CHITOSAN MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN KASTHURI TURMERIC (*Curcuma aromatica* Salisb.)

by

NIVYA J THENGUMPALLY
(2017-12-013)

THESIS
Submitted in partial fulfilment of the
requirement for the degree of

MASTER OF SCIENCE IN HORTICULTURE
Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF AGRICULTURE, VELLAYANI
THIRUVANANTHAPURAM-695 522
KERALA, INDIA

2019

DECLARATION

I, hereby declare that this thesis, entitled **"CHITOSAN MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN KASTHURI TURMERIC (*Curcuma aromatica* Salisb.)"** is a bonafide record of research work done by me during the course of research and 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 **NIVYA J THENGUMPALLY**

Date: 14.10.2019 **(2017-12-013)**

CERTIFICATE

Certfified that this thesis, entitled **"CHITOSAN MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN KASTHURI TURMERIC (*Curcuma aromatica* Salisb.)"** is a record of research work done independently by **Ms. Nivya J Thengumpally (2017-12-013)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Deepa 14/10/19

Vellayani
Date: 14.10.2019

Dr. Deepa S Nair
(Major advisor)
Assistant Professor Head,
Department of Plantation Crops and Spices
College of Agriculture, Vellayani
Thiruvananthapuram – 695 522.

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Nivya J Thengumpally (2017-12-013)** a candidate for the degree of **Master of Science in Horticulture,** with major in Plantation Crops and Spices agree that the thesis entitled **"Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)"** may be submitted by **Ms. Nivya J Thengumpally**, in partial fulfilment of the requirement for the degree.

Dr. Deepa S Nair
(Chairman, Advisory Committee)
Assistant Professor and Head
Department of Plantation Crops and Spices
College of Agriculture, Vellayani

Dr. G. S. Sreekala
(Member, Advisory Committee)
Assistant Professor
Department of Plantation Crops and Spices
College of Agriculture, Vellayani

Dr. T. Sajitha Rani
(Member, Advisory Committee)
Professor and Head
Instructional Farm
College of Agriculture, Vellayani

Dr. Beena. R
(Member, Advisory Committee)
Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani

ACKNOWLEDGEMENT

Thanks to Almighty 'GOD' who is more benevolent and merciful made me capable to complete this task.

It is with great reverence I place on record, my deepest sense of gratitude and indebtedness to my major advisor ***Dr. Deepa S. Nair,*** *Assistant Professor and Head, Department of Plantation Crops and Spices, College of Agriculture, Vellayani, for her meticulous supervision, soft and sincere suggestions, untiring help and constant encouragement throughout the progress of this study.*

My heartfelt thanks to ***Dr. G. S. Sreekala****, Assistant professor, Department of Plantation Crops and Spices, member of my advisory committee for her meticulous guidance, valuable suggestions, keen interest, wholehearted help and constructive criticism and also for the realization of the project.*

With great pleasure I express my heartiest and esteem sense of gratitude to ***Dr. P. C. Jessykutty****, Rtd. Professor and Head, Department of Plantation Crops and Spices and* ***Dr. Sonia N. S,*** *Assistant Professor, Department of Plantation Crops and Spices for their advice and support throughout the period of investigation during the period of endeavor.*

I would like to express my immense gratitude to farm managers and all labourers for their timely help and support.

I am thankful to my classmates ***Anargha, Krishnaveni*** *and* ***Pooja*** *for their friendship and kind help in times of need. I express my sincere gratitude to my seniors Manisha chechi, Shilpa and Maheswary chechi. I thankfully own up and express my thanks to Suresh chetan and Sugandhi chechi.*

I am extremely grateful to the members of advisory committee, ***Dr. T. Sajitha Rani,*** *Professor and Head, Instructional Farm and* ***Dr. Beena R.,*** *Assistant professor, Department of Plant Physiology, for their valuable suggestions and cooperation during the course of investigation.*

I acknowledge the boundless affection, timely help, companionship and moral support rendered by my friends ***Amala and Bincy****. I warmly remember their role in making the period of my study here a memorable and cherished one. Also, I am thankful to my beloved friends* ***Musi, Jyothis, Anjaly, Geethu, Pooja, Elzu and Sijo*** *for their love and support during my PG programme. I would like to express my sincere thanks to my beloved friend* ***Lintu*** *for his unsolicited help and moral support throughout my PG programme.*

Also I would like to thank my dear juniors ***Akhil, Namitha, Rakhi and Reddappa*** *for their affection, assistance and support and help during the period of my research work.*

At this time of thesis submission, no words to express my whole-hearted and affectionate gratitude to my Papa and Mummy for their immense love and copious encouragement throughout my career. Also, the unflinging support given by Nimmy chechi, Neenu chechi, Bellapi and other family members especially my chetai have been a precious source of strength throughout the course of my study here.

Once again I am thanking each and every person who helped me during my research programme.

