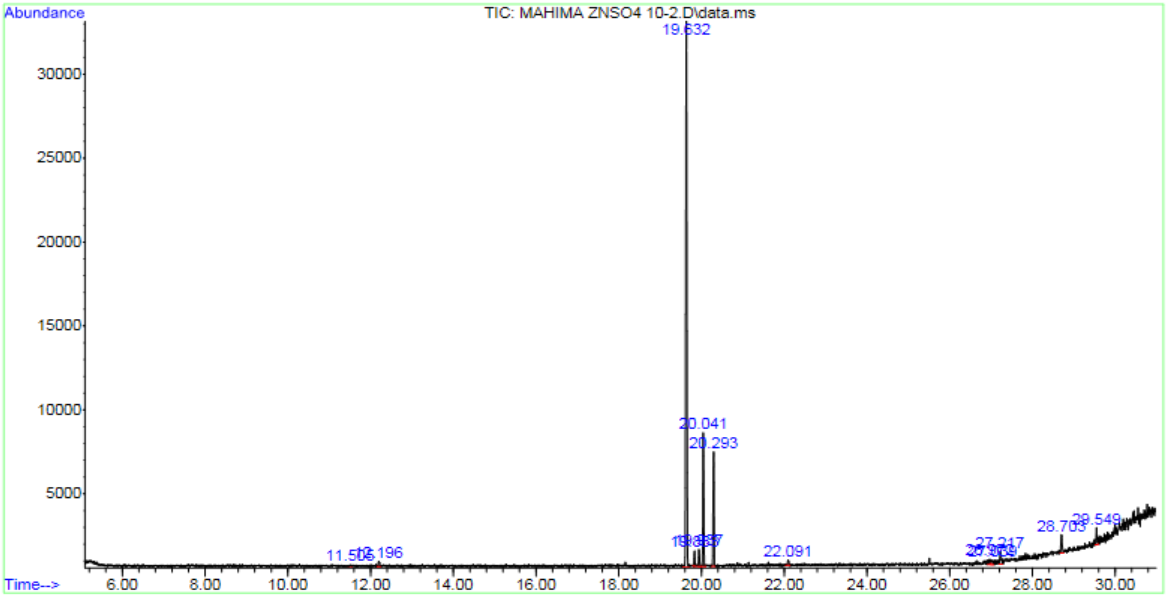

Figure 10.8 TIC of Hexane Fraction of *Z. officinale* cv-Mahima under foliar spray of $ZnSO_4.7\ H_2O$ (0.01M)

Table 10.18 The TIC analysis data of *Z. officinale* cv-Mahima under foliar spray of $ZnSO_4.7\ H_2O$ (0.01M)

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of Total
1	11.505	857	864	868	rBV	189	594	1.03	0.652
2	12.196	953	957	962	rVB	317	620	1.08	0.680
3	19.632	1947	1958	1966	rVB	32521	57631	100.00	63.220
4	19.833	1980	1985	1988	rVB	901	1089	1.89	1.195
5	19.944	1994	1999	2005	rVB2	1015	1500	2.60	1.645
6	20.048	2005	2013	2020	rBV	7963	11586	20.10	12.710
7	20.293	2041	2047	2051	rBV	6820	10573	18.35	11.598
8	22.091	2282	2289	2795	rBV2	309	589	1.02	0.646
9	22.217	2970	2979	2992	rBV4	5996	17541	18.79	8.503
10	26.972	3003	3007	3013	rBv	1091	1835	1.97	0.937
11	27.416	3174	3178	3185	rVV3	1591	3536	3.79	1.805
12	28.703	3288	3293	3296	rBV	972	2101	2.25	1.073
13	29.549	3314	3316	3320	rBV2	885	1681	1.80	0.858

Table 10.19 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Mahima under foliar spray of $ZnSO_4.7\ H_2O$ (0.01M)

Peak No	R.T.(min)	Area (%)	Name of the Compound	Ref no	CAS No	Quality Index
1	11.5053	0.6516	Decanal	29133	000112-31-2	98
2	12.1961	0.6801	Tridecane	51394	000629-50-5	96
3	19.6322	63.220	.alpha.-Curcumene	66865	000644-30-4	99
4	19.8328	1.1946	Nortricyclyl bromide	41891	1000342-32-2	2
5	19.9448	1.6455	Xylopropamine	34152	075659-60-8	9
6	20.0408	12.710	.beta.-Bisabolene	68571	000495-61-4	96
7	20.2933	11.598	.alpha.-Farnesene	68740	020307-83-9	87
8	22.0911	0.6461	Diethyl Phthalate	85001	000084-66-2	97
9	22.217	0.7865	Phenyl chloroformate	29677	001885-14-9	38
10	26.9725	0.6867	Cyclononasiloxane, octadecamethyl-	274624	000556-71-8	46
11	27.425	1.9044	3,4-dihydroxyphenylglycol, 4TMS	258664	056114-62-6	25

12	28.7025	2.4891	Phthalic acid, isobutyl octadecyl ester	262268	1000309-06-1	53
13	29.5494	1.787	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	71757	1000147-64-6	9

Table 10.20 The area percentage of common compounds present in the trial ($ZnSO_4$ 7.H_2O), in both the cultivars.

Sl.No.	Name of the Compound	Area% of Compound in *Z. officinale* cv- Varada ($ZnSO_4$ 7.H_2O)	Area% of Compound in *Z. officinale* cv- Mahima ($ZnSO_4$ 7.H_2O)
1	.alpha.- Curcumene	47.659%	63.220%
2	.beta- Bisobolene	13.448%	11.598%
3	.alpha.-Farnesene	12.751%	12.710%
4	Nortricyclyl bromide	1.842%	1.1946%
5	Xylopropamine	2.317%	1.6455%
6	3,4-dihydroxyphenylglycol,4TMS	0.937%	1.9044%
7	Phthalic acid, isobutyl octadecyl ester	0.858%	2.4891%
8	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	0.73%	1.787%

A total of 14 ccompounds identified from the GC-MS analysis of hexane fraction of ginger rhizome under $ZnSO_4$.7 H_2O foliar spray (0.01M) **(Table 10.18)**. And a total of 13 compounds for *Zingiber officinale* cv- Mahima under the same stress condition **(Table 10.19)**.

The common compounds identified in both varieties are .alpha.-Curcumene, .beta-Bisobolene, .alpha.-Farnesene, Nortricyclyl bromide, Xylopropamine, 3,4-dihydroxyphenylglycol, 4TMS, Phthalic acid, isobutyl octadecyl ester and -p-Nitrophenyl-oxadiazol-1,3,4-one-5.

The major compounds identified in its concentration are .alpha.- Curcumene (47.659% for Varada and 63.220% for Mahima), followed by .alpha.-Farnesene (13.448% for Varada and 11.598% for Mahima) .beta- Bisabolene (12.751% for Varada and 12.710% for Mahima), Nortricyclyl bromide (1.842% for Varada and 1.1946% for Mahima), Xylopropamine (2.317% for Varada and 1.6455% for Mahima), 3,4-dihydroxyphenylglycol, 4TMS (0.937% for Varada and 1.9044% for Mahima), Phthalic acid, isobutyl octadecyl ester (0.858% for Varada and 2.4891% for Mahima). -p-Nitrophenyl-oxadiazol-1,3,4-one-5 (0.73% for Varada and 1.787% for Mahima). **(Table 10.20)**.

10.6 GC- MS analysis of dried rhizome of *Zingiber officinale* cv-Varada and cv-Mahima under the foliar spray $ZnSO_4$ .7H_2O and Salicylic Acid (0.01 M each).

GC-MS analysis of dried rhizome of *Zingiber officinale* cv- Varada and cv-Mahima under the combination of foliar sprays ($ZnSO_4$. H_2O and Salicylic Acid 0.01M each) were analyzed to identify different phytoconstituents present in the trial. Under this combination of stress conditions, a Total Ion Chromatogram (TIC) of the hexane fraction of both varieties were created. The Total Ion Chromatogram demonstrated the presence of several phytochemicals at various retention times. The retention indices, area, and height of several compounds were displayed TIC analysis data. The proportion amount of these phytochemicals in the current sample under study is compared to the percentage quantity of the same phytochemicals in the ginger, which has been observed through library research. A total of 16 compounds has been identified for Varada and 11 compounds for Mahima.

