SALICYLIC ACID MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN LONG PEPPER (*Piper longum* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"SALICYLIC ACID MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN LONG PEPPER (*Piper longum* L.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani
Date:14-10-2019

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CERTIFICATE

Certified that this thesis entitled "**SALICYLIC ACID MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN LONG PEPPER (*Piper longum* L.)**" is a bonafide record of research work done independently by Ms. Krishna Veni Harish under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Krishna Veni Harish

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LIST OF ABBREVIATIONS AND SYMBOLS USED

^0C	Degree Celsius
%	per cent
µg	microgram
ABA	Abscisic acid
CA	Carbonic anhydrase
CAT	Catalase
CD	Critical difference
cm	centimetre
CRD	Completely Randomised Design
DNS	Dinitro Salicylic acid
DMSO	Dimethyl Sulfoxide
EC	Electrical conductivity
et al.	And others
Fig.	Figure
g	gram
g^{-1}	per gram
GA3	Gibberellic acid
H_2O_2	Hydrogen peroxide
HCL	Hydrochloric acid
HI	Harvest Index
IAA	Indole 3 acetic acid

K	Potassium
LAI	Leaf Area Index
MAP	Months after planting
mg	milligram
min^{-1}	per minute
ml^{-1}	per millilitre
mM	millimolar
N	Nitrogen
NAR	Net Assimilation Rate
nm	nanometer
NS	Non -significant
No.	Number
P	Phosphorous
$Plant^{-1}$	per Plant
POD	Peroxidase
Ppm	Parts per million
ROS	Reactive oxygen species
SA	Salicylic acid
SE	Standard Error
Sl.	Serial
SOD	Superoxide dismutase
sp. or spp.	Species (Singular and Plural)
viz.	Namely

Introduction

1. INTRODUCTION

Piper longum L. (long pepper, Piperaceae) is a perennial climber, commercially exploited for its pharmaceutical value. After black pepper and betel vine, it is the third most important species of the genus Piper. It is an important spice cum medicinal crop, native to Indo Malayan region. It was highly regarded as a flavour ingredient by the Romans. Long pepper of commerce is the dried spikes of female types. The spikes are harvested when it turns to dark green colour, before fully ripe. It is used for treatments in Ayurveda, Siddha and Unani systems of medicine. The spikes contain piperine and piplartine alkaloids. It is used in the treatment of respiratory tract disorders, arthritis, malaria, viral hepatitis, spleen disorders, tumors etc. (Sivarajan and Balachandran, 1994; Kumar *et al.*, 2011). Apart from the spikes, roots and thicker part of stem are also medicinally important which are dried and used as an important drug, called piplamool. It is one of the three ingredients of Trikadu, the most prescribed ayurvedic formulation against several respiratory complaints. Trikadu is a blend of dried powdered black pepper, long pepper and ginger in equal propotions.

Thippali is an ingredient in over 320 classical compound medicinal formulations and many herbal formulations (Singh *et al.*, 2004). It is a medicinal crop prioritized for cultivation and development by the National and Kerala State Medicinal Plants Boards and has a high demand in the indigenous drug industry. The approximate annual consumption of *P.longum* in terms of fruits and roots is 1737 t. The medicinally valuable part of the crop, is being imported to meet the market demand. About 9,067,191 kg of long pepper including fruits and roots was imported in the year 2004-2005 (Ved and Goraya, 2008).

In Kerala, *P. longum* is rarely cultivated and hence, extensively collected from the wild, threatening the very existence of the plant. Because of its great demand in the pharmaceutical industry, plants are being overexploited resulting in their extinction. It is now categorised under endangered category (Nair, 2000). There is an urgent need for conservation of this plant, through domestication and

culture. Due to the potent use of thippali in the field of pharmaceutical industry, yield as well as quality in terms of plant metabolites have to be enhanced.

Salicylic acid is a safe elicitor of metabolites and a plant growth enhancer. It is a natural phenolic compound and endogenous signal molecule that plays a key role in the regulation of plant growth, development, metabolism, defense mechanism, induction of specific plant responses to biotic and abiotic stresses (Kalarani *et al.*, 2002). It also acts as plant growth regulating substances and has a crucial role in physiological and biochemical processes during the entire lifespan of the plant (Vicente and Plasencia, 2011). Salicylic acid also alters the biosynthetic pathway of secondary metabolites leading to its enhanced production (Ranjbaran *et al.*, 2011; Rowshan and Bahmanzadegan, 2013).

Hence, the study entitled "Salicylic acid mediated metabolite elicitation and growth responses in long pepper (*Piper longum* L.)" has been proposed with the objective to study the effect of different concentrations of salicylic acid on plant growth, yield and metabolite production in *P. longum*.

Review of Literature

2. REVIEW OF LITERATURE

Piper longum L., popularly known as thippali or long pepper, belongs to the family Piperaceae. It is an economically important medicinal crop well adapted to the agro-climatic situations prevailing in the humid tropics. Long pepper is native to the Indo-Malayan region and is commonly seen in the tropical rain forests of India, Indonesia, Nepal, Sri Lanka, Malaysia, Philippines, and Timor. In India, it is seen in evergreen forests of Kerala, Tamil Nadu and Karnataka, lower hills of West Bengal, Assam, Madhya Pradesh, Khasi hills, Eastern Uttar Pradesh and Maharashtra. Nair (2000) categorised the plant under the endangered group in Tamil Nadu and under lower risk in Kerala. *P. longum* is well adapted for cultivation as an intercrop in arecanut, coconut, and rubber plantations of Kerala.

Every year large quantities of dry spikes of long pepper are required for meeting the demand of Ayurvedic industries in Kerala. The domestic production is quite insufficient to meet the increasing demand. Initiatives have to be undertaken for commercial cultivation and area expansion. Viswam, a high yielding selection from the geographical race, Cheemathippali., is an improved variety of *P. longum* released by Kerala Agricultural University.

The yield as well as quality in terms of plant metabolites in thippali, need to be enhanced, considering its demand in the field of the pharmaceutical industry. Salicylic acid has been established as a safe elicitor of metabolites and a plant growth enhancer in different plant species. The present study 'Salicylic acid mediated metabolite elicitation and growth responses in long pepper (*Piper longum* L.)" aims to study the effect of different levels of salicylic acid on plant growth, yield and metabolite production in *P. longum*.

In this chapter, literature on the effect of salicylic acid on plant growth and development in various horticulture and field crops has been reviewed.

2.1. Long pepper (*Piper longum*)

P. longum is a slender creeping climber, spreading on ground and rooting at nodes. Long pepper exhibits dimorphic branching patterns like orthotropic and plagiotropic branches (Ravidran and Balachandran, 2005). The plant is dioecious in nature and flowers throughout the year. Flowers are present in a short cylindrical spike. The male spikes are longer than the female ones. Fruits are small and are closely packed. Fruiting is apomictic hence no male plant is required for pollination (Ravindran and Balachandran, 2005; Sujatha and Nybe, 2007). The spikes are born opposite to the active leaves hence the more foliage will result in increased yield. The different parts of the plant namely roots, stems and fruits are exploited medicinally.

Khare (2006) reported that the fruits of *Piper longum* contain phytoconstituents such as volatile oil, other minor alkaloids such as piperine, piperlongumine, piplartine, piperidine, resin, and starch. Also reported that piperine is the principal pharmacological active compound and is the reason for the pungency in fruits. Dried spikes on steam distillation yielded 0.7 percent essential oil. Besides piperine, piper longumine (0.02 percent) and piper longuminine (0.2 – 0.25 percent) are alkaloids present in the roots (Khushbu *et al.*, 2011). *P. longum* contains important bioactive compounds *viz.* alkaloids, flavonoids, glycosides, tannins, phenols, and sterols (Rami *et al.*, 2013). Nair (2015) studied 41 accessions of *P. longum* and found that oleoresin, volatile oil and piperine contents ranged from 3.21 per cent to 20.21 per cent, 0.5 per cent to 1.60 per cent and 0.24 per cent to 1.10 per cent respectively.

P. longum is one of the most extensively used medicinal plants in the ayurvedic system of medicine. *Piper longum* is a powerful stimulant for both digestive and respiratory systems and has been shown to have a rejuvenating effect on the lungs (Sharma, 1996). Long pepper also has analgesic and diuretic effects and is useful in the relaxation of muscle tension and alleviation of anxiety (Sunila and Kuttan, 2004). It is also extensively used for enhancing the bioavailability and

bioefficacy of various drugs (Atal and Bedi, 2010). The extract of the root of *Piper* and its major compound, piperine exert anti-oxidant activity and are protective in the myocardial ischemic condition (Joshi *et al.*, 2013).

2.2. Salicylic acid

Plant growth regulators can be a natural or synthetic chemical that is applied to a seed or a plant to regulate various plant processes. The plant growth regulating substances are widely used in the agriculture sector. Salicylic acid (SA), chemically known as 2-hydroxy benzoic acid is a phenolic compound, consisting of an aromatic ring bearing a hydroxyl group or its functional derivative, which is synthesized by plants. Plant phenolics are categorised as secondary metabolites. (Hayat and Ahmad, 2007). (Hayat *et al.*, 2010) explained the role of interaction of SA in the induction of resistance to biotic (pathogen-associated) stress and tolerance to many abiotic stresses (chilling, drought, heat, UV radiation, heavy metal, and salinity/osmotic stress). In addition to this it also plays a crucial role in the plant growth and development, including seed germination, vegetative growth, flowering, fruit yield, stomatal closure, photosynthesis, respiration, thermogenesis, etc

Salicylic acid found to be very effective in enhancing cell division, cell elongation, biosynthesis of natural hormones such as IAA, cytokinins and GA_3, protect plant cells from senescence, nutrient uptake as well as biosynthesis of pigments and subsequently growth (Ding *et al.*, 2001). According to Dawood *et al* (2012), the positive effect of salicylic acid in growth of sunflower can be ascribed to increase the level of IAA, GA_3, zeatin and zeatin riboside, in the mean time decrease in ABA in shoot tissues.

2.2.1. Plant growth parameters

The growth and development of the plant is regulated by various internal and external stimuli. Enhancing effects of SA on growth parameters could be attributed to its stimulatory effects on physiological and biochemical processes. These processes led to ameliorate the vegetative growth and active assimilate

translocation from source to sink. This area briefly outlines the effect of the exogenous application of SA on plant growth parameters of various crops.

According to Gharib (2006), foliar application of SA at concentrations of 10^{-4} and 10^{-5} M significantly increased growth in terms of plant height, number of branches, number of spikes, number of leaves per plant, leaf area, fresh and dry weights of herb, and yield per plant in Basil and Marjoram.

Yildirim (2008) reported that in cucumber, foliar applications of SA 1.0 mM significantly increased number of leaves per plant, shoot fresh and dry weight, shoot diameter and fresh and dry weight of roots.

Mady (2009) studied the significance of foliar application with 50 and 100 ppm of SA on growth of tomato (*Solanum lycopersicum*) plants. Plant growth parameters *viz.,* number of branches, leaves per plant, leaf area per plant and leaves dry weight were significantly enhanced by the treatments.

According to Bekheta and Iman (2009), the exogenous application of SA on mung bean @ 15 mg l^{-1} significantly enhanced the plant height, number of branches per plant, total dry matter and yield.

Aftab *et al.* (2010) reported that in *Artemesia annua,* foliar application of SA 1.0 mM significantly enhanced the vegetative parameters *viz;* number of leaves, plant height and shoot biomass.

Idrees *et al.* (2011) studied the effect of SA on salinity stressed and unstressed plants of *Catharanthus roseus*. SA 10^{-5} M significantly enhanced the plant height, number of leaves, both fresh and dry weight of the shoot and roots in both stressed and unstressed plants, respectively.

Khandaker *et al.* (2011) found that the foliar application of SA 10^{-5} M in red amaranth significantly enhanced the growth parameters *viz;* plant height, stem length, leaf number, leaf size, root length, as well as fresh and dry matter yield.

Andrey *et al.* (2012) reported that the foliar application of SA under lead stressed condition showed a significant increase in the morphological characters like plant height, number of leaves and number of flowers in pea (*Pisum sativum*).

According to Dawood *et al.* (2012) sunflower plants treated with SA 75 mg l^{-1} significantly enhanced shoot length, number of leaves per plant, leaf area per plant, shoot fresh and dry weight.

According to Jadhav and Bhamburdekar (2012) the foliar application of SA 100 ppm enhanced the number of branches and total height of plant and SA 50 ppm significantly enhanced number of leaves, leaf area, fresh and dry weight per plant of groundnut.

Martin-Mexl *et al.* (2015) found that the micro propagated gloxinia (*Sinningia speciose* Benth.) seedlings transferred to greenhouse conditions were treated with different concentration of SA and found that all the treated plants showed a significant increase in the number of flowers per day by 25 to 37 per cent, flowered 6 days earlier and had higher leaf area compared to control plants.

Kaur *et al.* (2015) reported that the exogenous application of SA 50 mg l^{-1} significantly increased dry matter accumulation, plant height, number of branches per plant and stem girth in soybean.

Lingakumar *et al.* (2015) demonstrated that the foliar spray of SA at different concentrations (0.5 µM to 2.0 µM) significantly enhanced the growth parameters *viz.*, shoot length, root length, leaf area, shoot and root dry weight and root nodules respectively in 10 days and 20 days old seedlings of *Vigna radiata*.

Mohamed *et al.* (2017) reported that the exogenous application of SA at 3 mM concentration, on three different strawberry cultivars significantly enhanced the vegetative parameters *viz*; the number of leaves, number of crowns and leaf area per plant. It was also found to induce earliness in flowering and more flower clusters.

The inhibitory effect of SA has been reported by Pancheva *et al.* (1996) in barley seedlings wherein it inhibited the growth of leaves and roots. Scott *et al.* (2004) also reported the inhibition of growth in *Arabidopsis* due to the accumulation of salicylate under chilling stress.

2.2.2. Metabolic effects of salicylic acid

Senthil *et al.* (2004) observed that the exogenous application of SA 60 ppm significantly enhanced chlorophyll, soluble protein content and peroxidise activity of soybean var. CO-5.

Foliar application of SA found to enhance the accumulation of resveratrol in peanut (Chung *et al.* 2003) and in *Matricaria chamomilla* induced erniarin and umbelliferone alkaloids accumulation (Pastirova *et al.* 2004). Kang *et al.* (2004) reported that SA induced putrescine N-methyltransferase and hyoscyamine 6b-hydroxylase, the biosynthetic enzymes in tropane alkaloid pathway, in the adventitious roots of *Scopolia parviflora*.

Gharib (2006) reported that with the exogenous application of SA 10^{-4} M in case of basil and 10^{-3} M in marjoram significantly enhanced the oil percentage and yield per plant about two-fold on a fresh weight basis.

According to Karkar *et al.* (2007) exogenous application of SA 300 ppm along with brassinolide 10 ppm enhanced the kernel quality, total proteins, reducing sugar, non-reducing sugar, total soluble sugars, total carbohydrates and pod yield in groundnut.

Bekheta and Talaat (2009) reported that the foliar application of SA 15 mg L^{-1} significantly increased total carbohydrates, total proteins and pigments (chlorophyll and carotenoids) in groundnut plants.

Maity and Bera (2009) reported that the exogenous application of SA 1000 ppm in green gram found to enhance the plant pigments (chlorophyll a, chlorophyll

b and total chlorophyll), reducing and non-reducing sugars, starch and soluble protein content.

Dolatabadian *et al.* (2009) reported that the grain-soaking with SA on the salt stressed wheat plants significantly enhanced the activity of antioxidant enzymes such as catalyse (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), and proline oxidase (POX).

Aftab *et al.* (2010) reported that in *Artemesia annua,* foliar application of SA 1 mM significantly enhanced the biochemical parameters *viz;* plant pigments such as chlorophyll and carotenoid contents and also enhanced the content and yield of artemisinin.

Study conducted by Mandal (2010) in *Solanum melongena* L. demonstrated that the elicitors such as, salicylic acid (SA) and methyl jasmonate (MeJA) could induce cell wall strengthening of egg plant roots through lignin deposition and induction of several defense enzymes such as Phenylalanine Ammonia-Layase (PAL), peroxidase (POD), polyphenol oxidase (PPO), and catalyse (CAT).

Abdul *et al.* (2011) reported that in chickpea plants, application of SA 1.5 mM under both biotic and abiotic stress conditions was found to induce higher activities of POD, PPO, hydrogen peroxide, phenols and protein content.

Patel *et al.* (2011) reported that the foliar application of SA 1.5 mM significantly enhanced the activity of proline, CAT, POD and SOD even under drought conditions in chickpea.

Idrees *et al.* (2011) studied the effect of SA on salinity stressed and unstressed plants of *Catharanthus roseus*. They reported a significant increase in metabolites by the application of SA and the best results were obtained in SA 10^{-5} M, which significantly enhanced essential oil, chlorophyll content, activity of antioxidant enzymes such as CAT, POD and SOD and also it improved the activity of anticancer alkaloids such as vincristine and vinblastine.

Patel *et al.* (2012) reported that exogenous application of SA 1.0 mM and 1.5 mM at pre and post-flowering periods increased proline content and antioxidant enzyme activity such as SOD and POD in chickpea.

Sivanandhan *et al.* (2013) reported that the application of SA 150 µM significantly enhanced the production of secondary metabolites *viz;* withanolide A, withanone, and withaferin A in hairy root cultures of *Withania somnifera.*

Rowshan and Bahmanzadegan (2013), reported that the application of exogenous SA at 200 and 400 mg L^{-1} modified secondary metabolites and their pathways and improved the quality and quantity of the essential oil in yarrow.

Pirbalouti, *et al.* (2014) found that in thyme (*Thymus daenensis* Celak.) application of 3.0 M SA under reduced irrigation gave the highest values of oil content (3.2% v/w) and yield (14.9 gm^{-2}).

According to Li *et al.* (2014) the foliar application of SA effectively increased chlorophyll, photosynthesis and proline content. It also enhanced the activities of antioxidant enzymes *viz.*, SOD, POD and CAT enzymes in the seedlings of a medicinal tree, *Torreya grandis.*

Alyemeni *et al.* (2014) reported that the foliar application of SA 10^{-5} M resulted in a significant increase activity of CAT, POX and SOD in Cd stressed and unstressed chickpea plants.

Hadi *et al.* (2014) studied the effect of different concentrations of SA on bean under salt stress condition and among the different concentrations applied significantly higher results were obtained with SA 0.1 mM and SA 0.5 mM on chlorophyll a and total chlorophyll, proline, protein and soluble sugars. However, the higher concentration of SA 1 mM was not significantly different from the control treatments.

Manaa *et al.* (2014) found that the exogenous application of salicylic acid (SA 0.01 mM) and calcium sulphate ($CaSO_4$ 5 mM) singly or in combination

significantly enhanced the metabolic parameters *viz.*, total chlorophyll, carotenoids, soluble sugars and proline of two tomato cultivars (cv. Super Marmande and cv. Red River) exposed to salt stress (100 mM NaCl).

Nasiri (2018) reported that the application of SA along with ascorbic acid 1 mM concentration significantly increased the essential oil content and essential oil yield compared to control plants of dragonhead (*Dracocephalum moldavica* L.).

According to Ram *et al.* (1997) higher concentration of SA 100 ppm had no significant effect on the herbage and essential oil yields in *Pelargonium graveolens*, *Cymbopogon martini* and *Mentha arvensis*. They concluded that the amount of SA needed for the induction of synthesis of essential oil constituents may already be present in these plants or the synthesis of essential oil constituents occurs constitutively, without the intervention of SA.

2.2.3. Physiological parameters

SA has been established as an important regulator of photosynthesis, water relations and metabolic aspects of plants, depending on its analogues, concentrations, mode of application and plant type.

Chamarthy (2004) found that in rice foliar application of SA 50 ppm showed an increase in leaf area index (LAI), crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), dry mass and yield components.