Nivya J Thengumpally

TABLE OF CONTENTS

Chapter	Title	Page No.
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	4
3.	MATERIALS AND METHODS	17
4.	RESULTS	31
5.	DISCUSSION	69
6.	SUMMARY	80
7.	REFERENCES	86
	ABSTRACT	103

LIST OF TABLES

Table No.	Title	Page No.
1.	Details of the treatments	19
2.	Effect of foliar spray treatments on plant height (cm) and number of tillers in *C. aromatica*	34
3.	Effect of foliar spray treatments on number of leaves in *C. aromatica*	35
4.	Effect of foliar spray treatments on leaf area (cm^2) in *C. aromatica*	36
5.	Effect of foliar spray treatments on rhizome spread (cm) and rhizome thickness (cm) in *C. aromatica*	38
6.	Effect of foliar spray treatments on number of fingers in *C. aromatica*	39
7.	Effect of foliar spray treatments on root length (cm) and root spread (cm) in *C. aromatica*	41
8.	Effect of foliar spray treatments on root weight (g $plant^{-1}$) in *C. aromatica*	43
9.	Effect of foliar spray treatments on shoot weight (g $plant^{-1}$) in *C. aromatica*	44
10.	Effect of foliar spray treatments on chlorophyll content (mg g^{-1}) in *C. aromatica*	46
11.	Effect of foliar spray treatments on total protein content (mg g^{-1}) in *C. aromatica*	48
12.	Effect of foliar spray treatments on defense enzymes in *C. aromatica*	50
13.	Effect of foliar spray treatments on curcumin, volatile oil and oleoresin (per cent) in *C. aromatica*	52

14.	Effect of foliar spray treatments on carbohydrate, starch and reducing sugar content (mg g^{-1}) in *C. aromatica*	54
15.	Effect of foliar spray treatments on dry matter production (g $plant^{-1}$) in *C. aromatica*	56
16.	Effect of foliar spray treatments on leaf area index in *C. aromatica*	57
17.	Effect of foliar spray treatments on net assimilation rate (g m^{-2} day^{-1}) in *C. aromatica*	59
18.	Effect of foliar spray treatments on stomatal conductance (m mol m^{-2} s^{-1}) in *C. aromatica*	60
19.	Effect of foliar spray treatments on photosynthetic rate (μ mol CO2 m^{-2} s^{-1}) in *C. aromatica*	61
20.	Effect of foliar spray treatments on proline content (μ moles g^{-1}) in *C. aromatica*	62
21.	Effect of foliar spray treatments on cell membrane integrity (per cent) in *C. aromatica*	63
22.	Effect of foliar spray treatments on fresh and dry rhizome yield (g $plant^{-1}$) in *C. aromatica*	65
23.	Effect of foliar spray treatments on crop duration (days) and harvest index in *C. aromatica*	66
24.	Effect of foliar spray treatments on nutrient uptake (g $plant^{-1}$) in *C. aromatica*	68

LIST OF FIGURES

Figure No.	Title	Between Pages
1.	Effect of foliar spray treatments on curcumin content, volatile oil and oleoresin	74-75
2.	Effect of foliar spray treatments on fresh rhizome yield	77-78
3.	Effect of foliar spray treatments on dry rhizome yield	77 - 78
4.	Effect of foliar spray treatments on uptake of N, P and K	79-80

LIST OF PLATES

Plate No.	Title	Between pages
1.	Planting material of *C. aromatica*	18 - 19
2.	General view of experimental area	18 - 19
3.	*C. aromatica* plants at different stages of growth A) 1 MAP B) 3 MAP C) 5 MAP D) at harvest	32 - 33
4.	Harvested rhizome from *C. aromatica* plants exposed to foliar spray with A) Chitosan 3 g l^{-1} B) Chitosan 2.5 g l^{-1} C) Control	65- 66

LIST OF ABBREVIATIONS

%	Per cent
@	At the rate of
µg	Microgram
°C	Degree Celsius
CD	Critical Difference
cm	Centimetre
CMI	Cell membrane integrity
CRD	Completely Randomized Block Design
DNS	Dinitro Salicylic acid
DMP	Dry matter production
DMSO	Dimethyl Sulfoxide
EC	Electrical conductivity
EDTA	Ethylene diamine tetra acetic acid
et al.	And others
Fig.	Figure
g	Gram
ha^{-1}	Per hectare
HI	Harvest Index
i.e.	That is
K	Potassium
Kg	Kilogram
l^{-1}	Per liter
LAI	Leaf Area Index
MAP	Month After Planting

mg	Milligram
min^{-1}	Per minute
mM	Milli metre
N	Nitrogen
NAR	Net Assimilation Rate
nm	nanometre
NS	Non Significant
P	Phosphorous
PBS	Phosphate buffer saline
$Plant^{-1}$	Per plant
ROS	Reactive oxygen species
rpm	Rotations per minute
SE	Standard Error
SOD	Superoxide dismutase
UV	Ultra violet
Viz.	Namely
w/v	Weight per volume

Introduction

1. INTRODUCTION

Curcuma aromatica Salisb. (Kasthuri turmeric) belonging to the family Zingiberaceae, is a medicinal cum aromatic plant, cultivated for its characteristic aromatic rhizome. It is the second most important species of the genus *Curcuma* cultivated for its rhizomes, next to *Curcuma longa* (Sikha *et al.,* 2015). In comparison with *C. longa*, the rhizomes are less pigmented with characteristic camphoraceous odour. The aromatic rhizomes are exploited for its medicinal properties in India, China and other South East Asian countries. Its medicinal properties are being utilized in the traditional systems of medicine like Ayurveda, Siddha and Unani. It is known as *"Vanaharidra"* in Ayurveda and is used in the treatments of skin problems, cardiovascular and respiratory systems. It is widely distributed in South Asian regions, from China southwards to Srilanka. In India, it is found distributed in Himalayan region and Western Ghats and in southern parts of India and West Bengal (Shamim *et al.,* 2011; Anoop, 2015).