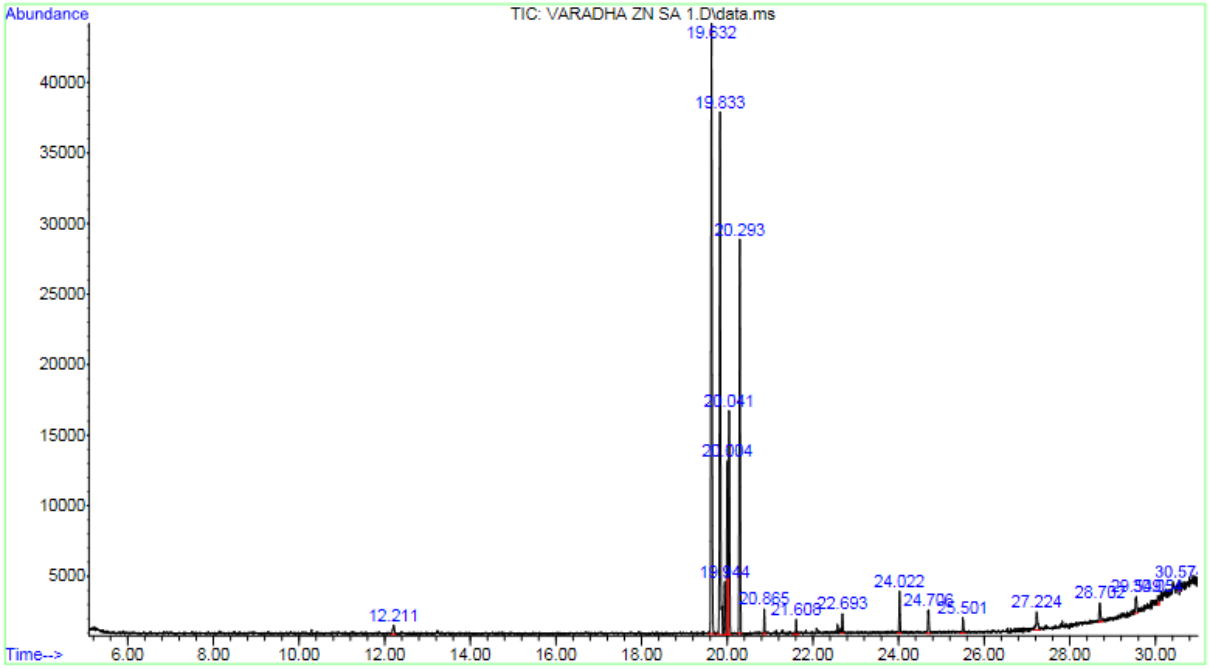

Figure 10.9 TIC of Hexane Fraction of *Z. officinale* cv-Varada under foliar spray of $ZnSO_4.7\ H_2O$ and Salicylic acid (0.01M each).

Table 10.21 The TIC analysis data of *Z. officinale* cv-Varada under foliar spray of $ZnSO_4.7\ H_2O$ and salicylic Acid (0.01M each)

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	12.204	951	959	964	rBV	640	1605	2.11	0.596
2	19.632	1951	1958	1966	rVB	43281	75992	100.00	28.205
3	19.833	1979	1985	1994	rVB	37067	64834	85.32	24.063
4	19.944	1994	2000	2004	rBV	3766	6410	8.44	2.379
5	20.041	2010	2013	2021	rVB	15891	27291	35.91	10.129
6	20.293	2041	2047	2052	rBV	27945	46693	61.44	17.330
7	20.865	2119	2124	2129	rBV	1770	2103	2.77	0.781
8	21.608	2215	2224	2232	rBV	1036	1412	1.86	0.524
9	22.693	2366	2370	2374	rVB	1371	2058	2.71	0.764
10	24.022	2544	2549	2554	rVB2	2978	4450	5.86	01.652
11	24.706	2635	2641	2645	rVB2	1652	3111	4.09	1.155
12	25.501	2740	2748	2755	rBV	1097	1893	2.49	0.703

13	27.224	2973	2980	2990	rBV3	1279	3571	4.70	1.325
14	28.702	3175	3179	3186	rBV	1329	2932	3.86	1.088
15	29.549	3289	3293	3298	rBV4	1183	2592	3.41	0.962
16	30.054	3360	3361	3366	rBV2	635	1190	1.57	0.442

Table 10.22 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Varada under foliar spray of $ZnSO_4.7\ H_2O$ and Salicylic Acid (0.01M each).

Peak No.	R.T.(min)	Area (%)	Name of the Compound	Ref no	CAS No	Quality Index
1	12.2109	0.5957	Acetonitrile, 2,2'-iminobis-	2686	000628-87-5	2
2	19.6321	28.2046	.alpha.- Curcumene	66865	000644-30-4	99
3	19.8327	31.6563	Nortricyclyl bromide	41891	1000342-32-2	2
4	19.9441	2.3791	Xylopropamine	4	19.9441	2.3791
5	20.0407	17.722	.beta.-Bisabolene	5	20.0407	17.722
6	20.2932	17.3302	.alpha.- Farnesene	68740	020307-83-9	87
7	20.8653	0.7805	N-Benzyl-N-ethyl-p-isopropylbenzamide	141055	015089-22-2	43
8	21.6081	0.5241	3-Methyl-3-hexene	3364	003404-65-7	9
9	22.6927	0.7638	p-Toluic acid, 2-octyl ester	109790	1000293-34-0	35
10	24.0224	1.6516	1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione	151730	10	24.0224
11	24.7059	1.1547	Carbamic acid, methylnitroso-, ethyl ester	14490	000615-53-2	3

12	25.5007	0.7026	Propanamide	735	000079-05-0	4
13	27.2242	1.3254	Cyclopentadecanone, 2-hydroxy-	13	27.2242	1.3254
14	28.7024	1.0882	Phthalic acid, isobutyl	14	28.7024	1.0882
15	29.5493	0.962	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	15	29.5493	0.962
16	30.0545	0.4417	Bicyclo[3.1.1]heptane,6-methyl-2-methylene-6-(4-methyl-3-pentenyl)-, [1R-(1 alpha- 5alpha- , 6 .beta)]-	71190	339352-50-0	47

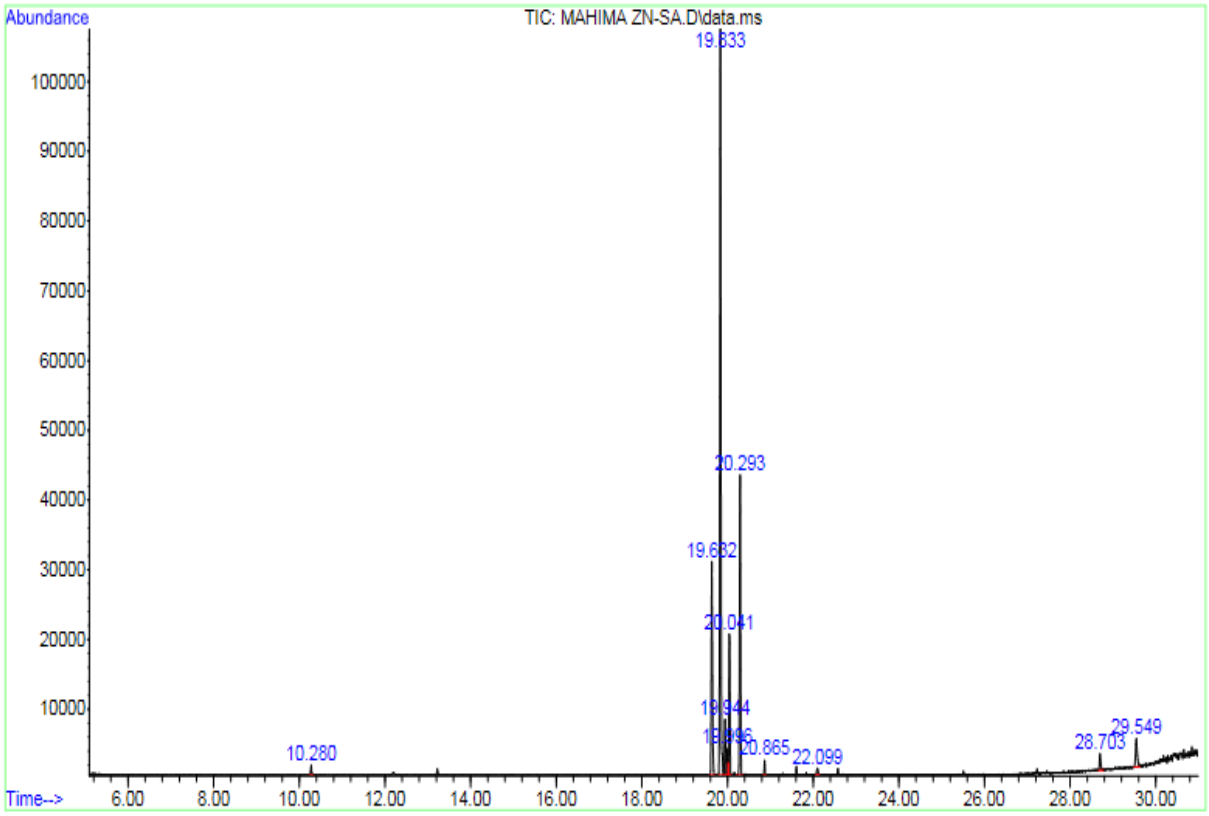

Figure 10.10 TIC of Hexane Fraction of *Z. officinale* cv-Mahima under foliar spray of $ZnSO_4.7\ H_2O$ and Salicylic acid (0.01M each).