Hayat *et al.* (2008) studied the growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress and concluded that when SA 10^{-5} M was applied at 45 days after sowing significantly enhanced net photosynthetic rate, stomatal conductance, internal carbon dioxide concentration, transpiration rate, chlorophyll content, membrane stability index, proline content and antioxidant enzyme activity.

Jadhav and Bhamburdekar (2012) reported that the application of SA 50 ppm showed a significant increase in the number of leaves, leaf area, fresh and dry weight per plant and NAR on a dry weight basis of groundnut.

Farjadi-Shakib *et al.* (2012) studied the effect of foliar application of salicylic acid on physiological and biochemical attributes of *Cyclamen persicum* and found that the SA application significantly enhanced the protein content and membrane stability index. It also showed an enhanced synthesis of antioxidant enzyme activity by increasing the activity of free radical scavengers such as CAT and SOD during all flowering stages.

Alyemeni *et al.* (2014) reported that in response to SA application 10^{-5} M showed a significant increase in stomatal conductance, transpiration rate, intercellular CO_2 concentration and net photosynthetic rate over the control in Cd stressed chickpea plants.

Hayat *et al.* (2014) revealed that the exogenous application of SA showed an increase in net photosynthetic rate and stomatal conductance and the maximum response was showed by the plants sprayed with 10^{-5} mol l^{-1} of SA, showing a statistically significant increase of 20.6 per cent stomatal conductance and 46.92 per cent net photosynthesis over that of the control in chickpea.

Ram *et al.* (2014) studied the effect of salicylic acid in *in vitro* grown seedlings of water melon (*Citrullus lanatus*) and found that the SA 0.25 mM significantly enhanced the chlorophyll a and b content, chlorophyll stability index and membrane stability index.

Narayanan *et al.* (2015) found that foliar spray of SA 100 ppm along with 1% Prosopis leaf extract recorded physiological parameters such as chlorophyll content, photosynthesis, transpiration, intercellular CO_2 concentration and stomatal conductance in black gram.

Sathishkumar *et al.* (2018) reported that in response to the foliar application of SA 40 ppm twice at pre and post-flowering stage in fingermillet significantly

enhanced the physiological attributes *viz.*, leaf area index, crop growth rate, relative growth rate and net assimilation.

2.2.4. Yield and yield components

The credibility of any exogenously sourced plant hormone depends on its effect on economical yield.

Zaghlool (2002) reported that SA 20 ppm as seed soaking and foliar spray significantly enhanced the yield by increasing number of pods per plant and 100-seed weight in blackgram.

Sharma and Kaur (2003) observed that the exogenous application of SA 50 ppm in soybean cv. SL-295 significantly enhanced the number of flowers per plant, number of pods per plant, number of seeds per plant, 100-seed weight, harvest index and yield.

San-Miguel *et al.* (2003) reported that the application of SA with one micromole or less is sufficient to enhance the root growth, as in *Pinus patula,* wherein SA 10^{-8} and 10^{-6} M increased root growth by 33 per cent and 30 per cent, respectively.

Kothule *et al.* (2003) found that in soyabean, application of SA 200 ppm resulted in significant increase in number of pods per plant, number of seeds per pod, weight of seeds per pod, grain yield per plant, 100 seed weight, and harvest index.

Murtaza *et al.* (2007) reported that the exogenous application of SA 10^{-4} M as seed treatment plus foliar spray significantly increased number of pods per plant, number of seeds per pod, 100 seed weight, biological yield and seed yield in pea.

Sharafizad *et al.* (2012) reported that in response to the exogenous application of SA 0.07 mmol resulted in the highest grain yield in wheat plants.

El-Hak *et al.* (2012) revealed that foliar application of salicylic acid at 200 ppm produced the highest plant dry weight, pod diameter, fresh seeds weight/pod, number of fresh seeds/pod, green pod yield, seeds weight/dry pod, dry seed yield and 1000-seed weight in pea (*Pisum sativum*).

Mohsen *et al.* (2014) reported that in response to the exogenous application of SA 1.0 mM showed an increase in biological yield, 100 seed weight, number of pods per plant, number of grains per pod, number of grains per plant, harvest index and grain yield in lentil (*Lens culinaris*).

Kaur *et al.* (2015) revealed that the exogenous application of SA 50 mg L^{-1} significantly increased number of pods per plant, seed index, straw yield and seed yield in soybean.

Karimian *et al.* (2015) reported that foliar application of SA 3 mM in groundnut under drought conditions significantly increased the dried herb yield, pod yield, seed yield, 100-seed weight and number of pods per plant.

2.2.5. Uptake of major nutrients

Mineral nutrients play a significant role in the growth and development of plants as they are important factors that regulate various physiological and biochemical processes. How each element influences a plant's physiological and biochemical procedures, (positively or negatively), is remarkable to each plant. This area briefly outlines the effect of the exogenous application of SA in nutrient uptake.

According to Gharib (2006) SA treatment increased the content of N (nitrogen), P_2O_5 (phosphorous), K_2O (potassium), Mn (manganese), Fe (iron), Zn (zinc), Na (sodium) and Cu (copper) in *Ocimum basilicum* and *Majorana hortensis*. The maximum mean values of all macro and micronutrient content of both species were obtained as a result of SA at 10^{-4} M and 10^{-5} M.

Gunes *et al.* (2005) reported that exogenously applied SA either with seed soaked (1.0 mM for 24 h) or soil incorporation (0.1 mM and 0.5 mM) under different abiotic stress condition enhanced the absorption of N, P_2O_5, K_2O, Mg and Mn content in maize.

Yildirim (2008) reported that in cucumber, foliar applications of SA 1.0 mM significantly enhanced the macro and micro nutrients in leaves of cucumber *viz;* N, P_2O_5, S, Fe, and Mn.

Karligag *et al.* (2009) studied the effect of exogenous application of salicylic acid on mineral content of strawberry plants grown under salt stress and greenhouse conditions. SA 1.00 mM significantly enhanced nutrients such as N, P_2O_5, K_2O, Mg, Fe, Mn and Cu uptake in leaves and roots of strawberry plants.

Mady (2009) studied the effect of foliar application of salicylic acid and vitamin E on tomato (*Lycopersicon esculentum*) plant. He concluded that the two concentrations of salicylic acid (50 and 100 ppm) applied along with vitamin E (200 ppm) enhanced the nutrient uptake of micro and macro nutrients such as N, P_2O_5, K_2O, Fe, Zn and Mn in leaves of treated plants as compared with those of untreated ones.

Khan *et al.* (2010) studied the effect of SA on mungbean (*Vigna radiata* L.) cultivar Pusa Vishal plants grown in medium supplemented with 50 mM NaCl. The application of 0.5 mM SA resulted in an increase of N, P_2O_5, K_2O and Ca content by 10.1, 31.6, 19.3 and 19.1 per cent, respectively, compared to the control.

Merwad (2015) reported that the application of 200 kg ha^{-1} of potassium in combination with salicylic acid foliar 1000 mg L^{-1} spray in sugar beet gave the highest nitrogen (N), phosphorous (P) and K content and uptake. Spraying SA increased the contents of N, P_2O_5 and K_2O by an average of 16.5%, 17.2% and 8.5%, respectively, and N, P_2O_5 and P_2O_5 uptake by an average of 28.9%, 29.2% and 20.3%, respectively, compared to that without SA.

Ali *et al.* (2016) reported that SA spraying at 100 ml l^{-1} increased concentrations of N, P_2O_5, P_2O_5, Mg and Ca in basil plants.

Youssef *et al.* (2017) studied the effect of salicylic acid on nutrient status of sunflower plants under salinity stress and he observed that, with the application of SA 1.4 mM significantly enhanced the uptake of N, P_2O_5, K_2O, Ca, Mg and Cl.

Materials and Methods

3. MATERIALS AND METHODS

The present study "Salicylic acid mediated metabolite elicitation and growth responses in long pepper (*Piper longum* L.)" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2017-19. The study aimed at assessing the effect of different levels of salicylic acid on plant growth, yield and production of metabolites in long pepper (*Piper longum* L.).

3.1 EXPERIMENTAL DETAILS

3.1.1. Location

The field experiment was conducted in the field located near the Department of Plantation Crops and Spices, College of Agriculture, Vellayani.

3.1.2. Planting material

The rooted cuttings of the variety Viswam (a promising selection of Kerala Agricultural University) maintained at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara were used for the study. The two month old rooted cuttings, procured from College of Horticulture, Vellanikkara were used for the study. The rooted cuttings were planted in polybags (Plate 1) filled with soil supplemented with 500g vermicompost. Cowdung slurry was applied at two months interval. PGMR mix I was applied at 100g per plant. Staking was done two weeks after polybag planting (Plate 2).

3.1.3. Design of the Experiment

The experiment was laid out in CRD with nine treatments and three replications. Each treatment consisted of twenty one plants. Destructive sampling was carried out at different periods of observation.

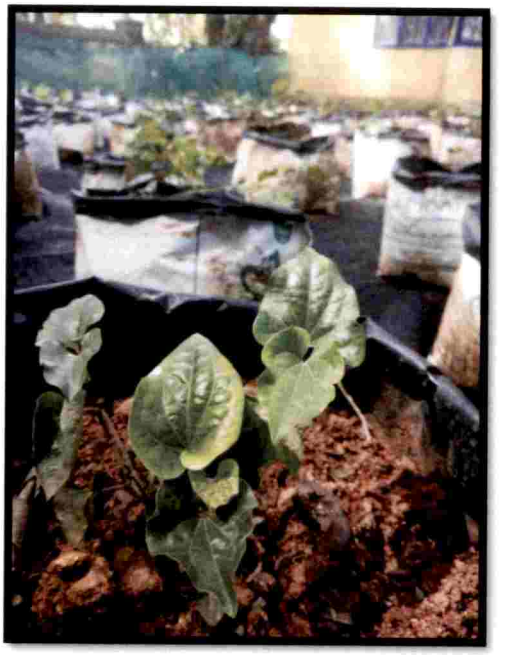

Plate 1. Two month old rooted cutting planted in grow bag

Plate 2. Staked plants (done after 2 weeks)

Plate 3. Field after attaining maturity

3.1.5. Treatment Details

Salicylic acid (SA) at different levels were applied as foliar spray at 2 months after planting (MAP), 4 MAP and 6 MAP. The foliar solution of SA was prepared by dissolving the specified amount of SA in 2 ml of ethyl alcohol and was made up to 1000 ml. The spray volume of SA solution varied at different periods of crop growth. The spray volume per plant being 15, 20, 30 ml at 2, 4 and 6 MAP, The treatments used are presented in Table 1.

3.2. OBSERVATIONS

3.2.1. Plant growth parameters

The following plant growth parameters were recorded at one month after each foliar application (*ie*, 3, 5, 7 MAP).

3.2.1.1. Plant height

Plant height was recorded by measuring the length of vine from the ground level to the tip of the shoot using a meter scale. The mean value was worked out and expressed in centimetre.

3.2.1.2. *Number of primary branches per plant*

The number of primary branches per plant was recorded and the mean value was worked out.

3.2.1.3. Number of spike bearing branches per plant

Number of spike bearing branches per plant was recorded by counting the spike bearing branches in the selected plants and the mean was worked out.

3.2.1.4. Number of inflorescence per plant

Total number of inflorescence produced per plant was recorded by counting and mean value was worked out.

Table 1. Different foliar spray treatments

Treatment No.	Foliar spray treatments
T_1	SA 0.1 mM
T_2	SA 0.5 mM
T_3	SA 1.0 mM
T_4	SA 1.5 mM
T_5	SA 2.0 mM
T_6	SA 2.5mM
T_7	Ethanol (0.2%)
T_8	Water spray
T_9	Control

3.2.1.5. Number of leaves per plant

Total number of leaves produced per plant was recorded by counting and the mean value was worked out.

3.2.1.6. Leaf area per plant

Ten leaves were randomly chosen and leaf area was measured by graphical method and the average value was calculated and expressed in cm^2.

Leaf area per plant = Average leaf area (10 leaves) x total number of leaves per plant

3.2.1.7. Shoot weight per plant (Fresh and dry weight)

The fresh and dry weight of the shoots were recorded. Shoots were oven dried at 60°C until constant weight was obtained. Mean value was worked out and expressed in gram.

3.2.1.8. Days to emergence of spike

This parameter was recorded by counting the number of days taken by the plant from the day of planting to the first emergence of spike.

3.2.1.9. Days to flowering

This parameter was recorded by counting the number of days taken by the plant from the emergence of spike to the initiation of flowers on the spike. The mean value was worked out.

3.2.1.10. Days from emergence to maturity of spike

This parameter was recorded by counting the number of days taken from spike emergence to the maturity of the spike (dark green colour). Ten spikes per plant were tagged at emergence and the number of days to maturity was recorded and mean value per plant was worked out.

3.2.2. Metabolite production

3.2.2.1. Chlorophyll content

Chlorophyll content of leaf samples were determined using the procedure described by Arnon (1949). The leaves were chopped into small bits and 0.5 g of the leaf sample was weighed out. The leaf bits were incubated overnight at room temperature in 10 ml DMSO (Dimethyl Sulphoxide): 80% acetone mixture (1:1 v/v), in test tubes. The coloured solution was then transferred into a measuring cylinder and made upto 25ml with DMSO - acetone mixture. The absorbance was measured using spectrophotometer (ELICO-SL 218 Double Beam) at 663 and 645 nm.

The chlorophyll content was determined by substituting the absorbance values in the formula given below and expressed in mg g^{-1} of fresh leaf.

$$\text{Total Chlorophyll} = 20.2\,(A_{645}) + 8.01\,(A_{663}) \times \frac{\text{Volume}}{1000 \times \text{Fresh weight}}\ \text{mg g}^{-1}$$

3.2.2.2. Total Proteins

The total soluble proteins of leaf samples were estimated using simple protein dye binding assay (Bradford, 1976) using bovine serum albumin (BSA) as the standard. Coomassie brilliant blue G 250 (100 mg) was dissolved in 50 ml of 95 per cent ethanol. 100 ml of concentrated (ortho) phosphoric acid 85% (w/v) was added to the above solution. The solution was then diluted to a final volume of 200 ml with distilled water. Leaf sample (0.1 g) was taken and was grounded to thin paste and soluble protein was extracted with 10 ml of phosphate buffer (pH 7.8).

The extract was centrifuged at 5000 rpm for 10 min. A known volume (5 ml) of diluted dye binding solution was added to 20 µl of the supernatant. The solution was thoroughly mixed and allowed the colour to develop for at least 5

min but no longer than 30 min, a blue colour developed and the absorbance was measured at 596 nm. The protein content was calculated using the BSA standard in the range of (10 – 100 µg). A standard curve was plotted using standard protein absorbance Vs concentration. The protein content was expressed as mg g^{-1} on fresh weight basis.

3.2.2.3. Estimation of Peroxidase

The peroxidase activity in plants was determined following the method described by Reddy *et al.* (1995). Leaf samples (200 mg) were homogenised in 1 ml of 0.1 M phosphate buffer (pH 6.5) and centrifuged at 5000 rpm for 15 min at 4°C. 0.1 g of this extract was added to 3.0 ml of pyrogallol solution, and adjusted to give a spectrophotometer (ELICO-SL 218 Double Beam) reading of zero at 430 nm. The enzyme reaction started on adding 0.5 ml of one per cent hydrogen peroxide (H_2O_2) into sample cuvettes and change in absorbance was measured every 30 s up to 3 min. One unit of peroxidase is defined as the change in absorbance $minute^{-1}$ at 430 nm.

3.2.2.4. Estimation of Catalase

The catalase (CAT) activity in plants was estimated following the method described by the Luck (1947). Leaf sample (200 mg) was ground in phosphate buffer using mortar and pestle. The homogenate thus obtained, was centrifuged at 5000 rpm for 15 min at 4°C and the supernatant was collected and used for the enzyme assay. The H_2O_2 phosphate buffer (3.0 ml) was taken in an experimental cuvette. This was followed by the rapid addition of 40 µl of enzyme extract and was mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm. The enzyme solution containing H_2O_2 – free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

3.2.2.5. *Estimation of Superoxide dismutase (SOD)*

Superoxide dismutase (SOD) activity was estimated following the method described by Karkkar *et al.* (1984). Leaf sample (1 g) was ground in pre-chilled pestle and motar with 10 ml ice cold 50 mM potassium phosphate buffer (pH 7.8) centrifuged at 10000 rpm for 10 minutes and supernatant collected and was used for estimation. 3 ml of reaction mixture containing 50 mM potassium phosphate buffer, 13 mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 75 µM NBT and 50 µL of crude enzyme extract, were mixed in duplicate and was made up with equal volume of double distilled water. Then set the blank without enzyme and NBT to calibrate the spectrophotometer. Set another control having NBT but no enzyme as the reference control. Expose all the tubes to 400 W bulb (4 x 100 W bulbs) for 15 min and then read the absorbance immediately at 560 nm spectrophotometer and then calculate percentage inhibition. The 50 per cent inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit in SOD activity.

3.2.2.6. *Estimation of Piperine*

The piperine content in the dried spikes was estimated spectroscopically as per the procedure described by Sowbhagya *et al.* (1990). Dried thippali spikes (10 mg) were powdered afresh and samples were extracted with 100 ml of acetone in a volumetric flask. The flasks were maintained at room temperature and shaken well for 2 h. Then 0.25 ml of clear solution from the flask was taken in a cuvette and made upto 5 ml with 4.75 ml acetone. The solution was shaken well and absorbance of the solution read at 337 nm in a UV spectrophotometer, with acetone as blank.

Preparation of the standard curve

Standard piperine solutions of concentrations *viz.*, 0.4, 0.8, 1.2, 1.6 and 2 mg l^{-1} were prepared and their absorbance values at 337 nm were recorded. These values were plotted on a graph against concentration. The concentration

corresponding to the absorbance of the sample was determined and piperine content in the samples were worked out.

The piperine yield per plant was worked out using the formula

Piperine yield per plant = Dry matter yield (g plant^{-1}) x piperine content (per cent)

3.2.2.7. *Volatile oil*

Extraction of oil was done by using modified Clevenger apparatus by hydro distillation method (AOAC, 1980). Twenty grams of dried spikes was powdered and was taken in a round bottomed flask, to which 200ml of distilled water was added. Initiate heating with heating mantle. The condensed volatile oil was collected in the graduated tube as top layer; being lighter than water. The volume of essential oil collected was noted and expressed in per cent volume per unit mass of the sample.

$$\text{Volume of essential oil (\%)} = \frac{\text{Volume of the volatile oil collected}}{\text{Total weight of the sample}} \times 100$$

The volatile oil yield per plant was worked out using the formula

Volatile oil yield per plant = Dry matter yield (g plant^{-1}) x volatile oil content (per cent)

3.2.2.8. Oleoresin

The oleoresin content in the spikes of *Piper longum* was estimated using Soxhlet apparatus by solvent extraction method [AOAC, 1980] with acetone as solvent. Two gram of dried spikes was powdered, packed in a timble and was placed in Soxhlet extraction tube. The solvent, acetone (150 ml) taken in the round bottom flask is then heated to reflex. The extraction cycle was repeated many times over three to four hours. During each cycle, a portion of non-volatile compound dissolved in the solvent. After completing the extractions (till no colour was observed in extraction tube), the non- soluble portion of the extracted sample remaining in the timble was discarded. Again, repeat the distillation process to remove all the solvents. After the distillation, the solution left in the

round bottom flask is transferred to weighed beaker and is kept overnight for vaporising the leftover acetone in the solution. The beaker along with the remaining contents is weighed next day. The difference in the weights gives the quantity of oleoresin, and is expressed in per cent.