A wide range of biological activities have been reported in this species which enables it to be utilized for the preparation of various formulations in the treatment of inflammation, wound and microbial infections or infection associated with conditions like diabetes and cancer (Li *et al*., 2009). The pharmacological value of the rhizome is due to the presence of secondary metabolites like alkaloids, curcuminoids, flavonoids, terpenoids and tannins (Anoop, 2015). The essential oil of *C. aromatica* has high demand in ayurvedic pharmaceutical and cosmetic industries. The rhizomes contains 4-8 per cent oil (Pant *et al.,* 2013). The antiseptic, antimicrobial and anti-inflammatory properties of the oil enables its use in embalmment and food preservation. The essential oil also has anti-tumour and anti-bacterial properties (Revathy and Malathy, 2013). The oil and extract of *C. aromatica* also serve as an important source of antioxidants being used in the food industries (Al-Reza *et al.,* 2010). Curcumin is a potential antioxidant extracted from *C. aromatica*, that helps in scavenging free radicals to give brighter skin complexion and to cure skin disorders and hence, is as a key ingredient in

Ayurvedic skin care formulations (Sikha *et al.,* 2015). The anti-carcinogenic activity of curcumin has been established in a variety of cell lines (Yu *et al.,* 2011).

The enhanced production strategies in terms of yield and secondary metabolites is inevitable to meet the high demand of pharmaceutical industries. Chitosan is confirmed as an effective biotic elicitor with non-toxic, biocompatibile and biodegradable properties to improve the production and biosynthesis of secondary metabolites in medicinal plants (Park and Kim, 2010; Mehregan *et al.*, 2017; Pliankong *et al.*, 2018). Chitosan is known to stimulate plant development processes associated with its metabolite profile and to elicit defense responses in plants. It is a natural, safe and cost effective biopolymer obtained from the deacetylation of chitin, a long-chain polymer of N-acetyl-glucosamine and easily extracted from fungal cell wall and crustacean shells (Malerba and Cerana, 2017; Yadav *et al*., 2019).

In this context, the study entitled "Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)" has been proposed with the objective to study the effect of different concentrations of chitosan on plant growth, yield and metabolite production in *Curcuma aromatica*.

Review of Literature

2. REVIEW OF LITERATURE

The present study entitled "Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)" has been taken up with the objective to study the effect of different concentrations of chitosan on plant growth, yield and metabolite production in *Curcuma aromatica*. The relevant literature on the effect of chitosan on various crops are reviewed in this chapter.

2.1 *Curcuma aromatica*

Curcuma aromatica Salisb. is a medicinally important herb that belongs to the family Zingiberaceae. A wide range of biological activities have been reported in this species which enables it to be utilized for the preparation of various formulations for the treatment of inflammation, wound and microbial infections or infection associated with conditions like diabetes, and cancer (Li *et al.*, 2009). The oil and extract of *C. aromatica* also serve as an important source of antioxidants being used in the food industries (Al-Reza *et al.*, 2010).

Curcuma aromatica is used as a substitute for *Curcuma longa* (Gopichand *et al.*, 2006). The rhizomes contains 4-8 per cent oil (Pant *et al.*, 2013). The oil has high demand in ayurvedic pharmaceutical and cosmetic industries. The essential oil of *C. aromatica* has anti-tumour and anti-bacterial properties (Revathy and Malathy, 2013). Curcumin content in kasthuri turmeric rhizomes is low and ranges from 0.05 to 0.10 per cent. Ampasavate *et al.* (2009) reported 15.3 per cent of curcuminoids in ethanolic extract of *C. aromatica* rhizome powder and on HPLC analysis percentage of curcumin was found to be 28.5 per cent of curcuminoids. A curcumin content of about 3 per cent has been recorded in *C. aromatica* by Jalgaonkar (2016).

2.2 Chitosan

Chitosan is a natural, safe and cost effective biopolymer obtained from the deacetylation of chitin, a long-chain polymer of N-acetyl-glucosamine and easily

extracted from fungal cell wall and crustacean shells (Domard and Domard, 2002). Its production cost is low. It is non-toxic, biocompatible and biodegradable (Park and Kim, 2010). These properties enables its utilization in various fields *viz.,* cosmetology, food, biotechnology, pharmacology and medicine (Choi *et al*., 2016). Chitosan acts as an elicitor of plant systemic immunity by the accumulation of defence related antimicrobial compounds and it plays an important role in the activation of induced resistance (Katiyar *et al*., 2014). Chitosan is also considered as biostimulant as it stimulates various plant responses, including induction of disease and abiotic stress resistance, enhancement of plant growth and yield, shelf life of flowers and fruits, and activation of secondary metabolite production (Pichyangkuraa and Chadchawanb, 2015).

2.2.1 EFFECT OF CHITOSAN ON PLANT GROWTH PARAMETERS

The enhancement in plant growth characters *viz.*, plant height, number of leaves, leaf area, shoot and root biomass, root length on application of chitosan has been reported in various horticultural crops. The improvement in plant growth characters could be attributed to increased enzyme activities of nitrogen metabolism by the application of chitosan (Ke *et al*., 2001).

Chitosan is a high potential biomolecule that increases plant growth and development (Chibu and Shibayama, 2001; Gornik *et al*., 2008). Hadwiger *et al.* (2002) reported that chitosan had molecular signals that served as plant growth promoters.

Tsugita *et al.* (1993) reported that when chitosan was applied to daikon radish, it enhanced shoot and root growth of the crop. Wanichpongpan *et al.* (2001) observed that chitosan had positive effect on the growth of gerbera plants. Chitosan treatment resulted in increase in number of leaves, leaf width and length of gerbera.

Barka *et al.* (2004) conducted a study to assess the effect of chitosan on grapevine and it was found that chitosan treatment significantly increased root and shoot biomass and also enhanced photosynthesis.