Table 10.23 The TIC analysis data of *Z. officinale* cv-Mahima under foliar spray of $ZnSO_4.7\ H_2O$ and salicylic Acid (0.01M each)

Peak No.	R.T.(min)	First Scan	Max Scan	Last Scan	PK TY	Peak height	Corr.area	Corr. %max	% of total
1	10.280	692	699	706	rBV3	1451	2266	1.19	0.572
2	19.632	1951	1958	1966	rBV	30686	57029	29.94	14.401
3	19.833	1978	1985	1994	rBV	107080	190480	100.00	48.099
4	19.944	1994	2000	2004	rVV	8044	13350	7.01	3.371
5	19.996	2004	2007	2010	rVV	3807	6332	3.23	1.599
6	20.041	2010	2013	2019	rVB	20251	33502	17.59	8.460
7	20.293	2036	2047	2054	rVB	43069	71928	37.76	18.163
8	20.865	2120	2124	3139	rVB	2153	2623	1.38	0.662
9	22.099	2285	2290	3396	rBV	1012	2173	1.14	0.549
10	28.703	3174	3179	3188	rBV	2349	5159	2.71	1.303
11	29.549	3286	3293	3302	rBV2	4059	11171	5.86	2.821

Table 10.24 The phytochemical components identified from the hexane fraction of ginger rhizome *Z. officinale* cv-Mahima under foliar spray of $ZnSO_4.7\ H_2O$ and Salicylic Acid (0.01M each).

Peak No	R.(min)	Area (%)	Name of the Compound	Ref no	CAS No	Quality Index
1	10.2796	0.5722	Dodecane	39972	000112-40-3	97

2	19.6322	14.4008	.alpha.-Curcumene	66865	000644-30-4	99
3	19.8328	48.0994	Nortricyclyl bromide	41891	1000342-32-2	2
4	19.9442	3.3711	Xylopropamine	34152	075659-60-8	9
5	19.9962	1.5989	trans- β-Ocimene	16094	003779-61-1	38
6	20.0408	8.4598	β-Bisabolene	68571	000495-61-4	96
7	20.2934	18.163	.alpha.- Farnesene	68740	020307-83-9	87
8	20.8654	0.6624	N-Benzyl-N-ethyl-p-isopropylbenzamide	141055	015089-22-2	43
9	22.0985	0.5487	Diethyl Phthalate	85001	000084-66-2	97
10	28.7026	1.3027	Phthalic acid, isobutyl octadecyl ester	262268	1000309-06-1	53
11	29.5495	2.8209	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	71757	1000147-64-6	9

Table 10.25 The area percentage of common compounds present in the trial (SA AND $ZnSO_4$ .7 H_2O 0.01M each), in both the cultivars.

Sl.No.	Name of the Compound	Area% of Compound in *Z. officinale* cv- Varada (SA and $ZnSO_4$. $7H_2O$)	Area% of Compound in *Z. officinale* cv- Mahima (SA and $ZnSO_4$. $7H_2O$)
1	.alpha.- Curcumene	28.2046%	14.4008%
2	Nortricyclyl bromide	31.6533%	48.0994%
3	Xylopropamine	2.3791%	3.3711%
4	.beta.-Bisabolene	17.722%	8.4598%
5	.alpha.- Farnesene	17.3302%	18.163%
6	N-Benzyl-N-ethyl-p-isopropylbenzamide	0.7805%	0.6624%
7	Phthalic acid, isobutyl octadecyl ester	1.0882%	1.3027%

8	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	0.962%	2.8209%

The common compounds identified for the combination of foliar sprays (Zinc Sulphate Heptahydrate and Salicylic Acid 0.01M each) were .alpha.-Curcumene (28.2046% for Varada and 14.4008% for Mahima), Nortricyclyl bromide (31.6533% for Varada and 48.0994% for Mahima), Xylopropamine (2.3791% for Varada and 3.3711% for Mahima), .beta.-Bisabolene (7.593% for Varada and 8.4598 for Mahima), alpha- -Farnesene (17.3302% for Varada and 18.163% for Mahima), N-Benzyl-N-ethyl-p-isopropylbenzamide(0.7805% for Varada and 0.6624% for Mahima), Phthalic acid, isobutyl octadecyl ester (1.0882% for Varada and1.3027% for Mahima), 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5 (0.962% for Varada and 2.8209% for Mahima). **(Table 10.25)**.

10.7 A Comparative Analysis of Phytoconstituents Present In Varada And Mahima Under Different Stress Trials Including Control

In the present study, GC-MS analysis of Hexane fraction of ginger rhizomes of two cultivars namely *Zingiber officinale* cv-Varada and *Zingiber officinale* cv- Mahima under different foliar sprays were done. A total of 44 compounds identified at different retention times for different trials for both the varieties. Some compounds were identified for both the varieties, and some compounds were found unique to single cultivar (i.e., Varada or Mahima) for a single stress trial or sometimes more than one stress trial.

Table 10.26 The list of compounds identified through GC-MS analysis in two varieties of ginger

Sl.No	Name of the Compound	Varada	Mahima
1	Dodecane	-----	+++
2	Decanal	-----	+++
3	Tridecane	-----	+++
4	Acetonitrile 2,2'-iminobis-	+++	----
5	Naphthalene	+++	-----
6	.alpha. – Phellandrene	+++	-----

7	Cyclohexanol, 3-(aminomethyl)-3,5,5-	+++	-----
8	.alpha.-Curcumene	+++	+++
9	Nortricyclyl bromide	+++	+++
10	Propanedioicacid, (hydroxyimino)-, diethyl ester	+++	-----
11	Xylopropamine	+++	+++
12	trans-.beta.-Ocimene	+++	+++
13	.beta.-Bisabolene	+++	+++
14	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	+++	-----
15	.alpha. -Farnesene	+++	+++
16	N-Benzyl-N-ethyl-p-isopropylbenzamide	+++	+++
17	2,6-Octadienal,3,7-dimethyl-,(*Z*)	+++	-----
18	3-Methyl-3-hexene	+++	-----
19	Diethyl Phthalate	+++	+++
20	Phenyl chloroformate	-----	+++
21	Acetamide, 2-chloro-	+++	-----
22	p-Toluic acid, 2-octyl ester	+++	-----
23	1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione	+++	-----
24	Carbamic acid, methylnitroso-, ethyl ester	+++	-----
25	Propanamide	+++	-----
26	N-(3-Methylbutyl) acetamide	+++	-----
27	Cyclononasiloxane, octadecamethyl-	+++	-----
28	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	+++	-----
29	Cyclododecanol, 1-aminomethyl-	+++	-----

30	Cyclopentadecanone, 2-hydroxy-	+++	-----
31	3,4- dihydroxyphenylglycol, 4TMS	+++	+++
32	2-Myristynoyl-glycinamide	+++	+++
33	Hexadecanoic acid, methyl Ester	-----	+++
34	1-(2-Adamantylidene) semicarbazide	-----	+++
35	6-Shogaol	+++	+++
36	Phthalic acid, isobutyl octadecyl ester	+++	+++
37	4-Hydroxyphenyl pyrrolidinyl thione	+++	+++
38	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	+++	+++
39	Copaene	+++	-----
40	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	+++	-----
41	Bicyclo [3.1.1]heptane, 6-methyl-2-methylene-6-(4-methyl-3-pentenyl)-,[1R-(1 alpha- , 5alpha- , 6 .beta)]-	+++	-----
42	2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino)pyridine	+++	+++
43	Silicic acid, diethyl bis(trimethylsilyl) ester	+++	-----
44	Arsenous acid, tris(trimethylsilyl) ester	+++	-----

• (+++) Present, (---) Absent

The **Table 10.26** shows the compounds identified in both the varieties. A GC-MS analysis of *Zingiber officinale* cv-Varada was found to have 38 compounds out of the identified 44 compounds. Out of 44 compounds 23 compounds were found unique to Varada. For *Zingiber officinale* cv-Mahima out of 44 compounds 21 compounds were identified. 15 compounds were found in both the cultivar varieties. Out of these 15 compounds 12 compounds were identified in the same trials of both the cultivars, and 3 compounds in both the cultivars but in different stress trials. All compounds were present in Varada except Dodecane, Phenyl Chloroforamate, Hexadecanoic acid, methyl ester and 1-(2-Adamantylidene) semicarbazide. And these 6 compounds were unique to Mahima

(Decanol, Tridecane, Dodecane, Phenyl Chloroforamate, Hexadecanoic acid, methyl ester and 1-(2-Adamantylidene) semicarbazide).