The oleoresin yield per plant was worked out using the formula

Oleoresin yield per plant = Dry matter yield (g plant^{-1}) x oleoresin content (per cent)

3.2.2.9. Estimation of total carbohydrate

The estimation of carbohydrate in plants was done following the method described by Sadasivam and Manickam (2008). 100 mg of sample was transferred into a boiling tube and hydrolysed by keeping it for three hours in boiling water bath with 5 ml of 2.5 N HCL and cooled to room temperature and neutralised it with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and then centrifuged. The supernatant was collected and 0.5 ml aliquots were taken for analysis. Standards prepared with concentrations 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' served as blank. Then the volume was made up to 1 ml by adding distilled water in all the tubes including the sample tubes. Anthrone reagent (4 ml) was added and then heated for 8 min in a boiling water bath. Then it was cooled and absorbance read at 630 nm. A standard graph was plotted using concentration of standard on X – axis versus absorbance on Y- axis.

$$\text{Amount of carbohydrate present in 100 mg of sample} = \frac{\text{mg of glucose}}{\text{Volume of test}} \times 100 \text{ mg g}^{-1}$$

3.2.2.10. Estimation of Starch

The estimation of starch in plants was done following the Anthrone method (Sadasivam and Manickam, 2008). To remove the sugars, a known quanity of leaf sample (0.1 g) was homogenized in hot 80% ethanol. The

homogenate was centrifuged and residue was retained. Thus, obtained residue was washed repeatedly with hot 80% ethanol till the washing does not give any colour with anthrone reagent. Then the residue was dried well over a water bath. The dried residue was then mixed with 5 ml water and 6.5 ml 52 % perchloric acid and was extracted at 0°C for 20 minutes. This solution was centrifuged and the supernatants after centrifugation were pooled and made up to 100 ml.

An aliquot of 0.1 ml of the supernatant was taken and again made up to 1 ml using distilled water. The standard was prepared by taking 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard solution and made up the volume to 1 ml in each tube using distilled water. Anthrone reagent (4 ml) was added to both the sample and standard test tubes. In a boiling water bath test tubes were heated for eight minutes and was then cooled rapidly. The intensity of colour change from green to dark green was measured at 630 nm. The glucose content in the sample was calculated using the standard curve. This value was multiplied by a factor of 0.9 arrive at the starch content.

3.2.2.11. Estimation of reducing sugars

The estimation of reducing sugars in plants was estimated using Dinitro Salicylic acid (DNS) method (Sadasivam and Manickam, 2008). The sample was weighed (100 mg) and the sugars were extracted with hot 80% ethanol, twice. The supernatant was collected and evaporated by keeping it on a boiling water bath at 80°C. The sugars were dissolved by adding 10 ml water. Aliquots of 0.5 to 3ml were pipetted out into test tubes and the volume was equalized to 3 ml with distilled water in all the test tubes. To this 3ml of DNS reagent was added. The test tubes were heated in a boiling water bath of 5 minutes.

Rochelle salt solution (40%, w/v) (1 ml) was added to the test tubes when the contents were hot. Then the test tubes were cooled and the intensity of dark red colour was red at 510 nm. A series of the standard, glucose, (0 to 500 µg) was run calculated from the standard graph and is expressed in mg g^{-1}.

3.2.3. Physiological parameters

3.2.3.1. Dry Matter Production

The entire plant was uprooted and oven dried at 70° ± 5°C until constant weight was obtained and mean value was expressed as g plant $^{-1}$.

3.2.3.2. Leaf area index

Leaf area was calculated by tracing the area of leaf on graph sheet and Leaf area index (LAI) was worked out as per the method suggested by William (1946).

$$LAI = \frac{\text{Total leaf area of the plant (cm}^2\text{)}}{\text{Area of land occupied by the plant (cm}^2\text{)}}$$

3.2.3.3. Net assimilation rate

The method proposed by Williams (1946) was used for calculating the net assimilation rate (NAR) on leaf dry weight basis and the values were expressed as mg cm^{-2} day^{-1}

$$NAR = \frac{(W_2 - W_1)}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1}$$

W_1 and W_2 = Plant dry weight (mg) at t_1 and t_2 respectively

L_1 and L_2 = leaf area (cm^2) at t_1 and t_2 respectively

$t_2 - t_1$ = time interval in days

3.2.3.4. Stomatal conductance

Stomatal conductance was measured in the morning between 9 am and 11 am, using Portable Photosynthetic System (Model: CIRAS-3 Ver. 1.06, Amesbury, U.S.A) and was expressed in mmoles m^{-2} s^{-1}.

3.2.2.5 Photosynthetic rate

Photosynthetic rate was measured in the morning between 9 am and 11 am, using Portable Photosynthetic System (Model: CIRAS-3 Ver. 1.06, Amesbury, U.S.A) and was expressed in μCO_2 moles m^{-2} s^{-1}.

3.2.2.6. Cell membrane stability index

Cell membrane stability index was estimated as per the procedure described by Blum and Ebercon (1981). Samples collected from both control and SA treated plants were washed three times in deionised water to remove electrolytes adhered on the surface. Samples were kept in a cappel vial (20 ml) containing 10 ml of deionised water and incubated in the dark for 24 hours at room temperature. The conductance was measured with a conductivity meter. After the first measurement, the vials were autoclaved for 15 min to kill the leaf tissue and release the electrolytes. After cooling, the second conductivity reading was taken. These two measurements were carried out individually for both control and stress treated plans. Cell membrane stability index was calculated by using following formula and expressed as per cent.

$$CMS\ (\%) = [\ 1-(T_1/T_2)/1-(C_1/C_2)] \times 100$$

Where, T and C refer to the treatment and control samples respectively. The subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

3.2.2.7. Proline

Proline content was quantified as per the procedure explained by Sadasivam and Manickam (2008). Plant sample (0.5g) was homogenized with 10 ml of 3% aqueous sulphosalicylic acid. Then it is centrifuged at 3000 rpm for 15 min. Thus obtained homogenate was filtered through Whatmann No. 2 filter paper. Aliquot (2 ml) was taken in a test tube and mixed with an equal amount of glacial acetic acid and acid ninhydrin. It is then heated in a boiling water bath at 100°C for one hour. The reaction was terminated by keeping the test tubes in an

ice bath for 10 minutes. Toluene (4 ml) was mixed with reaction mixture and stirred well for 20-30 seconds. The chromophore containing toluene layer was then separated from aqueous phase, warmed to room temperature. The red colour intensity was read at 520nm with toluene as blank. To prepare the standard curve, a series of standard with pure proline was run in similar method. A standard curve was drawn using concentration verses absorbance and the amount of proline in the sample was calculated from the standard curve.

The concentration of proline was determined from graph and expressed on fresh weight basis of sample

$$\mu M \text{ of proline /g tissue} = \frac{(\mu g \text{ proline / ml}) \times \text{ml toluene}}{115.5} \times \frac{5}{\text{g sample}}$$

3.2.4. Yield and yield components

The following observations on economic parts of the plant viz., spike (Plate 4A) and roots (Plate 4B) were recorded at /upto one year after planting.

3.2.4.1. Number of spikes per plant

Total number of spikes per plant was counted till one year and expressed as the total number of spikes per plant.

3.2.4.2. Length of spike

Five dark green mature spikes ready for picking were randomly selected from a plant and the length was measured using a ruler and the mean length worked out and recorded in cm.

3.2.4.3. Girth of spike

Five dark green mature spikes ready for picking were randomly selected from a plant and the girth was measured using a vernier caliper and the mean girth worked out and recorded in mm.

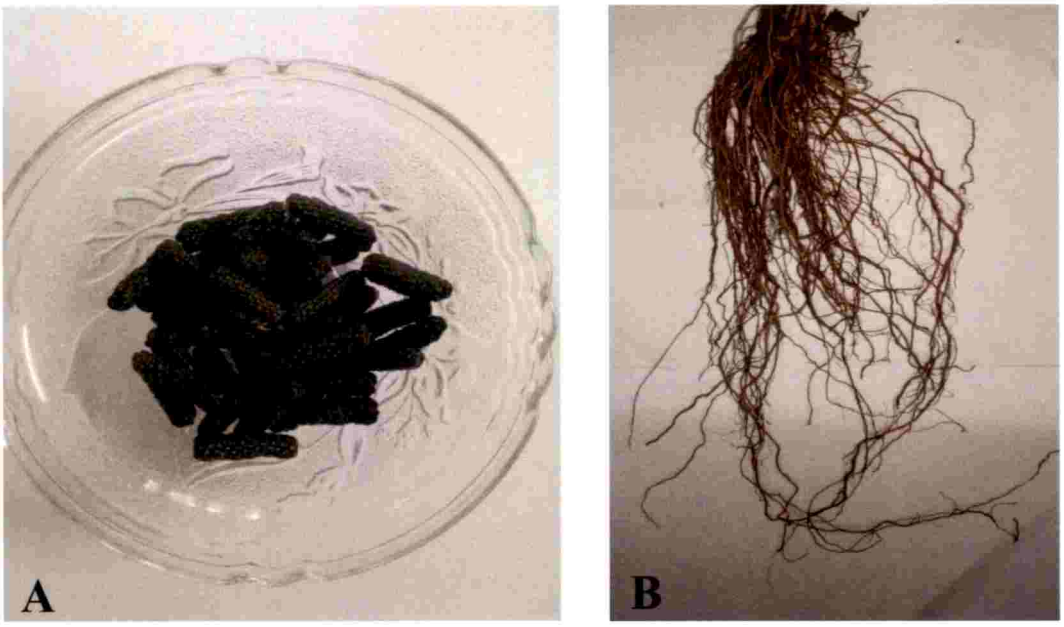

Plate 4. Economic parts of *P. longum* (A) Mature spike (B) Root

3.2.4.4. Fresh spike yield per plant

The spikes were picked on maturity and weight of freshly harvested spikes were taken at each picking upto one year after planting. The weight of the freshly harvested spikes were taken using electronic balance and was recorded in g.

3.2.4.5. Dry spike yield per plant

The freshly harvested spikes were oven dried for a period of four days at 60°C until constant weight was obtained and was recorded in g.

3.2.4.6. Driage of spikes

Fresh spikes were weighed and was oven dried until constant weight was obtained and dry weight was noted and driage was determined using the equation given below.

$$\text{Driage (\%)} = 100 - \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

3.2.4.7. Length of root

Root length was recorded at one year after planting by measuring the length using a meter scale. The mean value was worked out and expressed in cm.

3.2.4.8. Fresh Root yield

Weight of freshly harvested roots at one year after planting were taken using electronic balance and was recorded in g.

3.2.4.9. Dry root yield

The freshly harvested spikes were oven dried for a period of four days at 60°C until constant weight was obtained and was recorded in g.

3.2.4.10. Driage of roots

Fresh roots were weighed and was oven dried until constant weight was obtained and dry weight was noted and driage was determined using the equation given below.

$$\text{Driage (\%)} = 100 - \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

3.2.4.11. Harvest Index

Harvest Index was calculated at final harvest as the ratio of dry weight of spikes to the dry weight of whole plant.

$$\text{Harvest Index (HI)} = \frac{Y_{econ}}{Y_{bio}}$$

Y_{econ} = Total dry weight of spikes

Y_{bio} = Total dry weight of plant

3.2.5. Uptake of major nutrients

3.2.5.1.Nutrient uptake (Plant analysis)

The plant samples were chopped and dried in hot air oven at 70±2°C till constant weights were obtained. Samples were analysed for N, P, and K by adopting standard procedures.

Nitrogen content was estimated using microkjeldahl method (Jackson, 1973). Phosphorous content using Vanado molybdo phosphoric yellow colour method (Jackson, 1973) and potassium content using flame photometer method (Piper, 1976).

Uptake of nitrogen, phosphorous and potassium by the crop were computed by multiplying the nutrient content (per cent) with dry matter production and expressed as the uptake of nutrients in kg ha^{-1}.

3.2. STATISTICAL ANALYSIS

The result of various parameters obtained from the experiment was analysed statistically for the test of significance by standard procedure using OPSTAT software developed by Hariyana Agriculture University.

Results

4. RESULTS

An investigation entitled "Salicylic acid mediated metabolite elicitation and growth responses in long pepper (*Piper longum* L.)" was carried out during 2017-19 at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani. The data collected from the field experiment and laboratory analysis were statistically analysed and the results of the study are presented in this chapter.

The project was carried out to study the effect of different levels of salicylic acid on plant growth, yield and production of metabolites in *P. longum*

4.1. PLANT GROWTH PARAMETERS

The plants were exposed to foliar spray with different levels of salicylic acid at 2, 4 and 6 months after planting (MAP). The observations on plant growth parameters were recorded at 3, 5 and 7 MAP.

4.1.1. Plant height

The data on plant height as influenced by different levels of salicylic acid (SA) foliar spray at 3, 5 and 7 MAP are depicted in Table 2.

At 3 MAP, the foliar spray treatments had significant effect on plant height with T_1 recording the highest plant height (31.67 cm). This was followed by T_3 with a plant height of 29.00 cm, which was on par with T_2 and T_4. The least plant height (24.00 cm) was observed in water spray T_8 and control treatment T_9 and these were observed to be on par with T_5, T_6 and T_7.

At 5 MAP, significantly higher plant height (70.33 cm) was recorded in the treatment T_1 followed by T_2 (68.00 cm) and T_3 (65.67 cm). The treatment T_7 exhibited the lowest plant height (58.00 cm), which was on par with the treatments T_6, T_8 and T_9.

At 7 MAP, the highest plant height (112.33 cm) was recorded in the treatment T_1, which was on par with T_2. The treatment T_7 exhibited the lowest plant height (100.67 cm) which was observed to be on par with the treatments T_6, T_8 and T_9.

At all the stages of observation, it was found that the foliar spray treatment T_1 (SA 0.1 mM) gave maximum plant height among all the treatments tried.

4.1.2. Number of primary branches

Table 3 shows the effect of different foliar spray applications on number of primary branches of *P. longum* at 3, 5 and 7 MAP.

The number of primary branches did not show any significant variation among the different foliar spray treatments at all stages of observation. However, the higher number of primary branches were recorded in T_1 (SA 0.1 mM) and T_2 (SA 0.5 mM), at all stages of observation.

4.1.3. Number of spike bearing branches

The effect of different foliar spray applications on number of spike bearing branches of *P. longum* at 3, 5 and 7 MAP are illustrated in Table 4.

There was no significant variation among the treatments with respect to the number of spike bearing branches at all stages of observations. As in the case of spike bearing branches, higher number of spike bearing branches were observed in T_1 (SA 0.1 mM) and T_2 (SA 0.5 mM).

4.1.4. Number of inflorescence per plant

The data on number of inflorescence per plant as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 5.

At 3 MAP, there was no significant variation among the treatments with respect to the number of inflorescence per plant.

At 5 MAP, significantly higher number of inflorescence per plant (32.33) was recorded in treatment T_1, followed by T_2 (28.66) and T_3 (25.67). The lowest

Table 2. Effect of foliar spray treatments on plant height

Treatments	Plant height (cm)		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	31.67±0.88	70.33±0.88	112.33±0.88
T_2 (SA 0.5 mM)	28.67±0.67	68.00±0.58	109.67±0.88
T_3 (SA 1.0 mM)	29.00±0.58	65.67±0.88	107.00±0.58
T_4 (SA 1.5 mM)	27.33±0.88	62.67±0.33	106.33±0.88
T_5 (SA 2.0 mM)	26.00±0.58	62.00±0.58	104.00±0.58
T_6 (SA 2.5 mM)	25.33±0.88	60.00±0.58	102.67±0.88
T_7 (Ethanol)	24.33±0.88	58.00±0.58	100.67±1.20
T_8 (Water spray)	24.00±0.58	59.33±0.88	101.67±1.33
T_9 (Control)	24.00±0.58	59.00±0.58	100.67±0.88
SEm (±)	0.74	0.68	0.93
C.D (0.05)	2.190	2.008	2.762

Table 3. Effect of foliar spray treatments on number of primary branches

Treatments	Number of primary branches per plant		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	3.00±0.00	4.33±0.33	6.00±0.58
T_2 (SA 0.5 mM)	2.67±0.33	4.33±0.33	6.00±0.58
T_3 (SA 1.0 mM)	2.67±0.33	4.33±0.33	5.67±0.33
T_4 (SA 1.5 mM)	2.33±0.33	4.00±0.58	5.33±0.33
T_5 (SA 2.0 mM)	2.33±0.33	4.00±0.00	5.33±0.33
T_6 (SA 2.5 mM)	2.33±0.33	4.00±0.58	5.33±0.33
T_7 (Ethanol)	2.33±0.33	3.67±0.33	5.00±0.00
T_8 (Water spray)	2.33±0.33	3.33±0.33	4.33±0.33
T_9 (Control)	2.33±0.33	3.00±0.58	4.67±0.67
SEm (±)	0.31	0.42	0.43
C.D (0.05)	NS	NS	NS

Table 4. Effect of foliar spray treatments on number of spike bearing branches per plant

Treatments	Number of spikes bearing branches per plant		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	1.67±0.33	3.33±0.33	4.00±0.00
T_2 (SA 0.5 mM)	1.67±0.33	2.67±0.33	3.67±0.33
T_3 (SA 1.0 mM)	1.33±0.33	2.67±0.33	3.67±0.33
T_4 (SA 1.5 mM)	1.33±0.33	2.67±0.33	3.33±0.33
T_5 (SA 2.0 mM)	1.33±0.33	2.33±0.33	3.33±0.33
T_6 (SA 2.5 mM)	1.33±0.33	2.67±0.33	3.33±0.33
T_7 (Ethanol)	1.67±0.33	2.33±0.33	3.00±0.00
T_8 (Water spray)	1.00±0.00	2.33±0.33	2.67±0.33
T_9 (Control)	1.00±0.00	2.00±0.58	2.67±0.33
SEm (±)	0.29	0.31	0.29
C.D (0.05)	NS	NS	NS

Table 5. Effect of foliar spray treatments on number of inflorescence per plant

Treatments	Number of inflorescence per plant		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	5.33±0.33	32.33±0.88	65.67±0.88
T_2 (SA 0.5 mM)	4.67±0.33	28.67±0.88	61.33±0.88
T_3 (SA 1.0 mM)	4.33±0.33	25.67±0.88	57.67±0.88
T_4 (SA 1.5 mM)	4.33±0.33	24.33±0.88	53.00±0.58
T_5 (SA 2.0 mM)	3.67±0.33	22.67±0.67	48.00±0.58
T_6 (SA 2.5 mM)	4.00±0.58	21.00±0.58	45.33±0.88
T_7 (Ethanol)	4.00±0.58	21.33±0.67	46.00±1.53
T_8 (Water spray)	3.67±0.33	21.00±0.58	45.00±0.58
T_9 (Control)	4.00±0.00	21.33±0.33	45.67±1.20
SEm (±)	0.38	0.73	0.94
C.D (0.05)	NS	2.165	2.782

number of inflorescence (21.00) was recorded in treatment T_6 and ethanol spray T_8, which was statistically on par with T_5, T_7 and control treatment T_9.

At 7 MAP, inflorescence per plant were significantly higher in treatment T_1 (65.67), followed by T_2 (61.33) and T_3 (57.67). The lowest value was recorded in treatment T_8 (45.00) which was observed to be on par with T_6, T_7 and T_9.

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) gave maximum number of inflorescence among all the treatments tried.

4.1.5. Number of leaves

Total number of leaves as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 6.

At 3 MAP, salicylic acid had significant effect on number of leaves with T_1 recording significantly higher number of leaves (28.33) followed by T_2 (25.00) and T_3 (22.00). The lowest value was recorded in the control treatment T_9 (18.67) and was found to be on par with T_5, T_6, T_7 and T_8.

At 5 MAP, significantly higher number of leaves (55.00) was recorded in treatment T_1, followed by T_2 (50.33) and T_3(47.33). The lowest value (35.00) was recorded in the control treatment T_9. This was on par with water spray T_8.