Ohta *et al*. (2004) observed that 1.0 per cent chitosan soil mixture enhanced growth of several ornamental plants compared to inorganic water soluble fertilizers and control. In an experiment conducted by Kim *et al.* (2005), there was significant increase in weight and height of sweet basil by the application of chitosan.

The different concentrations of soluble chitosan were tried on *in vitro* plantlets of potato to study the effect on growth and yield of potato tubers. The application of chitosan at 500 mg l^{-1} enhanced the shoot weight while root weight was significantly enhanced by 5 and 15 mg l^{-1} of soluble chitosan. The higher concentrations of chitosan found to decrease the root weight significantly (Asghari-Zakaria *et al*., 2009).

The growth enhancement with chitosan was observed in *in vitro* cultures of Dendrobium (Kananont *et al*., 2010; Nge *et al*.,2006; Pornpienpakdee *et al*., 2010).

Application of chitosan (2 cm^3 l^{-1}) to strawberry plants resulted in growth enhancement in terms of plant height, number of leaves, fresh and dry weights of leaves. (Abdel-Mawgoud *et al.,* 2010).

Mondal *et al*. (2011) observed that foliar applications of chitosan at the optimal concentration of 75 mg l^{-1} on Indian spinach (*Basella alba* L.) resulted in an increase in plant height, leaf number, branch number, leaf area, and fresh and dry weight.

Chookhongkha *et al*. (2012) reported that the addition of 1.0 per cent chitosan to the soil enhanced plant height, canopy diameter and leaf area of chili pepper (*Capsicum annuum* L.).

Foliar application of 100–125 mg l^{-1} chitosan every 15 days increased fruit production, as well as plant height, leaf number, relative growth rate, photosynthesis rate and nitrate reductase activity in okra (Mondal *et al*., 2012).

Ahmed (2015) conducted a study on response of garlic plants to foliar application of chitosan. Chitosan extract were sprayed at three different

concentrations (2, 4 and 6 ml l^{-1}) at 30, 45, 60 and 75 days from sowing. He observed that foliar application of chitosan extract at 4 and 6 ml l^{-1} on "clone sids-40" garlic plants significantly increased leaf fresh weight, plant height, and the number of leaves per plant.

Malekpoor *et al.* (2016) observed considerable increase in the plant growth characters of basil (*Ocimum basilicum*) when it was sprayed with chitosan at varying concentration. However foliar application of chitosan at 0.4 g l^{-1} gave significant increase in plant growth characters both under stressed and non stressed conditions.

A study conducted by Rahman *et al.* (2018) observed that application of chitosan on the canopy of field grown strawberry plants significantly influenced leaf width, leaf length, leaf number per plant and canopy diameter compared to untreated control.

Kra *et al.* (2019) carried out an experiment in cassava (*Manihot esculenta*), to study the effect of chitosan on growth of vegetative parts and they observed that chitosan at 100 mg l^{-1} gave maximum vegetative growth. According to them, the increment in growth would result from increased cell division or their extension.

2.2.2 EFFECT OF CHITOSAN ON METABOLITE PRODUCTION

Chitosan is known to be a natural polymer which has the ability to induce the biosynthesis of secondary metabolites. The effect of chitosan on the activity of defense enzymes especially catalase, peroxidase and superoxide dismutase and on the production other metabolites have been reported in various crop species (Agrawal *et al.*, 2002; Ma *et al.*, 2014).

Chang *et al.* (1998) carried out an experiment in suspension cultures of *Mentha piperita* by adding chitosan at 200 mg l^{-1} in the culture media. The menthol content of *M. piperita* increased drastically due to the elicitation by chitosan.

Ben-Shalom *et al.* (2003) demonstrated that chitosan foliar spray at 0.1 per cent (w/v) could increase the peroxidase activity in cucumber plants and thereby controlling the grey mold disease.

Orlita *et al.* (2008) studied the effect of elicitors like chitin and chitosan on production of secondary metabolites in *Ruta graveolens* (Common rue). Application of chitin and chitosan at 0.01 per cent and 0.10 per cent showed a substantial increase in the production of secondary metabolites such as coumarins, furanocoumarins, acridone and quinolone alkaloids and flavonoids.

Stem cuttings of grapevine dipped in chitosan solution (2 per cent) resulted in increase in chlorophyll content and also induced stress tolerance to salt, drought and temperature stress (Gornik *et al.*, 2008).

In a study by Meng *et al.* (2008) investigated that application of chitosan as preharvest spray and postharvest coating on table grapes resulted in inhibition of SOD activity and increase in the accumulation of peroxidase enzyme.

Mandal (2010) conducted an experiment to study the effect of different elicitors such as chitosan, salicylic acid, methyl salicylate and methyl jasmonate in eggplant (*Solanum melongena* L.). The results explained that chitosan increased the activity of defense enzymes like peroxidase, catalase and also improved total phenolic content of the plant.

Beans plants were treated with different concentrations of chitosan solution in order to study its effect on chlorophyll content of the plant. It was found that the content of chlorophyll a, b and total chlorophyll is maximum when it is treated with chitosan solution at 2.5 per cent (Sheikha and Al-Malki, 2011).

In *Artemisia annua*, the foliar application of chitosan at 100 mg l^{-1} could increase the production of artemisinin by activating the genes responsible for the biosynthesis of artemisinin (Lei *et al.*, 2011).

Farouk and Amany (2012) opined that total content of chlorophyll and carbohydrate increased significantly as a result of foliar application of chitosan at 250 mg l^{-1}. But at higher concentrations of chitosan, these parameters were found to decline.