Out of the 44 compounds identified through GC-MS analysis of two cultivar varieties of ginger, 12 compounds found in both the cultivars in the same trials. And they include .alpha.-Curcumene, Nortricyclyl bromide, Xylopropamine, .beta.-Bisabolene , alpha- -Farnesene, N-Benzyl-N-ethyl-p-isopropylbenzamide, 3,4- dihydroxyphenylglycol, 4TMS, 2-Myristynoyl-glycinamide, 6-Shogaol, Phthalic acid, isobutyl octadecyl ester, 4-Hydroxyphenyl pyrrolidinyl thione, and 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5. Though these 12 compounds identified in both the varieties, its concentration in terms of area percentage was different in each treatment.

10.8 Comparative Analysis of area percentage common compounds identified through GC-MS in Varada and Mahima in the same trials

A comparative analysis of compounds identified in similar trials in both the varieties is done. A toatal of 12 compounds identified in similar trials of Varada and Mahima.

Table 10.27 Area Percentage of .alpha. -Curcumene in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
.alpha.-Curcumene	**20.82**	**44.57**	**31.78**	**66.57**	**47.26**	**59.44**	**47.65**	**63.2**	**28.2**	**14.4**

From the (**Table 10.27)** it is very clear that .alpha.-Curcumene is a compound which is present in both the cultivar varieties under all the stress trials including control. In the cultivar variety *Zingiber officinale* cv- Varada, every stress increased the area percentage of .alpha.-Curcumene content. The control plants had an area percentage of 20.82% of .alpha.-Curcumene in it. Highest area percentage was observed in the case of plant replicates which are sprayed with Zinc Sulphate Heptahydrate in a concentration of 0.01M (47.65%), followed by Salicylic Acid 0.01M concentration (47.26%). 31.78% area was observed in the case of plants which are sprayed with DMSO (Control for SA foliar spray).

In the combination study, that is plants which are sprayed with SA and $ZnSO_4$ 7.H_2O

in 0.1M each had 28.20% of .alpha.- -Curcumene, which is also higher than that of the control. But in the case of Mahima, every stress influenced the area percentage of .alpha.-Curcumene positively except the combination trial (SA+$ZnSO_4$ 7.H_2O 0.01M each). Control plants 44.57% area percentage of .alpha.-Curcumene content. The highest area percentage was observed in plants sprayed with DMSO (66.57%). Followed by $ZnSO_4$ 7.H_2O 0.01M (63.20%), then by the stress trial SA 0.01M (59.44%). Least area percentage was observed in the combination study (SA+$ZnSO_4$ 7.H_2O 0.01M each) and is 14.40%.

.alpha.- Curcumene is one of the common compound identified at a retention time 19.632 min. There are a lot studies in ginger which reported the presence of .alpha.-Curcumene in it. It was previously mentioned in several research that used *Zingiber officinale* (Raina *et al.,* 2005; Menon *et al.,* 2007; Choudhari and Kareppa, 2013). The therapeutic properties of curcumene have been reported to include antibacterial, antioxidant, anti-inflammatory, analgesic, antipyretic, antimicrobial, and immunomodulatory properties (Carrasco *et al.,* 2009; Jeena *et al.,* 2013; Mesomo *et al.,* 2013; Dhanik *et al.,* 2017; López *et al.,* 2017; Singh *et al.,* 2019). Study of the effect of drying methods in volatile components of ginger was given by Ding *et al.,* 2012 and found that drying methods effectively increased or positively regulated the .alpha.-Curcumene content in Chinese ginger (*Zingiber officinale* Roscoe). From this results it can be concluded that all stress factors had an effect percentage of .alpha.-Curcumene in ginger.

Table 10.28 Area Percentage of Nortricyclyl bromide in different trials (in %).

Name of the Compound	**V C**	**M C**	**V DMSO**	**M DMSO**	**V SA**	**M SA**	**V $ZnSO_4$**	**M $ZnSO_4$**	**V Zn+SA**	**M Zn+SA**
Nortricyclyl bromide	**31.85**	**2.50**	**18.11**	**4.13**	**8.81**	**14.13**	**1.84**	**1.19**	**31.65**	**48.09**

The Next compound identified is Nortricyclyl bromide at a retention time of 19.833. In the cultivar variety Mahima every stress influenced the production of this secondary metabolite in a positive manner except in the case of replicates which are sprayed with Zinc Sulphate heptahydrate in 0.01M concentration. In Mahima control plants showed an area

percentage of 2.50% of Nortricyclyl bromide. Highest concentration of Notricyclyl bromide was observed in the combination of both the foliar sprays (48.09%), followed by 14.13% for the trial SA 0.01M, then by 4.13% for DMSO and the least observed in the replicates sprayed with $ZnSO_4$ 7.H_2O. In the case of Varada control plants and foliar stress combination had almost a similar area percentage for Nortricyclyl bromide (31.85% for Control and 31.65% for Zn+SA 0.01M each). In Varada also least area percentage for Nortricyclyl bromide was observed in the replicates sprayed with $ZnSO_4$ 7.H_2O (1.84%) **(Table 10.28)**.

Nortricyclyl bromide was previously reported by many scientists. GC-MS analysis of *Amygdalus spinosissima* extract by Farahani *et al.* 2022. In a comparative analysis of essential oils of Curcuma longa L., *Zingiber officinale* Roscoe and *Xylopia aethiopica* (Dunal) A. Rich**.** by Okhale *et al.* (2021). In their study they reported the presence of Norticyclyl bromide in the Z. *officinale* essential oil. The same compound has been previously reported by different authors in different plants. In *Laggera pterodonta* and *Laggera aurita* by Dantanko & Malann, (2020).

Table 10.29 Area Percentage of Xylopropamine in different trials (in %).

Name of the Compound	**V C**	**M C**	**V DMSO**	**M DMSO**	**V SA**	**M SA**	**V $ZnSO_4$**	**M $ZnSO_4$**	**V Zn+SA**	**M Zn+SA**
Xylopropamine	**2.1**	**11.6**	**2.05**	**1.37**	**1.94**	**2.75**	**2.31**	**1.64**	**2.37**	**3.37**

Xylopropamine is the next compound identified at a retention time 19.944. It was previously reported in the study by Malathia *et al.* (2022). GC-MS analysis of bioactive compounds of *Curcuma longa* Linnaeus (*Zingiberaceae*) rhizome extract by Arivoli *et al.* 2019 reported the identification of Xylopropamine by GC-MS analysis. In the present study Xylopropamine was found to be higher in terms of its area percentage in the control plants of Mahima (11.64%). And the area percentage was found decreasing on every stress. So, it is very clear that in the case of *Zingiber officinale* cv-Mahima stress negatively regulated the production of Xylopropamine concentration in it (3.37% for Zn+SA, followed by 2.75% for SA 0.01M then 1.64 for $ZnSO_4$ and the last 1.37% for DMSO). But in the case of Varada there is only a slight difference noticed between the area percentage of control

and stress trials. 2.10% for control and for the stress trials 2.37% for combination of foliar sprays, 2.31% for $ZnSO_4$ 2.05% for DMSO and the last 1.94% for SA. **(Table 10.29)**.

Table 10.30 Area Percentage of .beta.-Bisabolene in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
.beta.-Bisabolene	**8.71**	**19.36**	**9.60**	**9.55**	**8.90**	**10.20**	**13.44**	**11.59**	**17.12**	**8.45**

.beta-Bisabolene was found to be positively regulated in the case of Varada **(Table 10.30)**. Control plants had an area percentage of 8.71%. Plants under the foliar spray of SA 0.01M had an area percentage of 8.90% which was followed by DMSO 9.60% then by the foliar spray of zinc Sulphate Heptahydrate 0.01M 13.44% and the highest area percentage was obtained in the combination of two foliar sprays, 17.12%. So it can be inferred that stress altered the production of .beta-Bisabolene in ginger rhizomes. Here, in, the case of the cultivar variety Varada every stress positively upregulated the production of .beta-Bisabolene in it.