At 7 MAP, significantly higher number of leaves (92.33) were recorded in treatment T_1, followed by T_2 (87.00) and T_3 (83.33). The lowest value (74.00) was recorded in treatment T_8 which was observed to be on par with T_7 and T_9.

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) gave maximum number of leaves among all the treatments tried.

4.1.6. Leaf area per plant

The data on leaf area as influenced by various foliar spray applications in *P. longum* at 3, 5 and 7 MAP are depicted in Table 7.

Table 6. Effect of foliar spray treatments on number of leaves per plant

Treatments	Number of leaves per plant		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	28.33±0.88	55.00±1.23	92.33±1.20
T_2 (SA 0.5 mM)	25.00±0.58	50.33±0.88	87.00±0.58
T_3 (SA 1.0 mM)	22.00±0.58	47.33±0.88	83.33±0.88
T_4 (SA 1.5 mM)	21.00±0.58	46.00±0.58	80.33±0.88
T_5 (SA 2.0 mM)	20.67±0.33	44.33±0.88	78.67±1.25
T_6 (SA 2.5 mM)	19.67±0.67	44.67±0.0.88	77.33±0.88
T_7 (Ethanol)	20.00±1.00	38.67±0.88	75.33±0.88
T_8 (Water spray)	19.00±0.58	36.33±0.88	74.00±0.58
T_9 (Control)	18.67±0.88	35.00±1.15	75.67±0.33
SEm (±)	0.71	0.98	0.91
C.D (0.05)	2.088	2.916	2.703

Table 7. Effect of foliar spray treatments on leaf area

Treatments	Leaf area (cm^2) per plant		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	1364.40±22.50	2729.17±59.96	4666.53±87.87
T_2 (SA 0.5 mM)	1207.73±15.02	2503.03±37.77	4387.87±55.47
T_3 (SA 1.0 mM)	1071.33±27.49	2356.73±32.14	4147.87±74.78
T_4 (SA 1.5 mM)	1045.10±28.12	2281.77±35.39	3971.23±52.25
T_5 (SA 2.0 mM)	1026.63±22.72	2179.57±39.87	3857.40±56.86
T_6 (SA 2.5 mM)	960.70±15.64	1975.01±24.31	3843.47±33.43
T_7 (Ethanol)	979.43±16.13	1856.00±31.53	3697.27±64.05
T_8 (Water spray)	931.27±20.56	1782.90±59.69	3645.60±29.53
T_9 (Control)	924.90±20.51	1717.80±55.60	3721.90±10.39
SEm (±)	31.91	57.96	64.63
C.D (0.05)	72.916	147.706	167.352

At 3 MAP, significantly higher leaf area (1364.40 cm^2) was recorded in treatment T_1, followed by T_2 (1207.73 cm^2) and T_3 (1071.33 cm^2). The lowest value (924.90 cm^2) was recorded in control treatment T_9, which was on par with T_6, T_7 and T_8.

At 5 MAP, significantly higher leaf area (2729.17 cm^2) was recorded in treatment T_1, followed by T_2 (2503.03 cm^2) and T_3 (2356.73 cm^2). The lowest value (1717.80 cm^2) was recorded in control treatment T_9 which was observed to be on par with T_8 and T_7.

At 7 MAP, leaf area (4666.53 cm^2) were significantly higher in the treatment T_1, followed by T_2 (4387.87 cm^2) and T_3 (4147.87 cm^2). The lowest value was recorded in the water spray treatment T_8 (3645.60 cm^2), which was found to be statistically on par with T_7 and T_9.

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) gave maximum leaf area among all the treatments tried.

4.1.7. Shoot weight per plant

Mean data on shoot weight as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 8.

At 3 MAP, significantly higher shoot weight per plant were recorded in treatment T_1, fresh weight being 23.68 g and dry weight 14.42 g. This was followed by T_2 (fresh-21.20 g; dry-13.91 g) and T_3 (fresh-20.41 g; dry-13.75 g). The treatment T_8 recorded the lowest values (fresh-18.58 g; dry-13.37 g) and was found to be on par with T_7 and T_9.

At 5 MAP, shoot weight per plant were significantly higher in treatment T_1, fresh weight being 45.96 g and dry weight 28.00 g. This was followed by T_2 (fresh-43.55 g; dry-27.48 g). Dry weight of T_2 was found to be on par with T_1. The treatment T_9 recorded the lowest values (fresh-35.43 g; dry-35.53 g) and was observed to be on par with T_7 and T_8.

At 7 MAP, shoot weight per plant were significantly higher in treatment T_1, fresh weight being 88.10 g and dry weight 52.62 g. This was followed by T_2 (fresh-85.74 g; dry-51.81 g) and T_3 (fresh-83.38 g; dry-51.30 g). The treatment T_8 recorded the lowest values (fresh-75.11 g; dry-49.63 g) and was found to be on par with T_6, T_7 and T_9.

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) gave maximum shoot weight among all the treatments tried.

4.1.8. Days to emergence of spike

The data on days to emergence of spike as influenced by different foliar spray treatments in *P. longum* is depicted in Table 9.

The foliar spray treatments had significant effect on days to emergence of spike. The minimum number of days (75 days) to emergence of spike was observed in T_1 which was observed to be on par with T_2. The maximum number of days (84.67 days) to spike emergence was observed in treatment T_6. This was on par with T_7, water spray T_8 and control treatment T_9.

4.1.9. Day to flowering

The effect of different foliar spray treatments on days to flowering of *P. longum* is presented in Table 9.

The foliar spray treatments had significant effect on days to flowering from the spike emergence. The lowest number of days to flowering was recorded in the treatment T_1 (22.67 days) followed by T_2 (24 days) and T_3 (26.33 days). Treatments, T_9 and T_8 recorded maximum number of days (27.67 days) which was observed to be on par with T_4, T_5, T_6 and T_7.

4.1.10. Days from emergence to maturity of spikes

Days from emergence to maturity of spikes as influenced by different foliar spray treatments in *P. longum* is shown in Table 9.

Table 8. Effect of foliar spray treatments on shoot weight

Treatments	Fresh and dry shoot weight (g plant^{-1})					
	3 MAP		5 MAP		7 MAP	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
T_1 (SA 0.1 mM)	23.68±0.22	14.42±0.07	45.96±0.61	28.00±0.07	88.10±0.64	52.62±0.04
T_2 (SA 0.5 mM)	21.20±0.08	13.91±0.05	43.55±0.45	27.48±0.09	85.74±0.87	51.81±0.16
T_3 (SA 1.0 mM)	20.41±0.16	13.75±0.06	41.93±0.37	27.16±0.13	83.38±0.67	51.30±0.15
T_4 (SA 1.5 mM)	19.96±0.09	13.65±0.03	39.96±0.38	26.70±0.08	81.30±1.04	50.90±0.21
T_5 (SA 2.0 mM)	19.26±0.11	13.51±0.05	38.81±1.05	26.48±0.22	80.65±0.57	50.72±0.09
T_6 (SA 2.5 mM)	19.03±0.04	13.46±0.04	37.36±0.28	26.17±0.06	76.76±0.56	50.01±0.12
T_7 (Ethanol)	18.86±0.06	13.43±0.05	36.85±0.41	26.08±0.08	75.66±0.91	49.73±0.20
T_8 (Water spray)	18.58±0.12	13.37±0.05	35.72±0.48	26.26±0.01	75.11±0.85	49.63±0.18
T_9 (Control)	18.75±0.06	13.40±0.05	35.43±0.57	25.93±0.11	75.93±0.77	49.77±0.13
SEm (±)	0.12	0.04	0.55	0.19	0.78	0.15
C.D (0.05)	0.345	0.152	1.642	0.591	2.233	0.447

Table 9. Effect of foliar spray treatments on days to emergence of spike, days to flowering and days from emergence to maturity of spike

Treatments	Days to emergence of spike	Days to flowering	Days from emergence to maturity of spike
T_1 (SA 0.1 mM)	75.00±0.58	22.67±0.33	61.33±0.33
T_2 (SA 0.5 mM)	76.67±0.33	24.00±0.58	61.67±0.88
T_3 (SA 1.0 mM)	78.67±0.88	26.33±0.33	61.33±0.67
T_4 (SA 1.5 mM)	80.00±0.58	26.67±0.33	60.67±0.33
T_5 (SA 2.0 mM)	81.67±1.15	27.33±0.33	60.67±0.33
T_6 (SA 2.5 mM)	84.67±0.88	27.00±0.58	60.33±0.67
T_7 (Ethanol)	84.00±0.58	27.33±0.33	61.67±0.33
T_8 (Water spray)	84.33±0.88	27.67±0.33	60.00±0.58
T_9 (Control)	83.67±0.67	27.67±0.33	60.00±0.58
SEm (±)	0.81	0.40	0.56
C.D (0.05)	2.426	1.190	NS

There was no significant variation among the treatments with respect to days from emergence to maturity of spikes.

4.2. METABOLITE PRODUCTION

4.2.1. Total chlorophyll

The data on total chlorophyll as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 10.

At 3 MAP, the foliar spray treatments had a significant effect on total chlorophyll with T_2 recording the highest chlorophyll content (1.62 mg g^{-1}) and was followed by T_1 (1.56 mg g^{-1}) and T_3 (1.53 mg g^{-1}). The lowest chlorophyll content was observed in control treatment T_9 (1.31 mg g^{-1}) and was observed to be on par with T_7 and T_8.

At 5 MAP, highest chlorophyll content (1.66 mg g^{-1}) was recorded in the treatment T_2 and was followed by T_1 (1.60 mg g^{-1}) and T_3 (1.57 mg g^{-1}). The control treatment T_9 (1.34 mg g^{-1}) exhibited the lowest chlorophyll content, which was statistically on par with T_7 and T_8.

At 7 MAP, significantly higher chlorophyll content (1.72 mg g^{-1}) was recorded in the treatment T_2, followed by T_1 (1.66 mg g^{-1}) and T_3 (1.63 mg g^{-1}). The control treatment T_9 (1.41 mg g^{-1}) exhibited the lowest chlorophyll content which was observed to be on par with T_7 and T_8.

At all the stages of observation, it was found that the treatment T_2 (SA 0.5 mM) gave significantly higher chlorophyll content among all the treatments tried.

4.2.2. Total proteins

The effect of different foliar spray applications on total protein content of *P. longum* at 3, 5 and 7 MAP are illustrated in Table 11.

At 3 MAP, significantly higher protein content (12.27 mg g^{-1}) was recorded in the treatment T_2 followed by T_1 (11.72 mg g^{-1}) and T_3 (11.43 mg g^{-1}). The

Table 10. Effect of foliar spray treatments on total chlorophyll

Treatments	Total chlorophyll (mg g^{-1})		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	1.561±0.01	1.601±0.01	1.662±0.01
T_2 (SA 0.5 mM)	1.624±0.01	1.664±0.01	1.724±0.01
T_3 (SA 1.0 mM)	1.530±0.00	1.571±0.00	1.631±0.00
T_4 (SA 1.5 mM)	1.497±0.00	1.538±0.00	1.588±0.00
T_5 (SA 2.0 mM)	1.488±0.01	1.528±0.01	1.579±0.00
T_6 (SA 2.5 mM)	1.436±0.00	1.477±0.00	1.530±0.01
T_7 (Ethanol)	1.324±0.01	1.364±0.01	1.425±0.01
T_8 (Water spray)	1.316±0.00	1.353±0.00	1.417±0.01
T_9 (Control)	1.310±0.00	1.340±0.00	1.410±0.00
SEm (±)	0.01	0.01	0.01
C.D (0.05)	0.021	0.020	0.021

Table 11. Effect of foliar spray treatments on total proteins

Treatments	Total Proteins (mg g^{-1})		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	11.72±0.02	12.83±0.03	13.86±0.02
T_2 (SA 0.5 mM)	12.27±0.03	13.28±0.01	14.62±0.06
T_3 (SA 1.0 mM)	11.43±0.06	12.57±0.03	13.66±0.02
T_4 (SA 1.5 mM)	10.90±0.03	11.94±0.06	13.29±0.03
T_5 (SA 2.0 mM)	10.04±0.03	11.39±0.03	12.83±0.03
T_6 (SA 2.5 mM)	9.76±0.03	10.46±0.05	12.20±0.05
T_7 (Ethanol)	9.27±0.04	9.94±0.03	11.45±0.02
T_8 (Water spray)	9.26±0.03	9.95±0.04	11.39±0.03
T_9 (Control)	9.24±0.03	9.97±0.06	11.40±0.04
SEm (±)	0.02	0.03	0.02
C.D (0.05)	0.105	0.121	0.106

treatment T_9 exhibited the lowest protein content (9.243 mg g^{-1}) which was observed to be on par with treatments T_7 and T_8.

At 5 MAP, the highest protein content (13.28 mg g^{-1}) was recorded in the treatment T_2 which was followed by T_1 (12.83 mg g^{-1}) and T_3 (12.57 mg g^{-1}). The treatment T_7 (9.94 mg g^{-1}) exhibited the lowest protein content, which was found to be on par with treatments T_8 and T_9.

At 7 MAP, significantly higher protein content (14.62 mg g^{-1}) was observed in treatment T_2. This was followed by T_1 (13.86 mg g^{-1}) and T_3 (13.66 mg g^{-1}). The lowest protein content (11.39 mg g^{-1}) was observed in water spray treatment T_8 and was found to be on par with the treatments T_7 and T_9.

At all the stages of observation, it was found that the treatment T_2 (SA 0.5mM) gave significantly higher protein content among all the treatments tried.

4.2.3. Peroxidase

Mean data on peroxidase activity as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are illustrated in Table 12.

At 3 MAP, significantly higher peroxidase activity (19.66 activity $g^{-1}min^{-1}$) was recorded in the treatment T_2 which was followed by T_1 (18.93 activity $g^{-1}min^{-1}$) and T_3 (18.51 activity $g^{-1}min^{-1}$). The control treatment T_9 exhibited the lowest peroxidase activity (16.78 activity $g^{-1}min^{-1}$) which was observed to be on par with T_7 and T_8.

At 5 MAP, the highest peroxidase activity (21.51 activity $g^{-1}min^{-1}$) was observed in the treatment T_2 and was followed by T_1 (19.92 activity $g^{-1}min^{-1}$) and T_3 (19.36 activity $g^{-1}min^{-1}$). The control treatment T_9 exhibited the lowest peroxidase content (17.56 activity $g^{-1}min^{-1}$) and was statistically on par with T_7 and T_8.

At 7 MAP, significantly higher peroxidase activity (23.93 activity $g^{-1}min^{-1}$) was recorded in the treatment T_2 and was followed by T_1 (22.08 activity $g^{-1}min^{-1}$)

and T_3 (21.62 activity $g^{-1}min^{-1}$). The lowest peroxidase activity (20.1 activity $g^{-1}min^{-1}$) was observed in treatment T_8 and was observed to be on par with T_7 and T_9.

At all the stages of observation, it was found that the treatment T_2 (SA 0.5 mM) gave significantly higher peroxidase activity among all the treatments tried.

4.2.4. Catalase

The data on catalase activity as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP is depicted in Table 13.

At 3 MAP, significantly higher catalase activity (912.33 U ml^{-1}) was recorded in the treatment T_2 followed by T_1 (891.00 U ml^{-1}) and T_3 (861.67 U ml^{-1}). The control treatment T_9 (703.00 U ml^{-1}) exhibited the lowest value which was observed to be on par with T_7 and T_8.

At 5 MAP, significantly higher catalase activity (1027.00 U ml^{-1}) was recorded in the treatment T_2, followed by T_1 (988.33 U ml^{-1}) and T_3 (959.33 U ml^{-1}). The control treatment T_9 exhibited the lowest catalase activity (803.00 U ml^{-1}) and was observed to be on par with the treatments T_7 and T_8.

At 7 MAP, significantly higher catalase activity (1154.67 U ml^{-1}) was recorded in the treatment T_2 followed by T_1 (1126.33 U ml^{-1}) and T_3 (1085.00 U ml^{-1}). The treatment T_8 exhibited the lowest catalase activity (907.33 U ml^{-1}) which was observed to be on par with T_7 and T_9.

At all the stages of observation, it was found that the treatment T_2 (SA 0.5 mM) gave significantly higher catalase activity among all the treatments tried.

4.2.5. Superoxide dismutase (SOD)

The effect of different foliar spray treatments on superoxide dismutase (SOD) activity of *P. longum* at 3, 5 and 7 MAP are illustrated in Table 14.

At 3 MAP, significantly higher SOD activity (1.093 activity $g^{-1}min^{-1}$) was recorded in the treatment T_2 followed by T_1 (0.971 activity $g^{-1}min^{-1}$) and T_3 (0.954

Table 12. Effect of foliar spray treatments on peroxidase activity

Treatments	Peroxidase (activity $g^{-1}min^{-1}$)		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	18.93±0.10	19.92±0.10	22.08±0.29
T_2 (SA 0.5 mM)	19.66±0.11	21.07±0.03	23.93±0.03
T_3 (SA 1.0 mM)	18.51±0.14	19.36±0.10	21.65±0.07
T_4 (SA 1.5 mM)	18.36±0.05	18.89±0.14	21.12±0.03
T_5 (SA 2.0 mM)	18.14±0.30	18.64±0.07	20.82±0.03
T_6 (SA 2.5 mM)	17.71±0.02	18.55±0.03	20.62±0.02
T_7 (Ethanol)	16.82±0.04	17.80±0.03	20.12±0.03
T_8 (Water spray)	16.78±0.04	17.76±0.05	20.10±0.02
T_9 (Control)	16.78±0.04	17.80±0.05	20.11±0.02
SEm (±)	0.10	0.16	0.08
C.D (0.05)	0.368	0.561	0.301

Table 13. Effect of foliar spray treatments on catalase activity

Treatments	Catalase (U ml^{-1})		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	891.00±1.53	988.33±1.34	1116.33±2.17
T_2 (SA 0.5 mM)	912.33±0.89	1027.00±0.58	1158.00±1.73
T_3 (SA 1.0 mM)	861.67±0.87	959.33±1.46	1088.33±2.59
T_4 (SA 1.5 mM)	859.00±1.00	926.33±0.89	1049.00±2.52
T_5 (SA 2.0 mM)	810.67±0.87	906.33±2.34	1006.67±2.02
T_6 (SA 2.5 mM)	762.00±2.08	871.67±2.02	957.67±1.66
T_7 (Ethanol)	712.00±1.76	810.67±2.02	908.11±1.45
T_8 (Water spray)	704.00±1.53	804.33±0.89	907.33±2.41
T_9 (Control)	703.00±2.08	803.00±2.08	911.33±2.61
SEm (±)	1.48	1.62	2.17
C.D (0.05)	4.439	4.867	6.502

activity g^{-1} min^{-1}). The treatment T_7 (0.853 activity $g^{-1}min^{-1}$) exhibited the lowest value which was observed to be on par with T_8 and T_9.

At 5 MAP, significantly higher SOD activity (1.201 activity $g^{-1}min^{-1}$) was recorded in the treatment T_2 followed by T_1(1.176 activity $g^{-1}min^{-1}$) and T_3 (1.098 activity $g^{-1}min^{-1}$). The lowest SOD activity (0.954 activity $g^{-1}min^{-1}$) was exhibited by the treatment T_8 which was observed to be on par with T_6, T_7 and T_9.

At 7 MAP, significantly the highest SOD activity (1.292 activity $g^{-1}min^{-1}$) was recorded in the treatment T_2 followed by T_1 (1.267 activity $g^{-1}min^{-1}$) and T_3 (1.240 activity $g^{-1}min^{-1}$). The control treatment T_7 (0.845 activity $g^{-1}min^{-1}$) exhibited the lowest SOD activity which was found to be on par with the treatments T_8 and T_9.

At all the stages of observation, it was found that the treatment T_2 (SA 0.5 mM) gave significantly higher SOD activity among all the treatments tried.