A study conducted by Baque *et al.* (2012) revealed that chitosan is a potential elicitor, as it improved the production of anthraquinones, phenolics and flavonoids, when adventitious root cultures of *Morinda citrifolia* were exposed to 0.2 mg ml^{-1} chitosan.

Srisornkompon *et al.* (2014) observed that in tea leaves (*Camellia sinensis*) the total phenolic content increased significantly by the application of chitosan. Both pre and post harvest treatment of chitosan had positive effect on the phenolic content of tea leaves compared to control treatment.

Chitosan applied as foliar spray at 0.1 per cent (w/v) to 30 days old tomato plants grown under glass house condition resulted in increase in protein content (up to two fold) of the crop (Sathiyabama *et al.*, 2014).

Anjusha and Gangaprasad (2014) reported that the content of curcumin is very low in *Curcuma aromatica* compared to other *Curcuma sp.* The content of curcumin in *C. aromatica* was found to be 0.0175 g per 100 g.

Gorelick *et al.* (2015) reported that aswagandha (*Withania somnifera*) plants cultivated for its roots and leaves, when treated with chitosan at 100 mg ml^{-1} showed higher percentage, about 69 per cent of withaferin A.

In turmeric Anusuya and Sathiyabama (2016) observed that foliar spray of chitosan at 0.1 per cent w/v resulted in induction of defense related enzymes like peroxidase, polyphenol oxidase etc. in leaves and rhizomes. Curcumin content of turmeric rhizomes also increased by chitosan spray.

The summer tomato (*Solanum lycopersicum*) was sprayed with different concentrations of chitosan *viz.*, 0 (control), 25, 50, 75 and 100 mg l^{-1}. It was found

that the morphological characters such as plant height, number of leaves and leaf area of the plant significantly enhanced up to 75 mg l^{-1} of chitosan compared to control treatment (Mondal *et al.,* 2016).

According to Sathiyabama *et al.* (2016), chitosan application at 0.1 per cent stimulated curcumin accumulation and plant growth in *Curcuma longa* thereby doubling curcumin production in the plant. It also increased the activity of peroxidase in both leaves and rhizomes.

Malayamana *et al.* (2017) conducted an experiment in *Phyllanthus debilis* grown in *in vitro* condition. Chitosan at 1.5 g l^{-1} of growth medium provided maximum hydrolysable tannins which is the main active constituent of this crop.

Zong *et al.* (2017) observed that application of chitosan to edible rape (*Brassica rapa* L.) under cadmium stress resulted in increase in plant growth characters such as root length, root weight and shoot weight, total chlorophyll content, photosynthetic activity etc. It also improved the activities of antioxidant enzymes such as SOD, catalase, peroxidase.

The oligo chitosan at 100 ppm when applied as foliar spray in tomato and egg plant 5 times up to harvest, resulted in considerable increase in the protein content of plants (Sultana *et al*., 2017). Stevia plants when sprayed with chitosan at 0.1 per cent gave significant increase in the production of secondary metabolite rebaudiosides A (Mehregan *et al*., 2017).

Rahman *et al.* (2018) conducted an experiment in strawberry and reported that the plants sprayed with chitosan at 1000 ppm showed maximum content of carotenoids, anthocyanins, flavonoids and phenolics compared to control.

Kra *et al*. (2019) reported that in *Manihot esculenta,* the peroxidase activity in leaves was found to be significantly increased with increase in chitosan concentration and maximum activity was recorded in plants treated with chitosan at 75 and 100 mg l^{-1}

2.2.3 EFFECT OF CHITOSAN ON PHYSIOLOGICAL PARAMETERS

Chitosan has been considered as a natural polymer which has the ability to induce various biological responses in plants. The positive effect of chitosan application on different physiological characters has been reported in several plant species (Mahdavi, 2013; Ray *et al.*, 2016; Yahyaabadi *et al.*, 2016). It has the ability to remove the reactive oxygen species and thereby protecting the functions of bio-membrane and improving the physiological activities of the crop (Song *et al.*, 2006). The chitosan also plays an important role in the alleviation of biotic and abiotic stress conditions in plants (Guo *et al.*, 2003; Yin *et al.*, 2008; Katiyar *et al.*, 2014; Sharif *et al.*, 2018).

Lee *et al.* (1999) demonstrated that application of chitosan 100 or 200 μg ml^{-1} reduced the stomatal aperture of tomato and *Commelina communis* and resulted in enhanced resistance to pathogen attack.

Bittelli *et al.* (2001) reported that pepper plants grown in pots in growth chambers when sprayed with chitosan shown reduced stomatal conductance which resulted in decrease in transpiration rates. Thus this study revealed that chitosan can be used as an effective anti-transpirant.

Khan *et al.* (2002) conducted an experiment in maize and soybean to find out the effect of chitosan oligosaccharides on the rate of photosynthesis. They observed a decrease in photosynthetic rate on the first day of foliar application of chitosan. But on the third day of application, about 10-18 per cent increase of net photosynthetic rate is noticed compared to control. This is due to the increase in stomatal conductance and transpiration rate.

Cucumber seedlings under salt stress were treated with chitosan at 150 mg l^{-1}, reduced the salt stress damage by increasing the proline content and decreasing the electrolyte permeability of the leaf cells (Song *et al.*, 2006).

Yang *et al.* (2009) reported that apple seedlings grown under drought stress when sprayed with chitosan at 100 mg l^{-1} enhanced cell membrane stability.