.beta-Bisabolene was identified at a retention time 20.04. There are many studies on this very valuable phytocompound. The essential oils from *Zingiber officinale* frequently include .beta-bisabolene. Murugesan *et al.* (2020) and Singh *et al.* (2008) had previously reported finding beta-Bisabolene in *Zingiber officinale*. It was also discovered to be in *Cuminum cyminum* essential oils (Singh *et al.*, 2006). According to several studies (Singh *et al.*, 2008; Jeena *et al.*, 2013; Deepak *et al.*, 2017; Murugesan *et al.*, 2020), .beta-Bisabolene has therapeutic properties that include anti-arthritic, antioxidant, antibacterial, anti-inflammatory, and ant nociceptive potential. Pandey *et al.*, reported in 2020 that it is found to be useful against common respiratory disorders. The anti-cancerous property of .beta-bisobolene was reported by Yeo *et al.* (2016).

Table 10.31 Area Percentage of .alpha.-Farnesene in different trials (in %).

Name of the Compound	V Control	M Control	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
.alpha.-Farnesene	**10.32**	**8.53**	**16.13**	**15.99**	**17.57**	**19.45**	**12.75**	**12.7**	**17.33**	**18.16**

In the case of .alpha.- Farnesene at a retention time of 20.293 was the one of the common compound reported in stress trials. Here also it is evident from the table that this compound has been upregulated, or its production has been increased on subject to various stress under study **(Table 10.31)**. A trend can be observed in both the cultivars. Control plants had an area percentage of 10.32% for Varada and 8.53% for Mahima. Plants under the foliar spray of Zinc Sulphate Heptahydrate 0.01M had an area percentage of 12.75% for Varada and 12.71% for Mahima. Next to this, a higher area percentage was noticed in the case of plants sprayed with DMSO; 16.13% for Varada and 15.09% for Mahima. Combination of foliar sprays allowed for the identification of the compound with an area percentage of 17.13% for Varada and 18.16% for Mahima. Salicylic Acid 0.01M had the highest area percentage for both the cultivars; 17.57% for Varada and 19.45% for Mahima. Ding *et al.*, (2012) earlier reported the presence of .alpha. -Farnesene in *Zingiber officinale*. According to Huang *et al.* (2010) and Lu *et al.* (2010), the chemical has antioxidant, anticancer, antibacterial, and hemolytic properties. Olaleye & Momoh, (2022) reported the presence of .alpha.-Farnesene in the secondary metabolite profiling of ginger rhizome using GC-MS analysis.

Table 10.32 Area Percentage of N-Benzyl-N-ethyl-p-isopropylbenzamide in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
N-Benzyl-N-ethyl-p-isopropylbenzamide	-	-	-	-	-	-	-	-	**0.780**	**0.66**

N-Benzyl-N-ethyl-p-isopropylbenzamide is a compound identified for both the Varada and Mahima under the combination both the foliar sprays (0.01M each) with an area percentage of 0.780% for Varada and 0.66% for Mahima. Apart from this, it is a common compound identified in Varada under all the trials except in the foliar spray of $ZnSO_4$ $7H_2O$. In the control plants it had an area percentage of 0.563% in plants sprayed with DMSO the area percentage is 0.7426% and in the case of variety under SA 0.01M stress it had an area percentage of 0.7238% **(Table10.32).**

Prior research has found that *Pandanus conoideus* and *Bulbine asphodeloides* both contain N-Benzyl-N-ethyl-p-isopropylbenzamide in their ethanolic extracts (Rohman *et al.*, 2012; Otang-Mbeng and Sagbo, 2019). Falowo *et al.* (2019) reported that the substance also exhibited antioxidant properties.

Table 10.33 Area Percentage of 3,4- dihydroxyphenylglycol, 4TMS in different trials (in %).

Name of the Compound	**V C**	**M C**	**V DMSO**	**M DMSO**	**V SA**	**M SA**	**V $ZnSO_4$**	**M $ZnSO_4$**	**V Zn+SA**	**M Zn+SA**
3,4-dihydroxyphenylglycol, 4TMS	**-**	**-**	**-**	**-**	**-**	**-**	**0.93**	**1.90**	**-**	**-**

A retention time of 27.425 allowed for the identification of the next compound 3,4-dihydroxyphenylglycol, 4TMS. It was identified in both the cultivars under the foliar spray of $ZnSO_4$ 7 H_2O at a concentration of 0.01M. 0.93% area percentage of 3,4-dihydroxyphenylglycol, 4TMS identified for Varada and 1.90% for Mahima. Apart from these two trials Varada under DMSO stress had an area percentage of 1.28% (**Table 10.33**). It had previously been documented in methanol extract of the aerial portions of *Ajuga orientalis* and hexane extract of the fruit of *Moringa oleifera* (Shunmugapriya *et al.*, 2017; Oran *et al.,* 2022). Antioxidant activity and cytotoxicity are two of the pharmacological properties of the 3,4-dihydroxyphenylglycol, 4TMS derivative (Oran *et al.*, 2022).

Table 10.34 Area Percentage of 2-Myristynoyl-glycinamide in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
2 Myristynoyl glycinamide	-	-	**0.33**	**1.47**	-	-	-	-	-	-

At a retention time of 27.82 allowed for the identification of 2-Myristynoyl-glycinamide in both Varada and Mahima. 0.33% of area identified for Varada and 1.47% for Mahima **(Table 10.34)**. Gajendiran *et al.* (2017) observed the presence of 2-myristynoyl-glycinamide in the methanol and n-hexane extract of *Mutinus elegans*. According to Liu & Tian, (2014); El-Zawawy *et al.* (2020); Ginting *et al.* (2021) and Poyil *et al.* (2022), this chemical compound has significant anti-asthmatic, antibacterial, antioxidant, anticancer, and antimicrobial effects.

Table 10.35 Area Percentage of 6-Shogaol in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
6-Shogaol	**1.52**	**1.29**	-	-	-	-	-	-	-	-

The next compound was 6-Shogaol with an area percentage of 1.52% for varada and 1.29% for Mahima in control plants **(Table 10.35)**. Shogaol is the dehydration product of gingerol and a major pungent principle of ginger. Varada under the foliar spray of Salicylic acid and its control DMSO also had 6-Shogaol with an area percentage of 1.7109% and 1.5047% and it has got a diverse therapeutic activity treating from Nausea to cancer Roli *et al.* 2020. Anti-cancerous activity of shogaol was studied by Qi *et al.* 2015.

Table 10.36 Area Percentage of Phthalic acid, isobutyl octadecyl ester in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
Phthalic acid, isobutyl octadecyl ester	-	-	-	-	-	-	**0.858**	**2.48**	**1.08**	**1.30**

At a retention time of 28.70 min. Phthalic Acid, isobutyl octadecyl ester has been identified for both Varada and Mahima under the foliar sprays of Zinc Sulphate Heptahydrate 0.01M and a combination of foliar sprays of Zn+ SA 0.01M each. An area percentage of 0.858% for Varada and 2.48% for Mahima for Zinc Sulphate Heptahydrate foliar spray and for combination of foliar sprays yielded 1.08% for Mahima and 1.30% Varada **(Table 10.36)**. It has previously been found in Methanoilic extracts of *Cardiospermum halicacabum* and *Cyperus iria* (Shaheed *et al.,* 2019; Bhagat & Bhuktar, 2020). According to Hailu *et al*. (2017), isobutyl octadecyl ester of phthalic acid has antibacterial properties.

Table 10.37 Area Percentage of 4-Hydroxyphenyl pyrrolidinyl thione in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
4-Hydroxyphenyl pyrrolidinyl thione	**1.46**	**1.85**	-	-	-	-	-	-	-	-

At a retention time of 29.54 yielded a common compound in control plants. 4-Hydroxyphenyl pyrrolidinyl thione has an area percentage of 1.46% in cv-Varada and

1.85% in Mahima **(Table 10.37)**. This compound was previously reported by Anyanwu *et al.* (2020) in *Ceratotheca sesamoides, Jatropha tanjorensis, Mucuna flagellipes, Pterocarpus mildbraedii and Piper guineense*.

Table 10.38 Area Percentage of 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5in different trials (in %).

Name of the Compound	V C	M C	V DMSO	M DMSO	V SA	M SA	V $ZnSO_4$	M $ZnSO_4$	V Zn+SA	M Zn+SA
2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	-	-	-	-	-	-	**0.73**	**1.787**	**0.962**	**2.8209**

A retention time of 29.549 allowed the identification of 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5. Which was identified in the Zinc Sulphate Heptahydrate foliar spray 0.01M (0.73% for cv-Varada and 1.787% for cv-Mahima) and combination of foliar sprays of Zinc Sulphate heptahydrate and Salicylic Acid in 0.01M each. 0.962% area percentage obtained for Varada and 2.8209% obtained for Mahima **(Table 10.38)**. It was previously reported by Ahuokpeme *et al.* 2020 in the GC-MS analysis of *Cinnamon* bark.