4.2.6. Piperine content and piperine yield

Piperine content and yield per plant as influenced by different foliar spray treatments of *P. longum* is illustrated in Table 15.

The foliar spray treatments had significant effect on piperine content (1.153 per cent) with T_2 recording the highest value and was followed by T_3 (1.057 per cent). T_3 was observed to be on par with T_1. The lowest piperine content (0.873 per cent) was observed in treatment T_8 and was observed to be on par with the treatments T_6, T_7 and T_9.

With respect to piperine yield T_2 recorded highest significant yield (0.84 mg $plant^{-1}$) and was followed by T_1 (0.79 mg $plant^{-1}$) and T_3 (0.77 mg $plant^{-1}$). The lowest piperine yield (0.61 mg $plant^{-1}$) was observed in control treatment T_8 and was observed to be on par with the treatments T_7 and T_9.

Table 14. Effect of foliar spray treatments on SOD (superoxide dismutase) activity

Treatments	SOD (activity $g^{-1}min^{-1}$)		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	0.971±0.01	1.176±0.00	1.267±0.00
T_2 (SA 0.5 mM)	1.093±0.01	1.201±0.00	1.292±0.00
T_3 (SA 1.0 mM)	0.954±0.00	1.098±0.01	1.240±0.00
T_4 (SA 1.5 mM)	0.927±0.00	1.067±0.01	1.190±0.01
T_5 (SA 2.0 mM)	0.896±0.00	0.991±0.02	1.144±0.00
T_6 (SA 2.5 mM)	0.878±0.00	0.973±0.00	1.120±0.00
T_7 (Ethanol)	0.853±0.00	0.957±0.00	1.019±0.00
T_8 (Water spray)	0.854±0.00	0.954±0.00	1.022±0.01
T_9 (Control)	0.855±0.00	0.956±0.00	1.023±0.00
SEm (±)	0.00	0.01	0.00
C.D (0.05)	0.015	0.022	0.013

Table 15. Effect of foliar spray treatments on piperine, piperine yield, volatile oil and volatile oil yield

Treatments	Piperine (per cent)	Piperine yield (mg $plant^{-1}$)	Volatile oil (per cent)	Volatile oil Yield (mg $plant^{-1}$)
T_1 (SA 0.1 mM)	1.05±0.00	0.796±0.00	1.26±0.01	0.953±0.00
T_2 (SA 0.5 mM)	1.15±0.01	0.847±0.01	1.32±0.01	0.972±0.01
T_3 (SA 1.0 mM)	1.06±0.01	0.768±0.00	1.28±0.01	0.932±0.01
T_4 (SA 1.5 mM)	1.01±0.01	0.723±0.01	1.25±0.01	0.895±0.00
T_5 (SA 2.0 mM)	0.95±0.01	0.680±0.01	1.23±0.01	0.879±0.01
T_6 (SA 2.5 mM)	0.90±0.01	0.638±0.01	1.21±0.01	0.852±0.01
T_7 (Ethanol)	0.88±0.01	0.617±0.01	1.17±0.01	0.815±0.01
T_8 (Water spray)	0.87±0.01	0.610±0.01	1.15±0.01	0.803±0.00
T_9 (Control)	0.88±0.01	0.615±0.00	1.18±0.01	0.822±0.00
SEm (±)	0.01	0.00	0.01	0.00
C.D (0.05)	0.03	0.021	0.026	0.019

4.2.7. Volatile oil content and volatile oil yield

The effect of different foliar spray treatments on volatile oil content and yield per plant of *P. longum* is illustrated in Table 15.

The different foliar treatments had significant effect on volatile oil content with T_2 recording highest volatile oil content (1.32 per cent). This was followed by T_3 (1.28 per cent) which was observed to be on par with T_1. The lowest volatile oil content was observed in treatment T_8 (1.15 per cent) and was observed to be on par with T_7 and T_9.

With regard to volatile oil yield T_2 recorded the highest volatile oil yield (0.97 mg plant^{-1}) and was followed by T1(0.95 mg plant^{-1}) and T3 (0.93 mg plant^{-1}). The lowest volatile oil yield was recorded in treatment T_8 (0.80 mg plant^{-1}) and was observed to be on par with the treatments T_7 and T_9

4.2.8. Oleoresin content and oleoresin yield

Influence of different foliar spray treatments on oleoresin content and yield per plant of *P. longum* is presented in Table 16.

The different foliar spray treatments had significant effect on oleoresin content with T_2 recording the highest significant oleoresin content (14.21 per cent). This was followed by T_3 (13.88 per cent) which was observed to be on par with T_1. The lowest oleoresin content (12.69 per cent) was observed in control treatment T_9 and was observed to be on par with the treatments T_7 and T_8.

With respect to oleoresin yield T_1 recorded highest oleoresin yield (10.55 mg plant^{-1}) and was found to be on par with the treatment T_2 (10.35 mg plant^{-1}), which was followed by T_3. The lowest oleoresin yield (8.84 mg plant^{-1}) was observed in treatment T_8 and this was observed to be on par with the treatments T_7 and T_9.

4.2.9. Carbohydrate

The effect of different foliar spray treatments on carbohydrate content of *P. longum* is illustrated in Table 17.

The different foliar spray treatments had a significant effect on carbohydrate content with T_2 recording the highest carbohydrate content (87.42 mg g^{-1}) and was followed by T_1 (85.83 mg g^{-1}) and T_3 (84.77 mg g^{-1}). The lowest carbohydrate content was observed in treatment T_8 (76.51 mg g^{-1}) and was on par with T_7 and T_9.

4.2.10. Starch

Mean total of starch content as influenced by different foliar spray treatments of *P. longum* is depicted in Table 17.

The different foliar spray treatments had a significant effect on starch content with T_2 recording the highest starch content (55.62 mg g^{-1}) and was followed by T_1 (53.84 mg g^{-1}) and T_3 (52.77 mg g^{-1}). The lowest starch content was observed in treatment T_7 (47.30 mg g^{-1}) and was observed to be on par with T_8 and T_9.

4.2.11. Sugar

Influence of different foliar spray treatments on sugar content of *P. longum* is illustrated in Table 17.

The different foliar spray treatments had a significant effect on sugar content with treatment T_2 recording the highest sugar content (29.56 mg g^{-1}) and was followed by T_1 (28.38 mg g^{-1}) which was found to be on par with T_3. The lowest sugar content was observed in treatment T_8 (23.41 mg g^{-1}) and was found to be on par with the treatments T_7 and T_9.

4.3. PHYSIOLOGICAL PARAMETERS

4.3.1 Dry matter production

The data on dry matter production per plant as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 18.

Table 16. Effect of foliar spray treatments on oleoresin and oleoresin yield

Treatments	Oleoresin (per cent)	Oleoresin yield (mg plant^{-1})
T_1 (SA 0.1 mM)	13.820±0.02	10.55±0.11
T_2 (SA 0.5 mM)	14.210±0.02	10.36±0.07
T_3 (SA 1.0 mM)	13.883±0.02	10.01±0.07
T_4 (SA 1.5 mM)	13.573±0.02	9.67±0.09
T_5 (SA 2.0 mM)	13.223±0.01	9.37±0.06
T_6 (SA 2.5 mM)	13.033±0.03	9.18±0.04
T_7 (Ethanol)	12.770±0.05	8.92±0.04
T_8 (Water spray)	12.767±0.06	8.84±0.04
T_9 (Control)	12.687±0.07	8.86±0.05
SEm (±)	0.04	0.06
C.D (0.05)	0.118	0.204

Table 17. Effect of foliar spray treatments on carbohydrate, starch and sugar content

Treatments	Carbohydrate (mg g^{-1})	Starch (mg g^{-1})	Sugar (mg g^{-1})
T_1 (SA 0.1 mM)	84.77±0.23	52.77±0.23	28.32±0.02
T_2 (SA 0.5 mM)	87.42±0.13	55.62±0.54	29.56±0.25
T_3 (SA 1.0 mM)	85.84±0.33	53.84±0.33	28.38±0.03
T_4 (SA 1.5 mM)	84.00±0.34	52.23±0.11	27.50±0.10
T_5 (SA 2.0 mM)	82.69±0.33	51.36±0.10	26.23±0.03
T_6 (SA 2.5 mM)	79.12±0.08	49.63±0.03	24.80±0.01
T_7 (Ethanol)	76.75±0.06	47.30±0.05	23.45±0.05
T_8 (Water spray)	76.51±0.16	47.32±0.04	23.41±0.02
T_9 (Control)	76.68±0.14	47.31±0.02	23.41±0.05
SEm (±)	0.12	0.17	0.10
C.D (0.05)	0.671	0.687	0.350

At 3 MAP, significant variation was observed on dry matter production with T_1 (18.42 g) recording the highest value, and was followed by T_2 (17.91 g) and T_3 (17.75 g). The lowest value was observed in treatment T_8 (17.43 g), which was on par with T_5, T_6, T_7 and T_9.

At 5 MAP, dry matter production was significantly higher in the treatment T_1 (35.77 g), which was followed by T_2 (35.08 g) and T_3 (34.66 g). The lowest value was recorded in the control treatment T_8 (33.43 g) which was observed to be on par with T_6, T_7 and T_9.

At 7 MAP, dry matter production (67.62 g) was significantly higher in the treatment T_1, followed by T_2 (65.48 g) and T_3 (64.64 g). The lowest value was recorded in the treatment T_8 (61.81 g) which was statistically on par with T_7 and T_9.

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) gave maximum dry matter production per plant among all the treatments tried.

4.3.2. Leaf area index (LAI)

Mean total of Leaf area index (LAI) as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 19.

At 3 MAP, foliar treatments showed significant effect on LAI with T_1 (0.758) recording the highest value, followed by T_2 (0.671) and T_3 (0.595). The lowest value (0.513) was recorded in control treatment T_9, which was on par with T_6, T_7 and T_8.

At 5 MAP, LAI was significantly higher in the treatment T_1 (1.516), followed by T_2 (1.390). The lowest value was recorded in the control treatment T_9 (0.960) which was observed to be on par with T8.

At 7 MAP, significantly higher LAI (2.592) was observed in the treatment T_1, followed by T_2 (2.437) and T_3 (2.304). The lowest value was recorded in the treatment T_8 (2.027), which was found to be on par with the treatments T_7 and T_9.

Table 18. Effect of foliar spray treatments on dry matter production

Treatments	Dry matter production (g plant^{-1})		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	18.42±0.07	35.77±0.07	67.62±0.04
T_2 (SA 0.5 mM)	17.91±0.05	35.08±0.08	65.48±0.03
T_3 (SA 1.0 mM)	17.75±0.06	34.66±0.13	64.64±0.03
T_4 (SA 1.5 mM)	17.65±0.03	34.20±0.08	63.83±0.03
T_5 (SA 2.0 mM)	17.51±0.05	33.98±0.22	63.29±0.02
T_6 (SA 2.5 mM)	17.46±0.04	33.67±0.06	62.60±0.03
T_7 (Ethanol)	17.43±0.04	33.58±0.08	61.82±0.20
T_8 (Water spray)	17.37±0.05	33.43±0.01	61.81±0.18
T_9 (Control)	17.40±0.04	33.54±0.51	61.84±0.012
SEm (±)	0.05	0.06	0.03
C.D (0.05)	0.152	0.176	0.089

Table 19. Effect of foliar spray treatments on leaf area index

Treatments	Leaf area index (LAI)		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	0.758±0.01	1.516±0.06	2.592±0.05
T_2 (SA 0.5 mM)	0.671±0.01	1.390±0.02	2.437±0.03
T_3 (SA 1.0 mM)	0.595±0.02	1.309±0.02	2.304±0.04
T_4 (SA 1.5 mM)	0.580±0.02	1.268±0.02	2.206±0.03
T_5 (SA 2.0 mM)	0.570±0.01	1.211±0.02	2.154±0.02
T_6 (SA 2.5 mM)	0.535±0.02	1.193±0.02	2.135±0.02
T_7 (Ethanol)	0.541±0.01	1.058±0.01	2.050±0.05
T_8 (Water spray)	0.514±0.01	0.983±0.03	2.027±0.03
T_9 (Control)	0.513±0.02	0.963±0.04	2.070±0.03
SEm (±)	0.01	0.03	0.03
C.D (0.05)	0.044	0.086	0.090

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) gave significantly higher leaf area index among all the treatments tried.

4.3.3. Net Assimilation Rate

The data on NAR as influenced by different foliar spray treatments in *P. longum* depicted in Table 20.

During the period from 3 and 5 MAP, significantly higher NAR (0.107 mg cm^{-2} day^{-1}) was obtained in the treatment T_2, which was found to be on par with T_1. The lowest value was observed in control treatment T_9 (0.094 mg cm^{-2} day^{-1}) which was observed to be on par with the treatments T_7 and T_8.

During the period from 5 MAP and 7 MAP, NAR were significantly higher in the treatment T_2 (0.077 mg cm^{-2} day^{-1}) which was on par with T_1 and T_3. The lowest value (0.072 mg cm^{-2} day^{-1}) was recorded in the treatments T_7, T_8 and T_9.

At both periods of observation, it was found that the treatment T_2 (SA 0.5 mM) gave maximum NAR among all the treatments tried.

4.3.4. Stomatal conductance

The result on the effect of foliar spray treatments on stomatal conductance in *P. longum* at 3, 5 and 7 MAP is depicted in Table 21.

At 3 MAP, stomatal conductance (318.67 mmoles m^{-2} s^{-1}) was significantly higher in the treatment T_1, followed by T_2 (300.67 mmoles m^{-2} s^{-1}) and T_3 (290.00 mmoles m^{-2} s^{-1}). The lowest value was recorded in the treatment T_8 (246.33 mmoles m^{-2} s^{-1}) which was observed to be on par with the treatments T_7 and T_9.

At 5 MAP, stomatal conductance was significantly higher in the treatment T_1 (407.67 mmoles m^{-2} s^{-1}), followed by T_2 (385.67 mmoles m^{-2} s^{-1}) and T_3 (376.00 mmoles m^{-2} s^{-1}). The lowest value was recorded in the control treatment T_8 (332.00 mmoles m^{-2} s^{-1}) which was observed to be on par with T_7 and T_9.

Table 20. Effect of foliar spray treatments on net assimilation rate

Treatments	Net assimilation rate (mg cm^{-2} day^{-1})	
	3 to 5 MAP	5 to 7 MAP
T_1 (SA 0.1 mM)	0.105±0.00	0.076±0.00
T_2 (SA 0.5 mM)	0.107±0.00	0.077±0.00
T_3 (SA 1.0 mM)	0.102±0.00	0.076±0.00
T_4 (SA 1.5 mM)	0.101±0.01	0.075±0.00
T_5 (SA 2.0 mM)	0.100±0.00	0.074±0.00
T_6 (SA 2.5 mM)	0.099±0.00	0.073±0.00
T_7 (Ethanol)	0.098±0.00	0.072±0.00
T_8 (Water spray)	0.098±0.00	0.072±0.00
T_9 (Control)	0.097±0.00	0.072±0.00
SEm (±)	0.001	0.000
C.D (0.05)	0.002	0.001

Table 21. Effect of foliar spray treatments on stomatal conductance

Treatments	Stomatal conductance (mmoles m^{-2} s^{-1})		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	318.67±1.45	407.67±2.19	467.00±2.08
T_2 (SA 0.5 mM)	300.67±1.45	385.67±2.91	456.00±2.08
T_3 (SA 1.0 mM)	290.00±1.53	376.00±2.31	446.33±1.45
T_4 (SA 1.5 mM)	278.00±2.08	360.33±1.20	437.33±2.60
T_5 (SA 2.0 mM)	263.33±2.03	352.00±2.08	428.33±2.73
T_6 (SA 2.5 mM)	258.33±0.88	349.00±2.89	411.67±1.76
T_7 (Ethanol)	247.33±2.33	334.00±4.04	382.33±3.28
T_8 (Water spray)	246.33±3.17	332.00±2.64	379.33±3.53
T_9 (Control)	251.67±2.33	337.67±2.03	382.00±2.08
SEm (±)	2.03	1.96	2.24
C.D (0.05)	8.093	7.679	9.309

At 7 MAP, foliar spray with SA had significant effect on stomatal conductance with T_1 (467.00 mmoles m^{-2} s^{-1}) recording the highest value, followed by T_2 (456.00 mmoles m^{-2} s^{-1}) and T_3 (446.33 mmoles m^{-2} s^{-1}). The lowest value was observed in control treatment T_8 (379.33 mmoles m^{-2} s^{-1}) which was observed to be on par with the T_7 and T_9.

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) was significantly superior with respect to stomatal conductance among all the treatments tried.

4.3.5. Photosynthetic rate

The data on photosynthetic rate as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 22.

At 3 MAP, the highest photosynthetic rate was recorded in treatment T_1 (7.50 μCO_2 moles m^{-2} s^{-1}), followed by T_2 (7.20 μCO_2 moles m^{-2} s^{-1}) and T_3 (6.93 μCO_2 moles m^{-2} s^{-1}). The lowest value (6.033 μCO_2 moles m^{-2} s^{-1}) was observed in treatment T_7 which is found to be on par with T_8 and T_9.

At 5 MAP, photosynthetic rate (8.43 μCO_2 moles m^{-2} s^{-1}) was significantly higher in the treatment T_1, followed by T_2 (8.10 μCO_2 moles m^{-2} s^{-1}) and T_3 (7.83 μCO_2 moles m^{-2} s^{-1}). The lowest value was recorded in the control treatment T_9 (6.87 μCO_2 moles m^{-2} s^{-1}) which was observed to be on par withT_7 and T_8.

At 7 MAP, photosynthetic rate was significantly higher in the treatment T_1 (8.93 μCO_2 moles m^{-2} s^{-1}), followed by T_2 (8.60 μCO_2 moles m^{-2} s^{-1}) and T_3 (8.40 μCO_2 moles m^{-2} s^{-1}). The treatment T_9 recorded the lowest value (7.40 μCO_2 moles m^{-2} s^{-1}) which was observed to be on par with the treatments T_7 and T_8.

At all the stages of observation, it was found that the treatment T_1 (SA 0.1 mM) recorded significantly higher photosynthetic rate among all the treatments tried.

4.3.6. Cell Membrane Stability Index

The data on cell membrane stability index as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are illustrated in Table 23. The foliar spray treatments significantly influenced cell membrane stability index in the crop.

At 3 MAP, cell membrane stability index (115.63 per cent) was significantly higher in treatment T_2, which was followed by T_1 (113.15 per cent). T_1 observed to be on par with T_3. The lowest value was observed in treatment T_8 (103.76 per cent) which was observed to be on par with T_6 and T_7.

At 5 MAP, significantly higher cell membrane stability index (110.54 per cent) was observed in the treatment T_2, followed by T_1 (107.47 per cent) and T_3 (105.93 per cent). The lowest value was recorded in the treatment T_8 (96.59 per cent) which was found to be on par with T_6 and T_7.

At 7 MAP, cell membrane stability index was significantly higher in the treatment T_2 (107.64 per cent), followed by T_1 (104.66 per cent) and T3 (102.35 per cent). The lowest value was recorded in the T_8 (92.57 per cent) and was on par with the treatments T_6 and T_7.

At all the stages of observation, it was found that the treatment T_2 (SA 0.5 mM) gave maximum cell membrane stability index among all the treatments tried.

4.3.7. Proline

Mean data on proline content as influenced by different foliar spray treatments in *P. longum* at 3, 5 and 7 MAP are depicted in Table 24.

At 3 MAP, significantly higher proline content (141.28 $\mu g\ g^{-1}$) was recorded in the treatment T_2. This was followed by T_1 (135.67 $\mu g\ g^{-1}$) and T_3 (132.75 $\mu g\ g^{-1}$). The lowest value was recorded in the treatment T_8 (117.41 $\mu g\ g^{-1}$), which was found to be on par with T_7 and T_9.