Guan *et al.* (2009) observed that chitosan treatment at 0.50 per cent showed significant increase in the proline content in two inbred lines of maize.

In rice, Phothi and Theerakarunwong (2017) studied the effect of chitosan on physiology and photosynthetic rate under elevated ozone. They reported that under elevated ozone the photosynthetic rate of rice decreased considerably. But when the plants were treated with chitosan, rate of photosynthesis and stomatal conductance increased.

Zong *et al.* (2017) evaluated the effect of chitosan in edible rape (*Brassica rapa*) grown under cadmium stress. They reported that foliar spray with chitosan 50 mg l^{-1} resulted in considerable increase in photosynthetic rate and stomatal conductance. The proline content of the plant was also brought to the normal level by the foliar application.

In *Thymus daenensis,* foliar application of chitosan at 400 $\mu l\ l^{-1}$ resulted in significant increase in dry matter production and proline content. It also improved the cell membrane integrity and thus reduced the membrane leakage (Bistgani *et al.*, 2017a).

2.2.4 EFFECT OF CHITOSAN ON YIELD AND YIELD COMPONENTS

The exogenous application of chitosan improved the yield of many crops (El-sawy *et al.*, 2010; Boonlertnirun *et al.*, 2011; Sheheta *et al.*, 2012). According to Abdel-Mawgoud *et al.* (2010) the increased crop yield might be resulted from higher dry matter production. The increased leaf area and number of leaves also contribute to increment in yield and yield attributes.

Chandrkrachang *et al.* (2005) conducted an experiment to study the effect of chitosan spray on orchid plants and it was observed that chitosan at 10 mg l^{-1} showed a noticeable increase in yield compared to control.

Foliar spray with chitosan at 0.1 g l^{-1} resulted in increased tuber yield and quality of micro propagated greenhouse-grown potatoes (Kowalski *et al.*, 2006).

According to Asghari-zakaria *et al.* (2009), application of chitosan at 500 mg l^{-1} resulted in an increase in growth characters and yield of potato (*Solanum tuberosum* L.) plants grown *in vitro*. The chitosan application gave maximum yield in terms of number and weight of mini tuber compared to control.

An experiment conducted by Zeng and Luo (2012) demonstrated that the yield of wheat plants can be increased by 13.6 per cent with the application of chitosan.

Salachna and Zawadzinska (2014) conducted an experiment in fressia plant to study the effect of chitosan on yield and the highest yield was recorded in plants which were treated with 0.50 per cent chitosan.

Janmohammadi *et al.* (2014) conducted investigations on effect of chitosan on the performance of lentil genotypes. Chitosan treatments were seed soaking with chitosan, foliar spraying with chitosan solution at 30 days after sowing and foliar spraying with chitosan solution at 50 per cent flowering stage. When the plants were treated with chitosan at the reproductive stage significant increase was observed in the yield and yield components viz., number of pods per plant, number of grains per pod and 100-seed weight. The economic yield and harvest index were also enhanced by chitosan application.

Yield of turmeric significantly increased by the application of chitosan as foliar spray. About 60% increase in yield was obtained in chitosan treated plants over the water sprayed control plants (Anusuya and Sathiyabama, 2016).

Mondal *et al.* (2016) opined that foliar spray with chitosan increased the fruit yield of *Solanum lycopersicum.* Among the various concentrations (0, 25, 50, 75 and 100 mg l^{-1}) of chitosan it was reported that maximum fruit yield obtained in treatment 75 mg l^{-1} compared to control.

Falcón-Rodríguez *et al.* (2017) found that foliar application of chitosan at 200 mg ha^{-1} enhanced tuber yield of potato (*Solanum tuberosum*) by increasing tuber size.

Bistgani *et al.* (2017b) opined that in *Thymus daenensis*, the essential oil content of the crop increased considerably with a chitosan spray of 400 μl l^{-1}.

In bell pepper, Mahmood *et al.* (2017) conducted a study using different biostimulants like chitosan, salicylic acid and putrescine and reported that foliar spray of chitosan had positive effect on yield and yield components of bell pepper.

Mutka *et al.* (2017) observed that two applications of chitosan at 250 ppm at pre-flowering and post-flowering stage on strawberry plants developed through tissue culture enhanced fruit production compared to non-treated control plants.

Salehi *et al.* (2017) reported that the foliar application of chitosan at 0.2 g l^{-1} on *Satureja isophylla* had a significant effect on yield and quality of essential oil.

Application of nano-chitosan through foliar spraying at 5ml l^{-1} on mango trees significantly increased yield in terms of number of fruits per tree and weight of fruit (Zagzog *et al.*, 2017).

2.2.5 EFFECT OF CHITOSAN ON UPTAKE OF PLANT NUTRIENTS (N, P, K)

Chitosan is considered as a biofertilizer which gets degraded enzymatically without affecting the soil borne beneficial microbes (Escudero *et al.*, 2017). The application of chitosan was found to improve nutrient uptake in plants. The enhanced nutrient uptake is favoured by the increased chlorophyll content and net photosynthetic rate due to chitosan application (Van *et al.*, 2013). According to O'Herlihy *et al.* (2003), the nutrient uptake was found to improve considerably with chitosan spray in potato (*Solanum tuberosum*) plants.

Dzung *et al.* (2011) conducted a study to evaluate the effect of chitosan on coffee plants and it was found that spraying with chitosan at 60 ppm resulted in

maximum nutrient uptake. The increase in nutrient uptake was about 9.49 per cent N, 11.76 per cent P and 0.98 per cent K.