10.9 Analysis of Area Percentage Common Compounds Identified through GC-MS in Varada And Mahima in the different Trials

trans-.beta.-Ocimene The first compound which is found in both the Varieties but under different stress trials. trans-.beta.-Ocimene was identified at a retention time of 19.966 min. In *Z. officinale* cv-Varada plants which are sprayed with DMSO (0.02%) showed an area percentage of 5.91% and trials which are sprayed with SA 0.01M had an area percentage of 6.2533%. So in Varada trans-.beta.-Ocimene was identified only in trials for DMSO which is a control for Salicylic acid trial, and is found that SA 0.01M has a higher area percentage than its control Mahima. In Mahima, it is not observed in SA trial and its control. Instead it is observed in combination trial ($ZnSO_4$ 7 H_2O+ SA 0.01M each) with an area percentage of 1.5989%. This compound was previously reported from Essential Oils of *Laggera pterodonta* and *Laggera aurita* by Dantanko & Malann, (2020).

Diethyl Phthalate is the next compound (R.T. 22.0985min) which is present in both the cultivar varieties but in different trials. In *Z. officinale* cv-Varada it is identified in DMSO and SA trials, with an area percentage of 0.4531% and 0.3385%. In *Z. officinale* cv-Mahima it is noticed in the plants which are sprayed with $ZnSO_4$ 7 H_2O 0.01M and $ZnSO_4$ 7 H_2O+ SA 0.01M each. Its area percentages were 0.6461% and 0.5487%.%. Diethyl phthalates were discovered during the phytochemical evaluation of different plants using GC-MS (Bhimba *et al.,* 2010; Gopu *et al.,* 2021). They are environmental pollutants and plasticizers (Hussein *et al.,* 2015).

2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino) pyridine is the compound identified in *Z. officinale* cv-Varada and *Z. officinale* cv-Mahima at a retention time of 30.114min. In control plants of Varada it had an area percentage of 0.3585% and in plants sprayed with $ZnSO_4$ 7 H_2O 0.01M had an area percentage of 3.462%. And this compound was previously reported as a bioactive constituent from *Morinda lucida* which also possess anti-leukemic potential (Adetutu *et al*., 2022).

10.10 Analysis of compounds present in Varada in different stress trials

Acetonitrile 2,2'-iminobis- is the first compound identified at a retention time of 12.04 in different trials in *Zingiber officinale* cv-Varada. In the control plants it had an area percentage of 0.529%. In plants sprayed with DMSO the area percentage has increased and is 0.6781%. Plants sprayed with Salicylic acid 0.01M had an area percentage of 0.5499% and cultivar which are sprayed with $ZnSO_4$ $7H_2O$ had an area percentage of 0.553%. All stress trials found to have increased the concentration of Acetonitrile 2,2'-iminobis- in the cultivar variety Varada. Acetonitrile 2,2'-iminobis- is previously reported from the Ethanol extract of *Chromolaena odorate* leaf (Aikoye, 2020). Another study by Gul *et al.* 2022 in the ethanolic root extract of *Berberis baluchistanica* by GCMS analysis also found to have Acetonitrile 2,2'-iminobis.

Naphthalene at a retention time of 12.6196 allowed for the identification of Naphthalene in Varada under the stress condition of plants which are sprayed with DMSO (0.2%). It gave an area percentage of 0.3358%. According to Azuma *et al.* (1996) and Senthilkumar *et al*. (2012), naphthalene was shown to be the main ingredient in the flower extracts of Magnolia sp. and *Trichilia connaroides,* respectively.

.alpha.- Phellandrene was identified at a retention time of 13.22min. It was found in the GC-MS analysis of hexane fractions of dried rhizomes of Varada under the trials DMSO (0.2%) and SA 0.01M. 0.402% of alpha- - Phellandrene was found in the case of DMSO trials, and 0.2969% was identified for plants which are sprayed with SA 0.01M. It was previously reported by Singh *et al.* 2010 in *Curcuma longa*. Volatile compound analysis of *Piper guineense* seeds and leaves by Ojinnaka *et al.* 2016 also reported this compound.

Cyclohexanol, 3-(aminomethyl)-3,5,5-trimethyl- identified at a retention time of 18.756 min, is a compound identified from the GC-M S analysis of *Zingiber officinale* cv-Varada under the foliar spray of DMSO (0.2%). In ethanol root extracts of *Rauwolfia vomitori*, Johnson *et al.* (2020) reported cyclohexanol, 3-(aminomethyl)-3,5,5-trimethyl-.

Propanedioicacid, (hydroxyimino)-, diethyl ester the next compound identified at a retention time of 19.871min with an area percentage of 0.721%. It was identified from the peak produced in TIC of *Zingiber officinale* cv-Varada under the stress condition of foliar spray of $ZnSO_4$ 7.H_2O (0.01M). It was reported in the study conducted by Kumar *et al.*,2015. The compound was found in the GC-MS analysis of endophytic microbes found in *Azadirachta indica*. This compound is though not a plant origin; this can be treated as an impurity in the sample.

1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene- is the compound identified at a retention time of 20.337 min. and with an area percentage of 10.3229% in control plants of the variety Varada. This compound was previously reported in ginger by Ding *et al.* (2012).

2,6-Octadienal,3,7-dimethyl-,(*Z*) A retention time of 21.2962 min allowed the identification of 2,6-Octadienal,3,7-dimethyl-,(Z) in *Zingiber officinale* cv-Varada under the foliar spray of DMSO. An area percentage 0.3185% has been detected for the hexane fraction. This compound was previously reported by Ding *et al*. (2012) in his study on Effect of drying methods on volatiles of Chinese ginger (*Zingiber officinale* Roscoe). Oyinlola *et al.* (2022) reported that the compound 2,6-Octadienal,3,7-dimethyl-,(Z) which is present in ginger has antibacterial activity against certain *Pneumococcal* bacteria

3-Methyl-3-hexene is a compound which is a compound identified at retention time of 21.60min. It was mainly found in the hexane fraction of Varada control plants, and plants which are sprayed with DMSO, and lastly in the combination study of two foliar spray stresses. With an area percentage of 0.3966% for control, 0.508% for DMSO, and 0.524%

for the combination studies. According to Rudziska *et al.* (2009), 3-methyl-3-hexene is one of the primary byproducts created during the thermo-oxidative breakdown of phytosterols. The 3-methyl-3-hexene found in the hexane ginger may therefore also be a degradation product which may be formed as a result of shade drying during the study.

Acetamide, 2-chloro- was identified at a retention time of 22.57 min in the GC-MS analysis hexane fractions of dried rhizomes of the variety Varada under the trials of DMSO and SA. Its area percentage was 0.5569% and 0.4996% each. It was previously reported in the study of bioactive screening of *Berberis baluchistanica* by Gul *et al.* (2022).

p-Toluic acid, 2-octyl ester identified at a retention time of 22.69min in the cv- Varada. The compound was identified in two trials, namely in plants which are sprayed with DMSO with an area percentage of 0.4431% and in the combination trial with an area percentage of 0.7639%. The compound was previously reported from the GC-MS analysis of *Syzygium calophyllifolium* Walp by Vignesh *et al.* (2013), they also studied its antimicrobial and cytotoxic activity.

1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione 1.656 area percentage of1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-dione was identified in the combination trial of Varada at a retention time of 24.02min. This compound was previously reported in the GC-MS analysis of *Zingiber officinale* by Olaleye & Momoh, (2022).

Carbamic acid, methyl nitroso-, ethyl ester was the compound identified at a retention time of 24.57min with an area percentage of 1.547% in the combination of foliar sprays. It is not usually found as a part of phytocompounds. So, it is considered as an impurity in the sample. It is usually found in agricultural chemicals; it is usually considered as a carcinogenic nitroso compound (Elespuru & Lijinsky, 1973).

Propanamide A retention time of 25.50 min allowed for the identification of Propanamide in different trials of Varada. 0.5725% was the area percentage in control, where as 0.737% was obtained from SA 0.01M, and in the combination of foliar sprays the area percentage was 0.7026%. Phenolic propenamide is previously reported by Oloyede *et al.* (2020) in *Acalypha hispida* (Burn. F).