Table 22. Effect of foliar spray treatments on photosynthetic rate

Treatments	Photosynthetic rate (μCO_2 moles m^{-2} s^{-1})		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	7.50±0.06	8.43±0.03	8.43±0.03
T_2 (SA 0.5 mM)	7.20±0.06	8.10±0.06	8.10±0.06
T_3 (SA 1.0 mM)	6.93±0.03	7.83±0.03	7.83±0.03
T_4 (SA 1.5 mM)	6.70±0.06	7.63±0.03	7.63±0.03
T_5 (SA 2.0 mM)	6.53±0.07	7.40±0.06	7.40±0.06
T_6 (SA 2.5 mM)	6.40±0.06	7.13±0.03	7.13±0.03
T_7 (Ethanol)	6.03±0.09	6.90±0.06	7.47±0.07
T_8 (Water spray)	6.07±0.09	6.90±0.06	7.43±0.03
T_9 (Control)	6.07±0.05	6.87±0.05	7.40±0.06
SEm (±)	0.06	0.04	0.05
C.D (0.05)	0.204	0.158	0.168

Table 23. Effect of foliar spray treatments on cell membrane stability index

Treatments	Cell membrane integrity (per cent)		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	113.15±0.09	107.47±0.38	104.66±0.25
T_2 (SA 0.5 mM)	115.63±0.20	110.54±0.38	107.64±0.05
T_3 (SA 1.0 mM)	111.51±0.13	105.93±0.17	102.35±0.10
T_4 (SA 1.5 mM)	110.49±0.14	104.67±0.20	100.91±0.28
T_5 (SA 2.0 mM)	109.17±0.22	102.10±0.16	98.75±0.15
T_6 (SA 2.5 mM)	105.73±0.14	96.59±0.26	92.74±0.20
T_7 (Ethanol)	105.04±0.14	97.92±0.21	92.65±0.21
T_8 (Water spray)	104.73±0.08	97.32±0.14	92.57±0.27
T_9 (Control)	0.61	0.21	0.35
SEm (±)	2.080	0.801	1.190

At 5 MAP, proline content (152.84 μg g^{-1}) was significantly higher in the treatment T_2, followed by T_1 (142.5 μg g^{-1}) and T_3 (139.88 μg g^{-1}). The lowest value was recorded in the treatment T_8 (123.11 μg g^{-1}) which was observed to be on par with T_7 and T_9.

At 7 MAP, significantly higher proline content (157.13 μg g^{-1}) was observed in treatment T_2, followed by T_1 (151.99 μg g^{-1}) and T_3 (149.58 μg g^{-1}). The lowest value was recorded in control treatment T_9 (137.35 μg g^{-1}) which was on par with the treatments T_6, T_7 and T_8.

At all the stages of observation, it was found that the treatment T_2 (SA 0.5 mM) gave significantly higher proline content among all the treatments tried.

4.4. YIELD AND YIELD COMPONENTS

4.4.1 Number of spikes

The effect of different foliar spray treatments on number of spikes of *P. longum* is illustrated in Table 25.

The different foliar spray treatments had significant effect on number of spikes with T_1 (177.33) recording significantly highest value, followed by T_2 (168.00) and T_3 (161.66). The lowest number of spikes was recorded in T_8 (144.00), which was on par with T_7 and T_9.

4.4.2. Length of spike

Influence of different foliar spray treatments on length of spikes of *P. longum* is illustrated in Table 25.

There was no significant variation among the treatments with respect to the length of spike.

4.4.3. Girth of spike

Girth of spikes as influenced by different foliar spray treatments on of *P. longum* is illustrated in Table 25.

Table 24. Effect of foliar spray treatments on proline content

Treatments	Proline ($\mu g\ g^{-1}$)		
	3 MAP	5 MAP	7 MAP
T_1 (SA 0.1 mM)	135.66±0.33	142.50±0.11	151.99±0.39
T_2 (SA 0.5 mM)	141.28±0.04	152.84±0.27	157.13±0.50
T_3 (SA 1.0 mM)	132.75±0.30	139.88±0.08	149.58±0.65
T_4 (SA 1.5 mM)	129.55±0.10	135.78±0.25	146.21±0.58
T_5 (SA 2.0 mM)	127.31±0.10	132.80±0.26	142.78±0.26
T_6 (SA 2.5 mM)	123.62±0.19	129.60±0.26	138.99±0.40
T_7 (Ethanol)	119.15±0.10	123.18±0.15	138.32±0.07
T_8 (Water spray)	117.41±0.21	123.11±0.08	138.10±0.06
T_9 (Control)	117.46±0.33	123.22±0.07	137.35±0.56
SEm (±)	0.40	0.37	0.55
C.D (0.05)	1.190	1.103	1.627

Table 25. Effect of foliar spray treatments on number of spikes, length and girth of spikes

Treatments	Number of spikes per plant	Length of spike(cm)	Girth of spike (mm)
T_1 (SA 0.1 mM)	177.33±0.88	2.22±0.07	6.57±0.03
T_2 (SA 0.5 mM)	168.00±0.58	2.30±0.06	6.63±0.03
T_3 (SA 1.0 mM)	161.67±1.45	2.30±0.06	6.70±0.06
T_4 (SA 1.5 mM)	155.33±0.88	2.27±0.03	6.63±0.03
T_5 (SA 2.0 mM)	151.00±0.58	2.30±0.06	6.67±0.03
T_6 (SA 2.5 mM)	148.00±0.58	2.30±0.03	6.57±0.03
T_7 (Ethanol)	146.00±0.58	2.33±0.03	6.60±0.06
T_8 (Water spray)	144.00±0.58	2.30±0.06	6.57±0.03
T_9 (Control)	145.33±0.33	2.33±0.03	6.57±0.07
SEm (±)	0.78	0.05	0.04
C.D (0.05)	2.311	NS	NS

There was no significant variation among the treatments with respect to the girth of spikes.

4.4.4. Fresh weight of spikes

The data on fresh spike yield per plant as influenced by different levels of salicylic acid concentrations for one year of *P. longum* are illustrated in Table 26.

The different foliar spray treatments had significant effect on fresh spike yield with T_1 (133.88 g plant^{-1}) recording significantly higher value, followed by T_2 (127.77 g plant^{-1}) and T_3 (123.77 g plant^{-1}). The lowest spike yield was recorded in the treatment T_7 (109.63 g plant^{-1}), which was found to be on par with the treatments T_8 and T_9.

4.4.5. Dry weight of spikes

The effect of different foliar spray treatments on dry weight of spikes of *P. longum* is illustrated in Table 26.

The foliar spray treatments had significant effect on dry spike yield (25.81 g plant^{-1}) with maximum dry spike yield recorded in treatment T_1, which was on par with T_2 (24.86 g plant^{-1}). T_2 was observed to be on par with T_3. The lowest value was recorded in the treatment T_8 (21.65 g plant^{-1}), which was observed to be on par with T_6, T_7 and T_9.

4.4.6. Driage of spikes

Influence of different foliar spray treatments on driage of spikes of *P. longum* is illustrated in Table 26.

There was no significant variation among the treatments with respect to the driage per cent. The driage was found to be 19 to 20 per cent.

4.4.7. Root length

Root length as influenced by different foliar spray treatments on of *P. longum* is presented in Table 27.

Table 26. Effect of foliar spray treatments on fresh and dry spike yiled and driage percent of spikes.

Treatments	Spike yield (g plant^{-1})		Driage (per cent)
	Fresh	Dry	
T_1 (SA 0.1 mM)	133.88±0.41	25.81±0.15	19.27±0.02
T_2 (SA 0.5 mM)	127.77±0.44	24.86±0.40	19.45±0.04
T_3 (SA 1.0 mM)	123.77±0.98	24.27±0.02	19.42±0.10
T_4 (SA 1.5 mM)	117.40±0.58	23.15±0.46	19.71±0.07
T_5 (SA 2.0 mM)	114.74±0.38	22.59±0.27	19.68±0.04
T_6 (SA 2.5 mM)	111.49±0.76	21.80±0.14	19.55±0.02
T_7 (Ethanol)	109.63±0.79	22.01±0.40	20.07±0.04
T_8 (Water spray)	110.11±0.30	21.65±0.42	19.66±0.06
T_9 (Control)	110.04±0.65	21.87±0.30	19.5±0.04
SEm (±)	0.62	0.32	0.24
C.D (0.05)	1.853	0.954	NS

Table 27. Effect of foliar spray treatments on length of root, fresh and dry root yield and driage per cent of roots.

Treatments	Length of root (cm)	Root yield (g plant^{-1})		Driage (per cent)
		Fresh	Dry	
T_1 (SA 0.1 mM)	63.27±2.11	28.62±0.85	11.96±0.08	41.86±1.20
T_2 (SA 0.5 mM)	70.61±0.35	30.15±0.44	12.01±0.14	40.30±0.32
T_3 (SA 1.0 mM)	64.22±1.18	28.33±0.06	11.71±0.10	41.33±0.33
T_4 (SA 1.5 mM)	58.04±0.88	27.97±0.12	11.63±0.05	41.59±0.18
T_5 (SA 2.0 mM)	53.25±0.60	27.85±0.10	11.58±0.02	41.60±0.08
T_6 (SA 2.5 mM)	52.67±0.74	27.76±0.07	11.60±0.04	41.78±0.05
T_7 (Ethanol)	51.98±1.36	26.93±0.16	11.32±0.01	42.04±0.24
T_8 (Water spray)	50.76±1.28	26.74±0.29	11.30±0.05	42.25±0.39
T_9 (Control)	50.32±2.08	26.92±0.14	11.29±0.07	41.95±0.06
SEm (±)	1.31	0.34	0.06	0.46
C.D (0.05)	3.895	1.026	0.220	NS

Foliar spray treatment on root length (70.27 cm) were significantly higher in the treatment T_2, followed by T_1 (63.27 cm) and T_3 (64.22 cm). The lowest value was recorded in the control treatment T_9 (50.76 cm) and which was observed to be on par with T_8 and T_7.

4.4.8. Fresh Root yield

The effect of different foliar spray treatments on fresh root yield of *P. longum* is illustrated in Table 27.

Foliar spray treatment on fresh root yield per plant (30.15 g plant^{-1}) were significantly higher in the treatment T_1, followed by T_2 (28.62 g plant^{-1}). T_2 was on par with T_3. The lowest value was recorded in the treatment T_7 (50.76 g plant^{-1}) and which was on par with T_9 and T_8.

4.4.9. Dry Root yield

Mean data on dry root yield as influenced by different foliar spray treatments of *P. longum* is depicted in Table 27.

The foliar spray treatments had significant effect on dry root yield (11.96 g plant^{-1}) with maximum dry root yield recorded in treatment T_2, which was on par with T_1. The lowest value was recorded in the treatment T_9 (11.29 g plant^{-1}), which was observed to be on par with T_7 and T_8.

4.4.10. Driage of roots

Influence different foliar spray treatments on driage of *P. longum* is illustrated in Table 27.

There was no significant variation among the treatments with respect to the driage per cent. The driage of root was found to be 40.00 to 42.50 per cent.

4.4.11. Harvest Index

The effect of different foliar spray treatments on harvest index of *P. longum* is illustrated in Table 28.

There was no significant variation seen among the treatments with respect to the harvest index of spikes, roots and spike cum root.

4.5. NURTIENT UPTAKE

4.5.1. Nitrogen

Result on different foliar spray treatments on nitrogen uptake of *P. longum* is presented in Table 29.

Foliar spray treatment on nitrogen uptake per plant (1.27 g plant^{-1}) were significantly higher in the treatment T_1 and followed by T_2 (1.18 g plant^{-1}) and T_3(1.183). The lowest nitrogen uptake was recorded in the treatment T_8 and T_9 (1.04 g plant^{-1}) and was observed to be on par with T_7.

4.5.2. Phosphorous

The data on phosphorous uptake per plant as influenced by different foliar spray treatments of *P. longum* is shown in Table 29.

The foliar spray treatments had significant effect on phosphorous uptake (0.059 g plant^{-1}) of the plant, with T_2 recording higher values which was found to be on par with T_1(0.056 g plant^{-1}) and was followed by T_3 (0.053 g plant^{-1}). The lowest phosphorous uptake (0.039 g plant^{-1}) was recorded in the treatments T_7 and T_9 and was found to be on par with T_8

4.5.3. Potassium

Potassium uptake per plant as influenced by different foliar spray treatments of *P. longum* is presented in Table 29.

The effect of foliar spray treatment on potassium uptake per plant (1.77 g plant^{-1}) was significantly higher in the treatment T_2, followed by T_1 (1.75 g plant^{-1}) and T_3 (1.60 g plant^{-1}). The lowest value (1.33 g plant^{-1}) was recorded in the control treatments T_7 and T_8, which were observed to be on par with control treatment T_9.

Table 28. Effect of foliar spray treatments on Harvest Index.

Treatments	Harvest Index		
	Spike	Root	Spike and root
T_1 (SA 0.1 mM)	0.276±0.00	0.139±0.00	0.414±0.00
T_2 (SA 0.5 mM)	0.278±0.01	0.140±0.00	0.419±0.01
T_3 (SA 1.0 mM)	0.275±0.00	0.137±0.00	0.413±0.01
T_4 (SA 1.5 mM)	0.270±0.00	0.136±0.00	0.407±0.00
T_5 (SA 2.0 mM)	0.267±0.01	0.137±0.00	0.403±0.00
T_6 (SA 2.5 mM)	0.264±0.00	0.138±0.00	0.403±0.01
T_7 (Ethanol)	0.266±0.00	0.137±0.00	0.405±0.01
T_8 (Water spray)	0.266±0.00	0.137±0.00	0.405±0.00
T_9 (Control)	0.265±0.00	0.137±0.00	0.406±0.00
SEm (±)	0.003	0.001	0.004
C.D (0.05)	NS	NS	NS

Table 29. Effect of different levels of salicylic acid on uptake of nutrients

Treatments	Uptake of N (g plant^{-1})	Uptake of P (g plant^{-1})	Uptake of K (g plant^{-1})
T_1 (SA 0.1 mM)	1.27±0.01	0.058±0.00	1.75±0.00
T_2 (SA 0.5 mM)	1.25±0.01	0.059±0.00	1.77±0.01
T_3 (SA 1.0 mM)	1.18±0.00	0.053±0.00	1.60±0.00
T_4 (SA 1.5 mM)	1.14±0.00	0.050±0.00	1.52±0.01
T_5 (SA 2.0 mM)	1.12±0.00	0.047±0.00	1.46±0.00
T_6 (SA 2.5 mM)	1.09±0.01	0.043±0.00	1.41±0.00
T_7 (Ethanol)	1.04±0.00	0.039±0.00	1.33±0.01
T_8 (Water spray)	1.04±0.01	0.040±0.00	1.33±0.01
T_9 (Control)	1.04±0.01	0.039±0.00	1.34±0.00
SEm (±)	0.00	0.00	0.00
C.D (0.05)	0.013	0.002	0.022

Discussion

5. DISCUSSION

The results of the present investigation entitled "Salicylic acid mediated metabolite elicitation and growth responses in long pepper (*Piper longum* L.)" are discussed in this chapter with reference to the observed data and available literature.

Salicylic acid (SA) is a phenolic phytohormone that acts as a key regulator of plant processes. It exerts stimulatory effects on various physiological and biochemical processes related to plant growth and development. SA plays a crucial role in the plant developmental processes *viz.*, seed germination, vegetative growth, flowering, fruit yield, photosynthesis, stomatal closure, respiration, thermogenesis, metabolic events and nutrient uptake (Chen *et al.*, 2013; Idrees *et al.*, 2010). The significant influence of salicylic acid on growth, yield and production of metabolites in *Piper longum* has been confirmed in the study.

5.1. EFFECT OF SALICYLIC ACID ON PLANT GROWTH PARAMETERS

In the study, foliar application of SA at different concentrations significantly influenced the plant growth in terms of plant height, number of leaves, leaf area, number of inflorescence and shoot weight in concentration dependent manner. All these parameters showed a decreasing trend with increasing concentration of SA. *P. longum* treated with salicylic acid 0.1 mM had significantly higher yield at all the stages of observation compared to untreated control plants. This result is in agreement with that of Gharib (2006) who reported that foliar spray with SA at 0.1 mM and 0.01 mM concentrations on *Ocimum basilicum* and *Origanum majorana* plants promoted growth in terms of plant height, number of branches, spikes and leaves per plant, leaf area and herbage yield. The plant growth promotion with application of SA has also been observed with different crop plants *viz.*, *Oryza sativa* (Chamarthy, 2004), *Cucumis sativus* (Yildirim *et al.*, 2008), *Solanum lycopersicum* (Mady, 2009), *Helianthus annuus* (Dawood *et al.*, 2012), *Vigna radiata* (Lingakumar *et al.*, 2015), *Olea europaea* (Rahman and Attia, 2016), *Fragaria* × *ananassa* (Mohamed *et al.*, 2017) etc.

The enhanced vegetative growth due to salicylic acid could be attributed to the increasing levels of cytokinins which promotes cell division and breaking of capillary sovereignty (Taiz and Zeiger, 1998), increasing the efficiency of photosynthesis by increased absorption of CO_2 in plastids (Khan *et al.*, 2003), thus providing the materials needed to build new tissues and increased vegetative growth. According to Shakirova *et al.* (2003) the positive effect of salicylic acid on growth and yield can be due to its influence on other plant hormones. SA caused marked increase in IAA (Indole 3 acetic acid), GA_3 (gibberellic acid), zeatin and zeatin riboside, in the mean time decrease in ABA (abscisic acid) content. The increase in endogenous hormones stimulates cell division and cell enlargement and subsequently growth.

In the study, the days to emergence of spike and days to flowering varied significantly among various treatments tried. SA 0.1 mM showed earliness with respect to spike emergence (75.00 days) and flowering (22.67 days). However, the higher concentration took more number of days to emergence of spike (84.67 days) and to flowering (27.00 days), which was found to be on par with the foliar spray treatments devoid of SA. Earliness in flowering due to SA application was reported in *Tagetes erecta* (Pacheco *et al.*, 2013), *Vigna mungo* (Narayanan *et al.*, 2015) and *Fragaria* × *ananassa* (Mohamed *et al.*, 2017). Foliar spray with 0.1 µM SA on to tissue culture derived *Gloxinia speciose*, plants exhibited six days of earliness in flowering (Martin-Mex *et al.*, 2015).

Oata (1975) and Pieterse and Muller (1977) reported that salicylic acid induced flowering by acting as a chelating agent. It functioned as endogenous growth regulator for flowering and florigenic effects (Raskin, *et al.*, 1987). According to Khurana and Cleland (1992) *Lemna paucicostata* LP6 did not normally flower when grown on basal Bonner-Devirian medium, but substantial flowering was obtained when 10 µM salicylic acid (SA) was added to the *in vitro* culture medium.

5.2. EFFECT OF SALICYLIC ACID ON METABOLITE PRODUCTION

In the study, it is observed that the chlorophyll pigments were significantly enhanced by the application of SA. The best results were obtained with SA 0.5 mM concentration in all the three stages of observation. Similar findings were obtained by Yildirim *et al.* (2008) in *Cucumis sativus*, Li *et al.* (2014) in *Torreya grandis*, Manaa *et al.* (2014) in *Solanum lycopersicum* and Al-Rubaye and Atia (2016) in *Cucurbita pepo*. Leaf chlorophyll, is important component of the photosynthetic system governing dry matter accumulation. It also acts as one of antioxidant substances, concentrated in the chloroplast and protect the photosynthetic apparatus, when a plant is subjected to stress, by scavenging the excess reactive oxygen species known as free radicals (Khodary, 2004). In *Artemisia annua*, foliar spray with SA 1.0 mM enhanced the chlorophyll content (Aftab *et al.*, 2010).