Farouk *et al.* (2011) reported that when chitosan and humic acid applied to radish (*Raphanus sativus*, L. var. *sativus*) under cadmium stress, application of Chitosan at 200 mg/ kg increased the efficiency of nutrient uptake than humic acid.

In common beans (*Phaseolus vulgaris* L.) Abu-Muriefah (2013) carried out an experiment using chitosan. Common beans plants subjected to water stress were sprayed with chitosan at different concentrations (100, 200 or 400 mg l^{-1}) at 40, 50 and 60 days from sowing. Water stressed plants showed low uptake of essential nutrients especially N, P and K. Foliar application of chitosan at 200 mg l^{-1} enhanced nutrient uptake of water stressed plants.

Van *et al.* (2013) studied the role of chitosan nanoparticles on Robusta coffee grown under greenhouse conditions. They reported that application of chitosan at 10-20 ppm enhanced nutrient uptake from 9.8 to 27.4 per cent N, 17.3 to 30.4 per cent P, 30 to 45 per cent K than untreated control.

Materials and Methods

3. MATERIALS AND METHODS

Studies on "Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)" were carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2017-2019. The objective of this experiment was to study the effect of different concentrations of chitosan on plant growth, yield and metabolite production in *Curcuma aromatica.*

The details of the materials used and methods adopted for the study are presented in this chapter.

3.1 EXPERIMENT DETAILS

3.1.1 Planting material

The planting material (Plate 1) for the experiment were procured from the Instructional Farm, College of Agriculture, Vellayani. Healthy, disease and pest free rhizome bits having 2-3 buds weighing 10-12 g were planted in growbags. The growbags were filled with potting mixture (sand: soil: compost-1:1:1). The crop was raised organically as per adhoc organic POP (KAU, 2013). The general view of the experimental area is presented in Plate 2.

3.1.2 Season

The experiment was carried out during June 2018 to January 2019.

3.1.3 Design of the experiment

The experiment was laid out in completely randomized block design (CRD) with nine treatments and three replications. Twenty one plants were maintained in each treatment.

Plate 1. Planting material of *C. aromatica* A) Rhizome B) 2-3 budded rhizome bits

Plate 2. General view of experimental area

3.1.4 Treatment details

The foliar spray of chitosan at different levels were given to the plants at 3 and 5 months after planting (MAP). The chitosan solution was prepared by dissolving the chitosan in 0.25 per cent glacial acetic acid. The spray volumes applied per plant at 3 MAP and at 5 MAP were 60 ml and 100 ml, respectively. The details of the treatments are depicted in Table 1.

Table 1. Details of the treatments

Treatments	Name of the treatment
T_1	Chitosan 0.5 g l^{-1}
T_2	Chitosan 1.0 g l^{-1}
T_3	Chitosan 1.5 g l^{-1}
T_4	Chitosan 2.0 g l^{-1}
T_5	Chitosan 2.5 g l^{-1}
T_6	Chitosan 3.0 g l^{-1}
T_7	Acetic acid (0.25 %) spray
T_8	Water spray
T_9	Control

3.2 OBSERVATIONS

3.2.1 Plant growth parameters

The observations on plant height, number of tillers, number of leaves, leaf area and shoot weight were taken one month after each foliar application i.e. 4 and 6 MAP. The observations on rhizome spread, rhizome thickness, number of fingers, root length, root spread and root weight were recorded at 4 MAP, 6 MAP and at harvest.

3.2.1.1 Plant height

The height of the longest tiller was measured from the base of the plant to the top of the young fully opened leaf. The mean value was recorded and expressed in centimeter (cm).

3.2.1.2 Number of tillers

The number of aerial shoots per plant was counted. The mean number was recorded.

3.2.1.3 Number of leaves

The number of fully opened leaves of each tiller from sample plants was counted and the total number of leaves per plant was recorded, mean value estimated.

3.2.1.4 Leaf area

Leaf area was calculated based on length and breadth method and expressed in cm^2.

The following relationship was used for computing leaf area (Randhawa *et al.*, 1985).

$Y = 4.09 + 0.564$ (Length × Breadth),

Where, Y = Leaf area

Length = Length of the leaf in cm

Breadth = Breadth of the leaf in cm

3.2.1.5 Rhizome spread

The horizontal spread of rhizome was measured using a meter scale. The mean value estimated and expressed in centimeter (cm).

3.2.1.6 Rhizome thickness

Rhizome thickness was measured using micrometer screw gauge. The mean value was recorded and expressed in cm.

3.2.1.7 Number of fingers

The total number of fingers including primary, secondary and tertiary from the mother rhizome were counted and mean value recorded.

3.2.1.8 Root length

The plants were uprooted with whole rhizome and maximum length of roots were measured and the mean length expressed in centimeter (cm).

3.2.1.9 Root spread

Root spread was measured by spreading the root system on a marked paper and measuring the spread of the root system at its broadest part. The mean value was calculated and expressed in cm.

3.2.1.10 Root weight

Roots were separated from individual plants and weighed to record the fresh weight. It was then dried in hot air oven at 70°C till constant weight was obtained and weighed again to record dry weight and expressed in g $plant^{-1}$.

3.2.1.11 Shoot weight

The above ground part of the plant was separated and weighed to record the fresh weight. It was then dried in hot air oven at 70°C till constant weight was obtained, weighed again to record the dry weight. The shoot weight was expressed in g $plant^{-1}$.

3.2.2 Metabolite production

The observations on chlorophyll content, total proteins and defense enzymes were recorded at 4 and 6 months after planting. The observations on

curcumin content, volatile oil, oleoresin and carbohydrate (starch, reducing sugar) were recorded at harvest.