The next compound **N-(3-Methylbutyl)acetamide**. This compound which is identified at a retention time of 25.508 and with an area percentage of 1.0918% in DMSO trials and

3.167% in plants which are sprayed with $ZnSO_4$ 7.H_2O. This compound was previously reported by Yang *et al.* 1998 in *Lentinus edodes*

Cyclononasiloxane, octadecamethyl- was the next compound at a retention time of 26.9725 with an area percentage of 0.686%. It is also tentatively identified from the aqueous extracts of *Catharanthus roseus* leaves and its antioxidant and antimicrobial activities were also studied by Syeda & Riazunnisa, (2020). The antibacterial activity was reported by Dehbashi *et al.* (2015) and the anti-inflammatory activity was reported by Hamid *et al.* (2018).

Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone was the next compound identified at a retention time of 27.15min in plants (*Zingiber officinale* cv-Varada) sprayed with 0.02% of DMSO. It has an area percentage of 1.1295%. It is a common volatile impurity reported from contaminated water Longo *et al.* (2021).

Cyclododecanol, 1-aminomethyl- This is another compound identified in Varada DMSO trials at a retention time of 27.1871min. with an area percentage of 1.0118%. This compound was previously reported from the fruit epicarp of *Cola rostrata* K. Shum by GC-MS analysis (Ajayi *et al.*, 2022).

Cyclopentadecanone, 2-hydroxy- This is a common compound identified at retention time of 27.2317 in all the stress trials of *Z. officinale* cv-Varada. In Control it has an area percentage of 1.1098% and found to be increasing its concentration in all the stress trials. 8.956% was observed in plants sprayed with $ZnSO_4$ $7H_2O$ 0.01M. DMSO trials showed an area percentage of 3.536% and SA foliar spray trials had an area percentage of 1.3476% and combination of foliar sprays had an area percentage of 1.3254%. It is a ketone compound previously reported in the genus *Grewia* (Tiliaceae) Kumar, 2022. It was also reported from *Albizia* spp. and found to have antioxidant and anti-bacterial activity Ghosh *et al.* (2021).

Copaene was identified at a retention time of 29.720 min. with an area percentage of 0.858% in the *Z. officinale* cv-Varada under foliar spray of $ZnSO_4$. 7 H_2O (0.01M). It is a phytocompound which is included in the class of sesquiterpenes and was previously reported by Ojinnaka *et al.* 2016 in he leaves and seeds of *Piper guineense* using gas chromatography-massspectrometry (GC-MS). It was also reported in *Z. officinale* by Ding *et al.* (2012); Shareef *et al.* (2016) and Okhale *et al.* (2021).

1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide A retention time of 30.0398 min allowed for the identification of 1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide in control plants of *Z. officinale* cv-Varada with an area percentage of 0.4575%. It is usually found as component of organic municipal solid waste (Lin *et al.,* 2010) and so it is considered as an impurity.

Bicyclo[3.1.1]heptane, 6-methyl-2-methylene-6-(4-methyl-3-pentenyl)-, [1R-(1 alpha-, 5alpha- , 6 .beta)]- identified at a retention time of 30.0545 min. with an area percentage of 0.4417% in the combination stress trial of *Z. officinale* cv-Varada. This compound previously reported by Ding et al. 2012 in *Z. officinale* under different drying conditions.

Silicic acid, diethyl bis(trimethylsilyl) ester is the next compound identified at a retention time of 30.4261min. in control plants with an area percentage of 0.6539%. It is a compound previously reported by Gul *et al.* (2022) in *Berberis baluchistanica* Ahrendt Root.

Arsenous acid, tris(trimethylsilyl) ester is a compound obtained at a retention time of 30.434 min. and with an area percentage of 0.683% in *Z. officinale* cv-Varada under SA 0.01M foliar spray. It was previously reported in bioactive compound analysis in *Salvia hispanica* by Al-Alwani & Mohammed, (2022).

10.11 Analysis of compounds present in Mahima in different stress trials

Dodecane which is identified at a retention time of 10.27 min was found in *Z. officinale* cv- Mahima under the combination of foliar sprays $ZnSO_4$. 7 H_2O and Salicylic Acid 0.01M each. An area percentage of 0.5722 was observed for this compound. Olaleye & Momoh, (2022) previously reported this compound in *Z. officinale*.

Decanal, the first compound found in the GCMS analysis of dried ginger rhizome (*Zingiber officinale* cv- Mahima) under the stress trial $ZnSO_4$. 7 H_2O foliar spray (0.01M) at a retention time of 11.5053 min and at an area percentage of 0.6516%. It was previously reported by Zhao *et al.* (2015) in the study of analysis of volatile oils from ginger by GC-MS. In a head space GC-MS analysis of ginger rhizome by Yu *et al.* 2022 also reported the presence of the compound Decanal in the ginger rhizome. The GC-MS analysis of baby ginger Paocai also found that the Decanal which is present in it is responsible for the aroma of baby ginger Paocai that had undergone a mild fermentation procedure (Luo *et al.*, 2017).

Tridecane is the second compound identified in Mahima under the stress trial $ZnSO_4$. 7 H_2O foliar spray (0.01M) trial. It has an area percentage of 0.6841% at a retention time of

12.1961 min. It was previously reported by Olaleye & Momoh, (2022). There are so many studies in ginger which identified the presence of tridecane in the ginger rhizome (; Bartley & Foley, 1994; Pizon *et al.*, 2018; Hamada *et al.*, 2018).

Phenyl chloroformate was another unique compound identified in Mahima under the stress trial $ZnSO_4$ 7.H_2O a retention time of 26.9717 min and with an area percentage of 0.7865. It was previously reported by Rehuman *et al.* (2020).

Hexadecanoic acid, methyl ester is the compound identified for *Z. officinale* cv-Mahima control plants at a retention time of 27.841min with an area percentage of 5.6394%. It was earlier discovered as a substantial component in the medicinal plant *Aframomum melegueta*, which is a member of the Zingiberaceae family (Agim *et al.*, 2017). It is also a bioactive component discovered in several plants. According to Chandrasekaran *et al.* (2011); Abubakar & Majinda, (2016); Shaaban *et al.* (2021) and Sharif *et al.* (2021), hexadecanoic acid, methyl ester has biological activities including anti-asthmatic, antibacterial, antifungal, and antimicrobial properties.

1-(2-Adamantylidene)semicarbazide is the compound identified at a retention time 28.279 min in control plants of *Z. officinale* cv-Mahima. It had an area percentage of 5.12%. This compound was previously reported by Saravanan *et al.* 2022 in their study on GC–MS Analysis, molecular docking and pharmacokinetic properties of phytocompounds from *Solanum torvum* unripe fruits and its effect on breast cancer target protein

Brief Summary

Stress played a crucial role in the production of different kinds of volatile compounds in ginger. With the help of area percentage quantity of different volatile compounds has been identified and recorded. A total of 44 compounds has been reported from all the trials including control and each phytocompounds under each treatment has been extensively studied with the help of libraries and available literatures. A strong comparative analysis between two selected cultivars was made on the basis of type and concentration of phytoconstituents were present in each trail.

SUMMARY AND CONCLUSIONS

In the present study a comparative analysis of stress induced changes in morphological attributes and in the production of secondary metabolites in two selected cultivar varieties of ginger (*Zingiber officinale* cv-Varada and *Zingiber officinale* cv-Mahima) was evaluated. All the data reported in this study were based on field experiments done under protective greenhouse condition. Under this controlled condition, the effect of applied stress signals (both foliar sprays and drought; later maintained by withholding the water supply) in the morphological characters as well as secondary metabolite production were precisely studied and analyzed.

Initially 15 trials were kept including controls (plants sprayed with DW is taken as one control and for SA, foliar spray of 0.02% DMSO was taken as another control). Stress considerably reduced the morphological attributes of both the ginger varieties. The morphological characters studied and it includes plant height, number of tillers per plant and number of leaves per tiller. Control plants of both the varieties had a greater plant height in Cm than the other stress attributes. One-way ANOVA was performed for all the trials for both the varieties to study the effect of foliar sprays and Homogeneity by Duncan test using SPSS software were conducted and the results were represented as Mean± Standard Error (S.E). *Zingiber officinale* cv- Mahima had a plant height of 85.98±0.538 Cm and *Zingiber officinale* cv-Varada 95.23±0.66 Cm. When comparing two varieties, cultivar Varada was fairly larger than Mahima. Plants which were completely withdrawn water supply during the stress application period were short plants compared to other trials. When it is combined with the foliar sprays, there was a significant increase in the plant height.

From the results it can be concluded that the foliar sprays had lessened the negative impact of drought stress. In the case of number of tillers per clump and number of leaves per clump, a same trend was observed. Number of tillers were manually counted and found that control plants had higher number of tillers and tiller number significantly decreased in each trial. The same was observed in the case of number of leaves also.