Total protein content in the leaves were enhanced by the application of salicylic acid and the best result was obtained with SA 0.5 mM concentration in all the three stages of observation. The enhancement of total proteins due to SA application has been reported in *Ocimum basilicum* and *Origanum majorana* by Gharib (2006), in *Cyclamen persicum* by Farjadi-Shakib *et al.* (2012) and in *Phaseolus vulgaris* by Hadi *et al.* (2014). The enhancement in protein content might be due to the increased nitrogen uptake.

Antioxidant enzymes such as CAT (catalase), POD (peroxidase) and SOD (superoxide dismutase) were significantly enhanced by the application of salicylic acid in all the three stages of observation in concentration dependant manner (Fig 1, 2 and 3). The highest value was obtained with SA 0.5 mM concentration. The above results are in line with the findings of Hayat *et al.* (2008) in *Lycopersicon esculentum*, Patel *et al.* (2011) in *Cicer arietinum* and Qudos (2015) in *Capsicum annum*. The increase in the activity of antioxidant enzymes might be due to the regulatory role of SA in antioxidant system. SA application seems to induce oxidative stress through increasing the production of H_2O_2 (hydrogen peroxide); induces resistance against abiotic or biotic stress *via* enhancing the antioxidant enzymes (Ganesan and Thomas, 2000). It signals the induction of antioxidative

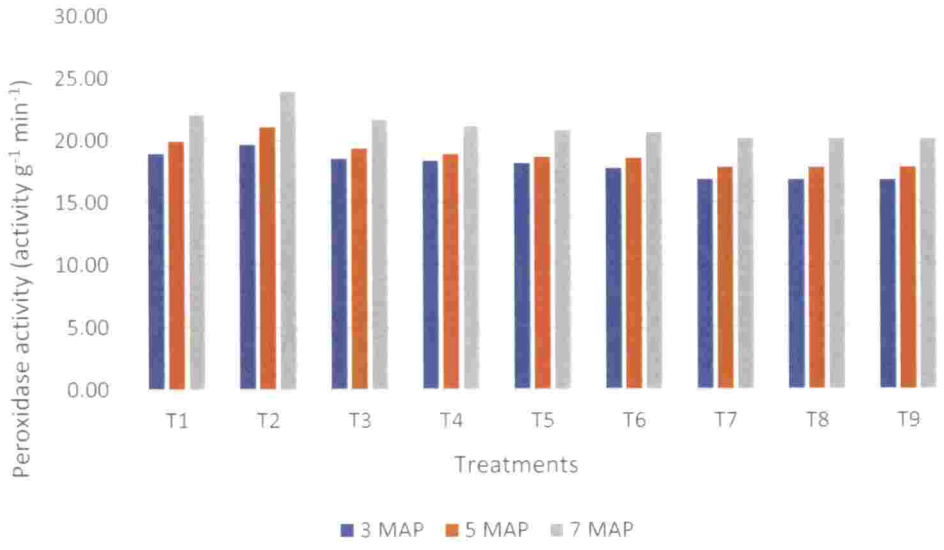

Fig. 1. Effect of foliar spray treatments on peroxidase activity in *Piper longum*

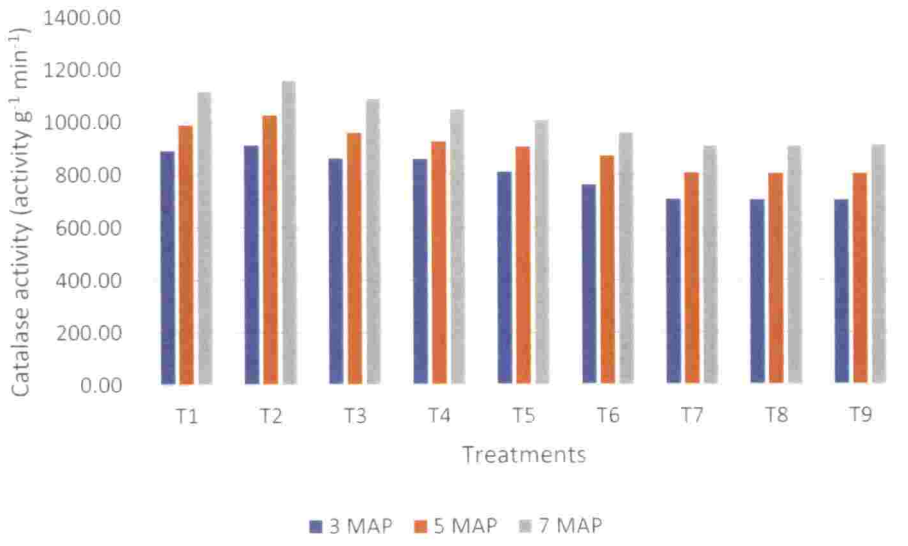

Fig. 2. Effect of foliar spray treatments on catalase activity in *Piper longum*

gene there by increases the activity of enzymatic antioxidants such as CAT, POD, SOD etc. It has been suggested that stimulation of antioxidants might be achieved by SA induced protein synthesis (Kovacik *et al.*, 2009).

There is 31 per cent increase in piperine content was observed in the plants exposed to foliar spray with SA 0.5 mM concentration over the control. The piperine content of 1.153 per cent was reported in this treatment (Fig 4). Exogenously applied SA caused enhanced accumulation of resveratrol in *Arachis hypogaea* (Chung *et al.*, 2003) and induced herniarin and umbelliferone alkaloid accumulation in *Matricaria chamomilla* (Pastirova *et al.*, 2004). This increase in alkaloid content might be due to enhanced enzyme activities, uptake of nutrients, photosynthesis and translocation of photosynthates and other metabolites, in response to exogenous application of SA (Khan *et al.*, 2007). According to Malarz *et al.* (2007), SA induces gene expression related to biosynthesis of secondary metabolites in plants.

The volatile oil and oleoresin increased by 12 per cent in plants exposed to foliar spray with SA 0.5 mM over the control. The higher volatile oil content (1.32 per cent) was obtained with SA 0.5 mM concentration (Fig. 5). Similar enhancement in the essential oil content and yield per plant in response to the exogenous application of SA has been reported in *Ocimum basilicum* and *Origanum majorana* (Gharib, 2006). According to Idrees *et al.* (2010), the increase in vegetative growth, nutrient uptake, increase in leaf oil gland population and synthesis of monoterpene has enhanced oil yield in lemon grass. In contrast to this, Ram *et al.* (1997) reported inhibitory effect of SA application (100 ppm) on the herbage and essential oil yields in *Pelargonium graveolens, Mentha arvensis* and *Cymbopogon martini.* The higher value (14.21 per cent) of oleoresin was recorded in plants subjected to foliar spray with SA 0.5 mM concertation (Fig. 6). The above results are in harmony with the findings of Rodrigues *et al.* (2009) where the salicylic acid based stimulant paste enhanced the production of oleoresine in *Pinus elliotti.*

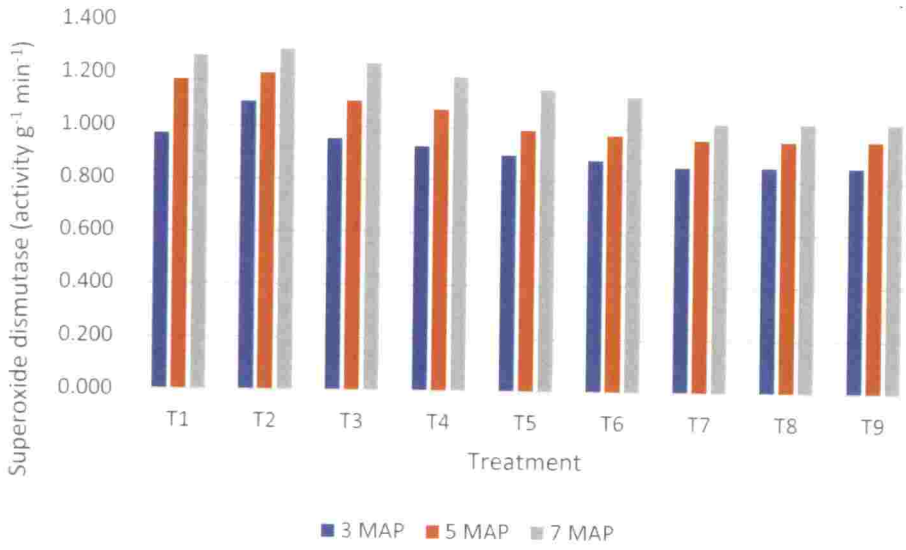

Fig. 3. Effect of foliar spray treatments on SOD activity in *Piper longum*

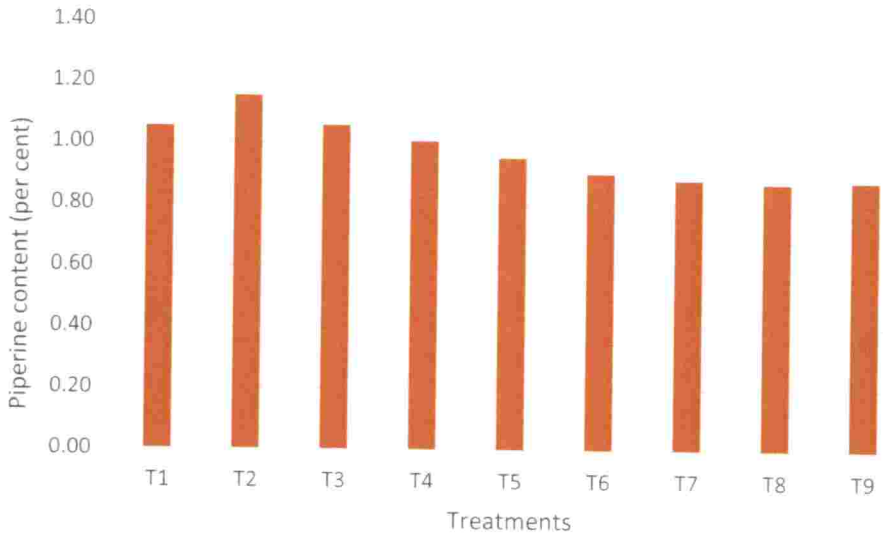

Fig. 4. Effect of foliar spray treatments on piperine content in *Piper longum*

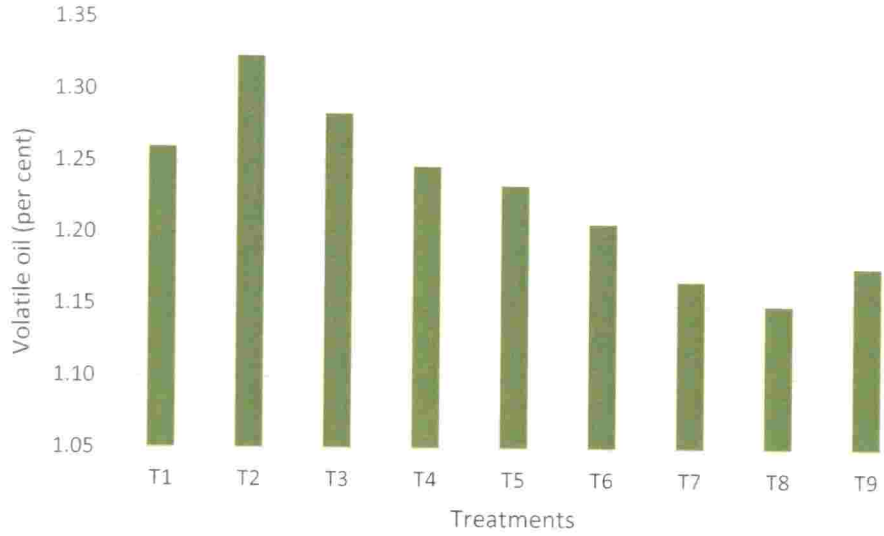

Fig. 5. Effect of foliar spray treatments on volatile oil content in *Piper longum*

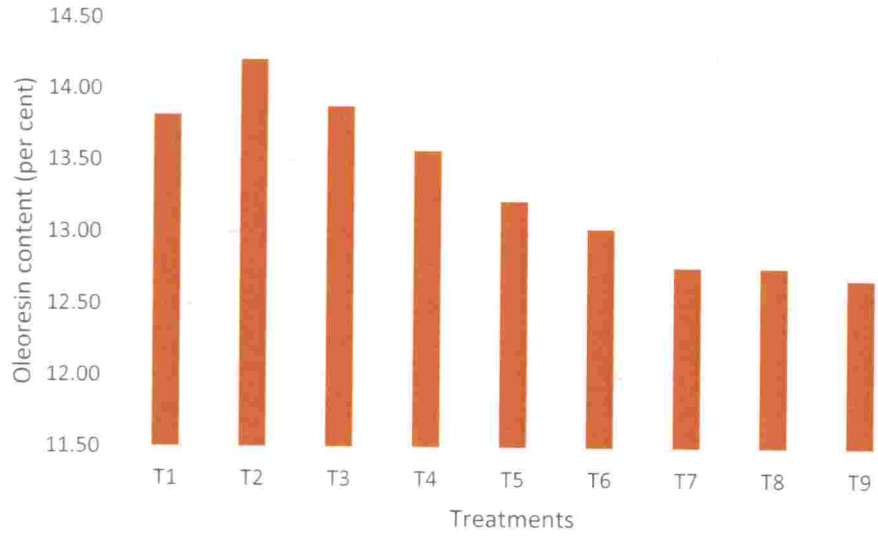

Fig. 6. Effect of foliar spray treatments on oleoresin content in *Piper longum*

The present study revealed that the foliar application of SA at different concentrations significantly enhanced the total carbohydrate, starch and sugar content in plants. The best results were obtained with SA 0.5 mM concentration. These results are in line with the findings of Bekheta and Talaat (2009) in *Vigna radiata*, Ghai *et al.* (2002) in *Brassica napus* and Al-Rubaye and Atia (2016) in *Cucurbita pepo*. In consensus with our finding, Dawood *et al.* (2012) is of the view that photosynthetic pigments increased in response to salicylic acid treatments which in turn enhanced biosynthesis of total carbohydrates significantly, resulting in enhanced growth of plants. SA contributes to the movement and transmission of nitrates in the internal plant tissue and to the synthesis of chlorophyll, thus improving the photosynthetic mechanism in plants leading to the production of carbohydrates (Kumar *et al.*, 2010).

5.3. EFFECT OF SALICYLIC ACID ON PHYSIOLOGICAL PARAMETERS

The results indicated that the application of salicylic acid had a positive effect on dry matter production over the control at all the stages of observation in concentration dependant manner. Among the treatments, the highest dry matter content was obtained in SA 0.1 mM concentration. This result is in confirmation with that obtained in *Ocimum basilicum* and *Origanum majorana* on foliar spray using SA at 0.1 mM and 0.01 mM concentrations (Gharib, 2006). The dry matter production in *Sesamum indicum* (Radhamani *et al.*, 2002), *Cucumis sativus* (Yildirim *et al.*, 2008), *Solanum lycopersicum* (Mady, 2009), *Artemisia annua* (Aftab *et al.*, 2010), *Cucurbita pepo* (Al-Rubaye and Atia, 2016) etc. also showed an enhancement on SA application. Salicylic acid facilitated absorption and utilization of mineral nutrients and transport of assimilates, which in turn enhanced the biomass (Basra *et al.*, 2007). The exogenous application of SA promoted the total dry matter production, reflected by higher photosynthetic rate (Narayanan *et al.*, 2015).

The Leaf Area Index (LAI) was enhanced in all the SA treatments compared to the untreated control plants. Among the different foliar spray treatments higher LAI

was observed in SA 0.1 mM concentration. Higher LAI with application of SA has also been observed with different crop plants *viz., Ocimum basilicum* and *Origanum majorana* (Gharib, 2006), *Oryza sativa* (Chamarthy, 2004), *Arachis hypogaea* (Naz, 2006). The exogenous application of SA increased the LAI, reflected by increased number of leaves.

A significant increase in the net assimilation rate (NAR) was observed in the SA treatments. This could be ascribed to the increased dry matter production in the SA treated plants. According to Hayat *et al.*, (2005) the increase might be due to stimulated vegetative growth through enhancement of cell division and cell enlargement. Similar findings were reported by Chamarthy (2004) in *Oryza sativa*, Jadhav and Bhamburdekar (2012) in *Arachis hypogaea*. A decrease in the NAR during the period between 5 and 7 MAP would be probably due to the decrease in the rate of vegetative growth after the crop had reached physiological maturity.

Exogenous application of salicylic acid resulted in increased stomatal conductance. Maximum efficiency was shown by SA at 0.1 mM. These results are in line with the findings by Hayat *et al.* (2008) in *Lycopersicon esculentum*, Najafian *et al.* (2009) in *Thymus vulgaris* and Narayanan *et al.* (2015) in *Vigna mungo.* Increase in stomatal conductance would result in increased uptake of carbon dioxide which would further improve the photosynthetic mechanism of the plants contributing to increased vegetative growth.

The photosynthetic rate also increased in response to the foliar application with SA, though with a decreasing trend with higher concentration. The highest photosynthetic rate was obtained in foliar spray treatment with SA 0.1 mM. These results are in line with the findings in *Lycopersicon esculentum* (Hayat *et al.*, 2008), *Thymus vulgaris* (Najafian *et al.*, 2009), *Cicer arietinum* (Alyemeni *et al.*, 2014; Hayat *et al.*, 2014) and *Vigna mungo* (Narayanan *et al.*, 2015). The photosynthetic enhancing effect of SA could be attributed to its stimulatory effects on rubisco activity and pigment contents (Khodary, 2004).

The effects of exogenous SA on photosynthesis parameters differ depending on the dose and plant species tested. High SA concentrations (1–5 mM) cause a reduction in the photosynthetic rate in barley plants (Pancheva *et al.*, 1996), and reduced chlorophyll contents in cowpea, wheat and Arabidopsis (Rao *et al.*, 1997; Chandra and Bhatt, 1998; Moharekar *et al.*, 2003). According to Mateo *et al.* (2006), when exogenous SA was more than 1 mM, plants usually showed oxidative bursting and cell death. SA was effective in inducing H_2O_2 in rice leaves at a concentration of 2 mM. H_2O_2 was proposed to function downstream of SA on the basis of evidence that high levels of SA can bind and inhibit H_2O_2 removing enzymes. At higher concentration, the plant simultaneously produces more ROS (Reactive oxygen species) and at the same time diminishes its own capacity to scavenge H_2O_2, resulting in the over accumulation of ROS and inhibiting the physiological processes necessary for plant growth and production.

In the study, it was observed that the application of salicylic acid had a positive effect on the cell membrane stability index and the highest value was observed in SA 0.5 mM concentration in all the three stages of observation. Similar findings were also reported in *Lycopersicon esculentum* (Hayat *et al.*, 2008), *Cyclamen persicum* (Farjadi-Shakib *et al.* 2012) and *in vitro* grown seedlings of *Citrullus lanatus* (Ram *et al.*, 2014). The decreased membrane damage in response to exogenous application of SA could be related to the increased production of proline and induction of antioxidant responses that protect the plant from oxidative damage (Senaratna *et al.*, 2000; El-Tayeb, 2005).

Proline content were significantly enhanced by the application of SA and the best results were obtained by salicylic acid at 0.5 mM concentration in all the stages of observation. These results are in conformity with the findings of Hayat *et al.*, (2008) in *Lycopersicon esculentum*, Patel *et al.* (2012) in *Cicer arietinum*, Li *et al.* (2014) in *Torreya grandis* and Manaa *et al.* (2014) in *Solanum lycopersicum*. According to Hayat *et al.* (2010) the exogenous application of SA increased the de novo synthesis of proline thereby increasing the endogenous proline content in leaves. The increase in proline content in SA treated plants could be attributed to

the activation of enzymes associated with proline metabolism and subsequent enhancement in proline accumulation. Proline besides acting as an excellent osmolyte is also known for stabilizing the complex II electron transport, membranes and 3-D structure of proteins and enzymes such as Rubisco and CA (carbonic anhydrase) thereby increasing photosynthetic rate (Hayat *et al.*, 2012).