A preliminary phytochemical screening of both the varieties were done to have a peripheral knowledge about the classes of phytocompounds were present in ginger rhizome. Phytochemical screening revealed that ginger rhizomes possess a great number of

phytochemicals. Keller Killiani's test for glycosides and the foam tests for saponins both yielded negative findings out of the twelve tests were performed for the analysis of preliminary phytochemicals. The presence of alkaloids, coumarins, flavonoids, quinines, phenols, tannins, terpenoids, steroids, carbohydrates, and proteins in the methanolic extract was evident.

The quantitative estimation of Total Phenolics Content (TPC), Total Flavonoid Content (TFC) and the comparative analysis of antioxidant assay by DPPH showed that stress signals increased the concentration of both TPC and TFC in both the varieties when comparing with the control plants which eventually resulted in the increase in antioxidant activity of the same. Increasing concentration of foliar spray of Salicylic Acid had a positive impact on the Total Phenolic Content when comparing with the foliar application of Zinc Sulphate of same concentration. A common trend was observed in both the cultivar varieties. Biological replicates sprayed with Salicylic Acid (SA) 0.01 M showed higher total phenolic content in both cv-Varada and cv-Mahima (43.86±0.441 mg GAE/g and 59±0.598 mg GAE/g each). Increasing concentration of both SA and $ZnSO_4.7H_2O$ in cv-Mahima and cv-Varada showed an increase in the concentration of total phenolic content in response to an increase in the concentration of respective foliar sprays. While in combination with drought, both the foliar sprays showed a negative cross tolerance. It is clear from the experiment that total flavonoid was found to be increasing while increasing the concentration of both the foliar sprays. But the highest total flavonoid content was observed in the trials which were sprayed with both the foliar sprays in its highest concentration 12.83±0.23 mg QE/g and 13.02±0.25 mg QE/g for cv-Mahima and cv-Varada respectively. In combination with drought, the total flavonoid content was found to be higher in comparison with solitary application of both the sprays. Antioxidant activity estimated by the radical scavenging activity by DPPH was found to be higher in the case of cv-Varada than that of cv-Mahima. When coming in to the comparison with respect to stress treatments, trials which were sprayed with SA in its higher concentration was found to have higher antioxidant activity than that if any other trials (70.667±0.05% for cv-Mahima and 66.66±0.33% unit for cv-Varada). Increasing concentration of both the foliar sprays had a positive impact on the antioxidant activity. The trend was similar to that of total phenolic content estimated for different stress trials. From this it can be inferred that

total phenolic content and antioxidant activity is directly proportional in this case. In combination with drought both the stress treatment showed a decrease in the antioxidant activity. In foliar spray combination trials both the foliar sprays in its higher concentration showed high antioxidant activity. Here, a positive relationship between antioxidant activities and total phenolic contents was also observed.

HPLC analysis of 15 trials of both varieties were done to estimate the quantity of 6-gingerol in rhizomes. Here also, the results indicated that stress considerably increased the concentration of 6-gingerol in trials. The control kept for SA, i.e., plants which are sprayed with 0.02% of DMSO had highest percentage of 6-gingerol in both the varieties than any other trials. 2.45% of gingerol was observed for cv-Mahima and 1.56% was obtained for cv-Varada. When comparing two varieties cv-Mahima contained higher quantity of 6-gingerol than that of cv-Varada. The percentage of 6-gingerol was lowest in the case of plants which are completely withdrawn the water supply (Drought control). Combination of foliar sprays with drought decreased the effect of negative impact of drought by increasing the concentration of 6-gingerol in their respective rhizomes.

The results were narrow down to perform the estimation of total chlorophyll content, to quantify the PAL enzyme activity and for GC-MS analysis to list down components and its concentration in different trials. The estimation of carotenoid content under different trails were monitored. Comparing Carotenoids with chlorophyll content in the sample, Carotenoids was found to be less in control plants under study. Carotenoids was found to be high in plants under the foliar spray of DMSO, which is taken as a control for Salicylic Acid stress, 878±67 µg/g for cv-Varada and 880±52 µg/g for Mahima, followed by SA 0.01M 823±89 µg/g for cv-Varada and 800±124 µg/g for cv-Mahima which is almost similar to that of plants which were sprayed with $ZnSO_4.7H_2O$. As expected all crude extracts of control as well as the stress trials showed PAL activity. The results of these assays demonstrated that the PAL activity was found to be higher in leaves rather than rhizome. In addition, the highest activity was found in leaves which was kept as control. That is the one which has not undergone any stress treatment (47.824±0.6 U/mg and 74.496±0.03 U/mg respectively). Among the two ginger varieties the PAL activity was higher in cv-Varada as compared to cv-Mahima. This high level of PAL activity in developing ginger leaves was unexpected because the shoots and rhizomes should have

significant PAL activity because lignin is produced in the growing xylem, whereas ginger leaves are not known to contain high levels of flavonoids, lignins, or other commonly found phenylpropanoid pathway derived compounds.

GC-MS analysis of 5 trials of two varieties indicated that there was a total of 44 compounds identified at different retention times in both the varieties. GC-MS analysis of *Zingiber officinale* cv-Varada was found to have 38 compounds out of the identified 44 compounds. Out of 44 compounds 23 compounds were found unique to cv-Varada. For *Zingiber officinale* cv-Mahima out of 44 compounds 21 compounds were identified. 15 compounds were found in both the cultivar varieties. Out of these 15 compounds 12 compounds were identified in the same trials of both the cultivars, and 3 compounds in both the cultivars but in different stress trials. All compounds were present in Varada except Dodecane, Phenyl Chloroforamate, Hexadecanoic acid, methyl ester and 1-(2-Adamantylidene) semicarbazide and these 6 compounds were unique to Mahima (Decanol, Tridecane, Dodecane, Phenyl Chloroforamate, Hexadecanoic acid, methyl ester and 1-(2-Adamantylidene) semicarbazide). GC-MS analysis revealed that there is a difference in concentration of phytochemicals present in each trial. Higher number of phytochemicals observed in Varada than Mahima, and number of phytocompounds was lesser in stress trials comparing with the control.

Out of the 44 compounds identified through GC-MS analysis of two cultivar varieties of ginger, 12 compounds found in both the cultivars in the same trials. And they include .alpha.-Curcumene, Nortricyclyl bromide, Xylopropamine, .beta.-Bisabolene , .alpha.-Farnesene, N-Benzyl-N-ethyl-p-isopropylbenzamide, 3,4- dihydroxyphenylglycol, 4TMS, 2-Myristynoyl-glycinamide, 6-Shogaol, Phthalic acid, isobutyl octadecyl ester, 4-Hydroxyphenyl pyrrolidinyl thione and 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5. Though these 12 compounds identified in both the varieties, but their concentration in terms of area percentage was different in each treatment.

The present study emphasized on how stress alters the physiological and morphological aspects of two closely related cultivars of ginger. The combined stress treatments also revealed that there are promising results in which one stress can be used to mask/reduce the negative impacts of another stress. A synergistic mechanism was found to operate in the combination trials, especially in combination of drought and foliar sprays.

Drought which is considered as a major menace for ginger, this combination stress analysis was found to be hopeful for further studies to reveal more insights in the field of stress physiology of potential crop plants which are directly connected to the day to day needs of human beings.

RECOMMENDATIONS

Plants which are growing under its natural condition are subjected to a variety of environmental stresses. Plant response to solitary stress will be different from that of the same plant which is subjected to a multitude of stress factors. Understanding the mechanism of stress communication in plants is of great significance. It helps us to understand the production and expression of different array of secondary metabolites in response to different stress signals. Ginger, which is considered as '*Maha Aushadha*' since ancient times, is well known for its secondary metabolites. In the present study, response of two cultivars of ginger to different stress signals was evaluated. Solitary as well as combined foliar sprays of Salicylic Acid and Zinc Sulphate Heptahydrate in two different concentrations and drought was taken as the stress factors. The present study gave an idea about the varied expression of two cultivars of ginger in terms of secondary metabolite production, volatile non-volatile composition of ginger in response to the different stressed environmental conditions, it was also found that some stress combinations may altered the production of secondary metabolites and other phytoconstituents and a significant level of stress crosstalk has been noted during the study. The negative impacts of reduced irrigation were minimized by the foliar sprays. The synergistic effect of stress combination had different results that are evaluated for the production of higher quantity of ginger volatiles and non-volatiles. The methodology gap or empirical gap which does not come under the purview of the present study can be taken as a future line of work in the form of recommendations.

- Evaluating and understanding the molecular mechanism behind stress perception, signal transduction, transcriptional activation of stress- responsive target genes and synthesis of stress related proteins and other molecules.
- Signal cross talk in the candidate plant can be manipulated and it may use for the production of drought tolerant ginger varieties to cultivate the same in drought affected areas.

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