5.4. EFFECT OF SALICYLIC ACID ON YIELD AND YIELD COMPONENTS

The foliar application of SA at lower concentrations significantly increased the yield in terms of number of spikes and spike yield. *P. longum* treated with salicylic acid 0.1 mM concentration recorded higher yield than all the treatments tried (Fig 7). This accounted for 18 per cent increase in spike yield over the control. In *P. longum*, the spike are normally born opposite to the active leaves. The number of leaves hence, is an indirect determinant of spike yield. In this study, higher number of leaves and spike yield are recorded in plants subjected to foliar spray with SA 0.1 mM. The increase in yield parameters were reported in various crop plants *Solanum lycopersicum* (Mady, 2009), *Arachis hypogaea* (Karimian *et al.*, 2015), *Vigna mungo* (Narayanan *et al.*, 2015) and *Fragaria* × *ananassa* (Mohamed *et al.*, 2017). Higher yield under exogenous application of salicylic acid could be ascribed to its effect on photosynthetic parameters, increased assimilate transport from source to sink and their ultimate conversion into final reserved food.

In the study it was observed that the application of salicylic acid had a significant effect on the root weight over the control plants (Fig. 8). SA 0.5 mM recorded higher root yield in terms of root weight and root length. These results are in harmony with the findings of San-Miguel *et al.* (2003) in *Pinus patula,* Yildirim (2008) in *Cucumis sativus* and Kong *et al.* (2014) in *Arachis hypogaea.* Similar results were obtained by Askari and Ehsanzadeh (2015) in fennel, where the root yield enhanced with the exogenous application of SA 1.0 mM. The root growth promotion due to SA application has profound influence on the productivity of plant, due to its role in water and nutrient uptake.

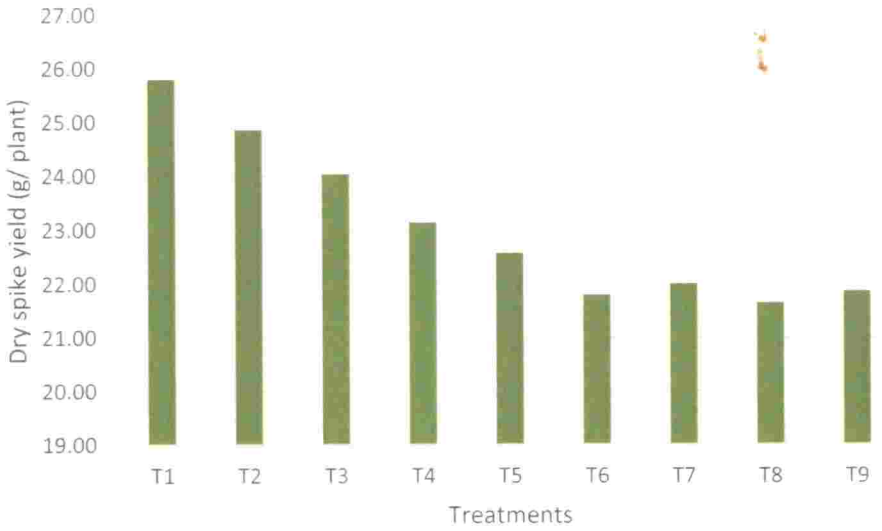

Fig. 7. Effect of foliar spray treatments on dry spike yield in *Piper longum*

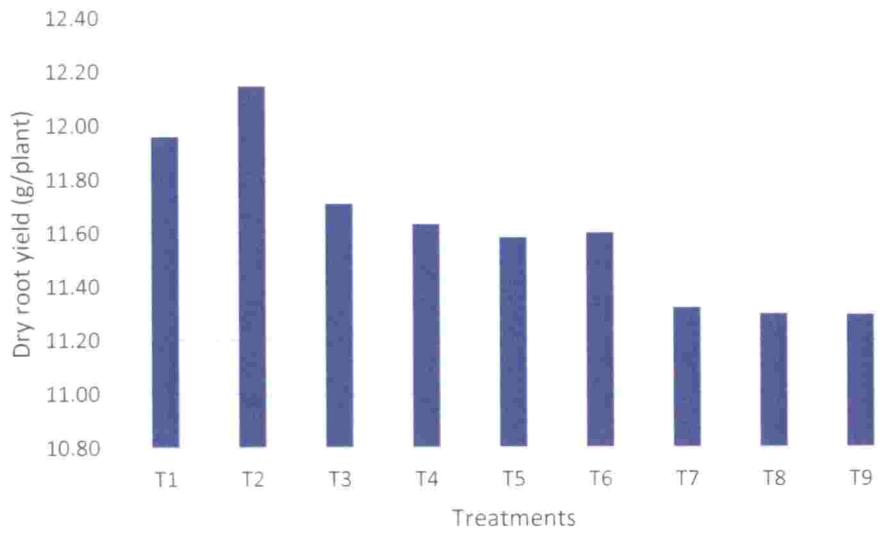

Fig. 8. Effect of foliar spray treatments on dry root yield in *Piper longum*

The harvest indices with respect to spike yield, root yield and spike cum root yield did not vary significantly among the treatments, though significant variation was observed with respect to spike and root yield. In *P. longum*, spikes are formed opposite to the leaves, hence a proportion is maintained between the economical yield and the biological yield. With respect to root yield, only a six per cent increase was observed over the control plants. The harvest index with respect to root yield also did not show any significant variation between treatments.

5.5.EFFECT OF SALICYLIC ACID ON UPTAKE OF MAJOR NUTRIENTS

Uptake of major nutrients such as N, P and K were significantly enhanced by the application of salicylic acid and the best positive result was obtained with SA 0.1 and SA 0.5 mM concentrations. The above results were in line with the findings of Gunes *et al.* (2005) in *Zea mays*, Gharib (2006) in *Ocimum basilicum* and *Origanum majorana*, Yildirim *et al.* (2008) in *Cucumis sativus* and Khan *et al.* (2010) in *Vigna radiata*. Significant enhancement in production of photosynthates, proteins, and yield, could be ascribed to increased nutrient uptake in SA treated plants. The plant stimulants were found to affect the nutrient uptake and availability, which influenced physiological processes in plants, leading to improved production and quality of secondary metabolites (Rioba *et al.*, 2015; Du-Jardin, 2015).

In the present study, it was observed that lower concentrations of SA (0.1 mM and 0.5 mM) elicited plant growth responses and metabolite production. The same treatments also evoked superior performance with respect to physiological parameters, activity of defence enzymes and nutrient uptake. In the parameters studied including spike yield and piperine content, the plants treated with higher concentration of SA 2.5 mM gave inferior performance. A decreasing trend was observed in these parameters with increasing concentration of SA.

In the study, foliar spray with either concentration of SA 0.1 mM and 0.5 mM. at 2, 4 and 6 MAP could effectively elicit plant growth, yield and metabolite production in *Piper longum*.

Summary

6. SUMMARY

The present study entitled "Salicylic acid mediated metabolite elicitation and growth responses in long pepper (*Piper longum* Linn.)" was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2017-19. The study aimed at assessing the effect of different levels of salicylic acid on plant growth, yield and production of metabolites in long pepper (*Piper longum* L.).

Two month old rooted cuttings of variety Viswam (a promising selection of Kerala Agricultural University) procured from the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara was used for the study. The rooted cuttings were planted in polybags filled with soil supplemented with 500g vermicompost. Cowdung slurry was applied at two months interval. PGMR mix I was applied at 100 g per plant.

The rooted cuttings of promising variety, Viswam of long pepper plants were exposed to nine foliar spray treatments with varying salicylic acid concentration and control treatments *viz*, SA 0.1 mM (T_1), SA 0.5 mM (T_2), SA 1.0 mM (T_3), SA 1.5 mM (T_4), SA 2.0 mM (T_5), SA 2.5 mM (T_6), ethanol (0.20 per cent) spray (T_7), water spray (T_8) and control (T_9) at 2, 4 and 6 months after planting (MAP). The study was conducted in completely randomized block design (CRD) with three replications. The plant growth parameters, metabolite production, physiological parameters, yield parameters and major nutrient uptake in response to various foliar spray treatments were studied.

The plant growth parameters *viz.*, plant height, number of primary branches, number of spike bearing branches, number of inflorescence, number of leaves, leaf area and shoot weight per plant (fresh and dry weight) were recorded at 3, 5 and 7 MAP. Plant height, number of inflorescence, number of leaves, leaf area, and shoot weight per plant (fresh and dry weight) varied significantly among the treatments tried. The foliar treatment with SA 0.1 mM (T_1) recorded higher plant height (31.67 cm, 70.33 cm and 112.33 cm, respectively) at 3, 5 and 7 MAP. At 7 MAP, T_1 was

found to on par with T_2 (109.67). Number of inflorescence was observed to be significantly higher in T_1 and the values recorded were 32.33 and 65.67 at 5 and 7 MAP, respectively. However, at 3 MAP no significant variation was seen among the different treatments. With respect to number of leaves and leaf area, T_1 recorded significantly higher values at all stages of observation. In T_1, the number of leaves recorded were 28.33, 55.00 and 92.33 and leaf area recorded were 1364.40 cm^2, 2729.17 cm^2 and 4666.53 cm^2, at 3, 5 and 7 MAP, respectively. The shoot weight was also found to be significantly higher in T_1, with respect to fresh and dry weight at all periods of observation. The fresh weight recorded were 23.68 g, 45.96 g and 88.10 g and dry weight 14.42 g, 28.00 g and 52.62 g at 3, 5 and 7 MAP, respectively. All these parameters showed a decreasing trend with increasing concentration of SA. However, no significant variation among the treatments was observed with the number of primary branches and spike bearing branches.

The days to emergence of spike and to flowering (from spike emergence) varied significantly among various treatments tried. SA 0.1 mM (T_1) showed earliness with respect to spike emergence (75 days) and flowering (22.67 days). However, the higher concentration of SA 2.5 mM (T_6) took more number of days to emergence of spike (84.67 days) and with respect to flowering control spray (T_9) and water spray (T_8) took more days (27.67 days) to flower, which was found to be on par with the foliar spray devoid of SA. However, the days from emergence to maturity of spike did not show any significant variation among the treatments.

The plant metabolites, *viz.*, total chlorophyll, total proteins and defense enzymes (peroxidase, catalase and superoxide dismutase) recorded significantly higher values in plants subjected to foliar spray SA 0.5 mM (T_2). T_2 obtained the best results with respect to total chlorophyll (1.62 mg g^{-1}, 1.66 mg g^{-1} and 1.72 mg g^{-1}), total proteins (12.27 mg g^{-1}, 13.28 mg g^{-1} and 14.62 mg g^{-1}), peroxidase (19.66 activity g^{-1} min^{-1}, 21.07 activity g^{-1} min^{-1} and 23.93 activity g^{-1} min^{-1}), catalase (912.33 U ml^{-1}, 1027.00 U ml^{-1} and 1158.00 U ml^{-1}) and superoxide dismutase (1.093 activity g^{-1} min^{-1}, 1.201 activity g^{-1} min^{-1} and 1.292 activity g^{-1} min^{-1}) at 3, 5 and 7 MAP, respectively.

The mature dark green oven dried spikes were analysed for carbohydrates, piperine, volatile oil and oleoresin content, which varied significantly among the different treatments. The treatment, T_2 recorded significantly higher carbohydrate (87.42 mg g^{-1}), starch (55.62 mg g^{-1}) and sugar contents (29.66 mg g^{-1}). The same treatment reported the highest values with respect to piperine (1.15 per cent), volatile oil (1.32 per cent) and oleoresin (14.21 per cent). This treatment gave approximately 31 per cent increase in piperine content, 12 per cent increase each in volatile oil and oleoresin content over the control. However, at higher concentration of SA, T_6 (SA 2.5 mM), piperine content was found to be significantly lower and on par with foliar spray treatments devoid of SA. With regards to piperine yield (0.85 mg $plant^{-1}$) and volatile oil yield (0.97 mg $plant^{-1}$), T_2 recorded significantly higher value. However, higher oleoresin yield (10.55 mg $plant^{-1}$) was obtained in treatment T_1.

The physiological parameters showed significant variation with regards to the foliar treatments applied. The SA treatment T_1 (0.5 mM) reported the highest values with respect to dry matter production (18.42 g, 35.77 g and 67.62 g), leaf area index (0.76, 1.52, 2.59), stomatal conductance (318.67 mmoles m^{-2} s^{-1}, 407.67 mmoles m^{-2} s^{-1} and 467.00 mmoles m^{-2} s^{-1}) and photosynthetic rate (7.50 μCO_2 moles m^{-2} s^{-1}, 8.43 μCO_2 moles m^{-2} s^{-1} and 8.93 μCO_2 moles m^{-2} s^{-1}) at all the stages of observation. However, T_2 (0.5 mM) recorded significantly higher values with respect to other physiological parameters *viz.*, NAR (0.107 mg cm^{-2} day^{-1} and 0.077 mg cm^{-2} day^{-1}), cell membrane stability index (115.63 per cent, 110.54 per cent, 107.64 per cent) and proline (141.28 $\mu g\, g^{-1}$, 152.84 $\mu g\, g^{-1}$ and 157.13 $\mu g\, g^{-1}$) at all stages of observation. T_2 found to be on par with T_1 with respect to NAR at both periods of observation.

The yield parameters *viz.*, number of spikes, fresh and dry spike yield, fresh and dry root yield and root length varied significantly with different foliar spray treatments. With respect to number of spikes (177.33) and fresh (133.88 g) and dry (25.81 g) spike yield, T_1 recorded significantly higher values. T_1 was found to be on par with T_2 with respect to dry spike yield. The root parameters such as fresh

(30.15 g) and dry root yield (12.01 g) and root length (70.61 cm) were significantly higher in treatment T_2. With regards to dry root yield T_1 (11.96) found to be on par with T_2. However, length and girth of spike and driage per cent of spike and root did not showed any significant variation among the treatments.

The nutrient uptake by the crop was studied and the results revealed that the plants exposed to T_1 showed significantly higher uptake of N (1.27 g plant^{-1}). However, with respect to P (0.059 g plant^{-1}) and K (1.78 g plant^{-1}), treatment T_2 recorded higher values.

In the study, it was observed that foliar spray with SA 0.1 mM (T_1) gave better performance with respect to plant growth parameters and physiological parameters followed by that with SA 0.5 mM (T_2). Foliar spray with SA 0.5 mM gave superior performance with respect to plant metabolites, while high spike and root yield were recorded in foliar spray with SA 0.1 mM and 0.5 mM. Hence, it can be inferred from the study that foliar spray with either concentration of SA 0.1 mM and 0.5 mM. at 2, 4 and 6 MAP could effectively elicit plant growth, yield and metabolite production in *Piper longum*.

Future line of work

- Optimization of SA application with respect to concentration and frequency have to be studied in *Piper* sp.
- The stress alleviation response and mechanism of SA has to be taken in *Piper* sp.
- Elicitation behaviour of SA has to be studied in other medicinal plant species and spices.

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Abstract

SALICYLIC ACID MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN LONG PEPPER (*Piper longum* L.)

by

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ABSTRACT

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ABSTRACT

A study on "Salicylic acid mediated metabolite elicitation and growth responses in long pepper (*Piper longum* L.)" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2017-19. The study aimed at assessing the effect of different concentrations of salicylic acid on plant growth, yield and metabolite production in *P. longum*.

The rooted cuttings from the promising variety of long pepper, Viswam plants were exposed to nine foliar spray treatments with varying salicylic acid (SA) concentrations and control treatments *viz*, SA 0.1 mM (T_1), SA 0.5 mM (T_2), SA 1.0 mM (T_3), SA 1.5 mM (T_4), SA 2.0 mM (T_5), SA 2.5 mM (T_6), ethanol (0.20 per cent) spray (T_7), water spray (T_8) and control (T_9) at 2, 4 and 6 months after planting (MAP). The study was conducted in completely randomized block design (CRD) with three replications. The plant growth parameters, metabolite production, physiological parameters, yield parameters and major nutrient uptake in response to various foliar spray treatments were studied.

The plant growth parameters *viz.*, plant height, number of leaves, leaf area, number of primary branches, number of spike bearing branches and shoot weight per plant (fresh and dry weight) were recorded at 3, 5 and 7 MAP. The foliar treatment with SA 0.1 mM (T_1) recorded significantly higher values with respect to plant height, number of leaves, leaf area and shoot weight at all stages of observation. All these parameters showed a decreasing trend with increasing concentration of SA. However, no significant variation among the treatments was observed with respect to the number of primary branches and spike bearing branches. The days to emergence of spike and to flowering varied significantly among the treatments tried. SA 0.1 mM (T_1) showed earliness with respect to spike emergence (75 days) and flowering (22.67 days). However, the higher concentration took more number of days to emergence of spike (84.67 days) and to flowering (27 days), which was found to be on par with the foliar spray treatments

devoid of SA. However, the days from emergence to maturity of spike did not show any significant variation among the treatments.

The plant metabolites, *viz.*, total chlorophyll, total proteins and defense enzymes (peroxidase, catalase and superoxide dismutase) at 3, 5 and 7 MAP, recorded significantly higher values in plants subjected to foliar spray with SA 0.5 mM (T_2). The mature dark green oven dried spikes were analysed for carbohydrates, piperine, volatile oil and oleoresin content, which varied significantly among the different treatments. The treatment, T_2 recorded significantly higher carbohydrate, starch and sugar contents. The same treatment reported the highest values with respect to piperine (1.15 per cent), volatile oil (1.32 per cent) and oleoresin (14.21 per cent) content. This treatment gave approximately 30 per cent increase in piperine content, 12 per cent increase in volatile oil and oleoresin content over the control. However, at higher concentration of SA, T_6 (SA 2.5 mM), piperine content (0.90 per cent) was found to be significantly lower and on par with foliar spray treatments devoid of SA.

The physiological parameters at 3, 5 and 7 MAP, showed significant variation with regards to the foliar treatments. The foliar spray treatment with SA 0.1 mM (T_1) recorded significantly higher values with respect to dry matter production, leaf area index, stomatal conductance and photosynthetic rate at all the stages of observation. The physiological parameters *viz.*, NAR, cell membrane stability index and proline recorded significantly higher values in T_2 at all stages of observation.

The yield parameters *viz.*, number of spikes, fresh and dry spike yield, fresh and dry root yield and root length varied significantly with different foliar spray treatments. With respect to number of spikes (177.33), fresh (133.88 g) and dry (25.81 g) spike yield, T_1 recorded significantly higher values. T_1 was found to be on par with T_2 with respect to dry spike yield. The root parameters such as fresh (30.15 g) and dry (12.15 g) root yield and root length (70.61 cm) were significantly higher in treatment T_2. With regards to dry root yield, T_2 was found to be on par

with T_1. However, harvest index, spike length, spike girth and driage did not showed any significant variation among the treatments.

The nutrient uptake by the crop was studied and the results revealed that the plants exposed to T_1 showed significantly higher uptake of N (1.27 g plant^{-1}). However, with respect to P (0.059 g plant^{-1}) and K (1.78 g plant^{-1}), treatment T_2 recorded higher values.

In the study, it was observed that SA 0.1 mM (T_1) gave better performance with respect to plant growth parameters and physiological parameters followed by SA 0.5 mM (T_2). Foliar spray with SA 0.5 mM gave superior performance with respect to plant metabolites, while high spike and root yield were recorded in foliar spray with SA 0.1 mM and 0.5 mM. Hence, it can be inferred from the study that foliar spray with either concentration of SA 0.1 mM and 0.5 mM. at 2, 4 and 6 MAP could effectively elicit plant growth, yield and metabolite production in *Piper longum*.