3.2.2.1 Chlorophyll content

Leaf bits (0.1g) collected from both control and treatment plants were washed in distilled water and used for the estimation of chlorophyll. Pigments were extracted from leaf bits by using acetone: DMSO (1:1) mixture. The leaf sample were incubated in acetone: DMSO solution in the dark for overnight. The coloured solution was decanted into measuring cylinder and made up to 10 ml. The absorbance was recorded at 663 and 645 nm using UV- visible spectrophotometer. Chlorophyll 'a', Chlorophyll 'b' and Total chlorophyll was estimated as described by Arnon (1949) and expressed in mg g^{-1} of fresh weight. The formula for calculating Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll is as given below.

$$\text{Chlorophyll a} = [\{12.7\,(A663) - 2.69\,(A645)\} \times \text{volume}/\,(\text{weight} \times 1000)]$$

$$\text{Chlorophyll b} = [\{22.9\,(A645) - 4.68\,(A663)\} \times \text{volume}/\,(\text{weight} \times 1000)]$$

$$\text{Total chlorophyll} = [\{20.2\,(A645) + 8.01\,(A663)\} \times \text{volume}/\,\text{weight}] \times 1000$$

3.2.2.2 Total proteins

Total protein content was estimated as per procedure described by Bradford (1976) using bovine serum albumin as the standard. 0.5 g of sample was taken and grinded in 10 micro liter of PBS solution. After centrifuging, supernatant was collected. Four milliliter of Coomassie brilliant blue dye was added to the solution. Blank solution was also prepared and absorbance was measured at 595 nm using spectrophotometer. Values were calculated using standard graph and expressed in mg g^{-1}.

3.2.2.3 Defense enzymes

3.2.2.3.1 Catalase

Catalase activity was measured as per procedure described by Luck (1974). 200 mg of leaf sample was prepared in phosphate buffer. The homogenate was centrifuged at 5000 rpm for 15 min at 4°C and the supernatant was used for the enzyme assay. The H_2O_2-phosphate buffer (3 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 and it is expressed as units ml^{-1}.

3.2.2.3.2 Peroxidase

Peroxidase activity was measured according to the procedure described by Peru (1962). Leaf sample (200 mg) was homogenized in 1 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The supernatant was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was used as the enzyme extract for the assay.

Reaction mixture containing 1 ml of 0.05 M pyrogallol and 50 µl of enzyme extract was taken in both reference and sample cuvettes, mixed and kept in spectrophotometer (ELICO-SL 218 Double Beam), reading was adjusted to zero at 420 nm. The enzyme reaction was started by adding 1 ml of 1 per cent hydrogen peroxide (H_2O_2) in to sample cuvettes and change in absorbance was measured every 30 sec up to 3 min. One unit of peroxidase is defined as the change in absorbance/min at 420 nm and it is expressed as activity g^{-1} min^{-1}.

3.2.2.3.3 Superoxide Dismutase (SOD)

Superoxide dismutase activity was measured by the method described by Beauchamp and Fridovich (1971). Grind 1 g of clean tissue in 10 ml ice cold 50 mM potassium phosphate buffer, pH 7.8 in a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was used for assay. Mix a 3 ml reaction mixture containing 50 mM potassium phosphate buffer, 13 mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 75 µM NBT (Nitroblue tetrazolium) and µl of crude enzyme extract, in duplicate. Make up the volume equal by adding double distilled water. Set a blank without enzyme and NBT to calibrate the spectrophotometer (ELICO-SL 218 Double Beam). Set another control having NBT but no enzymes as reference control. Expose all the tubes to 400 W bulb for 15 min. Read the absorbance immediately at 560 nm. Calculate the percentage inhibition. The 50 per cent inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity and it is expressed as activity g^{-1} min^{-1}.

3.2.2.4 Curcumin content

Dissolve 0.2 g of powdered dry rhizome of *C. aromatica* in 250 ml of absolute ethanol. Reflux the contents in the flask fitted with an air-condenser over a heating mantle for 3-5 h, compensate alcohol loss if any due to evaporation by adding alcohol freshly in to the flask. Cool and decant the extract into a volumetric flask and make up the volume. Dilute a suitable aliquot (1 ml) to 10 ml with absolute alcohol. Measure the intensity of yellow colour at 425 nm in a spectrophotometer (Sadasivam and Manickam, 2008).

$$\text{Percentage of curcumin} = \frac{0.0025 \times A_{425} \times \text{volume made up} \times \text{dilution factor} \times 100}{0.42 \times \text{weight of sample (g)} \times 1000}$$

Since 0.42 absorbance at 425 nm = 0.0025g curcumin

3.2.2.5 Volatile oil

Coarsely ground powder of dried rhizome was used for estimating volatile oil. The method adopted was hydro distillation using Clevenger distillation apparatus for 3-4 h. The oil content was expressed in percentage (v/w) on dry weight basis.

Percentage of volatile oil (v/w) = (Volume of oil (ml)/ Weight of sample (g))*100

3.2.2.6 Oleoresin

Finely ground powder of dried rhizome was used for estimating oleoresin. The method adopted was solvent extraction using Soxhlet apparatus for 1 h. The content was expressed in percentage (v/w) on dry weight basis.

Percentage of oleoresin (v/w) = (Volume of extract (ml)/Weight of sample (g))*100

3.2.2.7 Carbohydrate

The carbohydrate content present in the rhizome of *C. aromatica* was estimated by using the procedure given by Hedge and Hofreiter (1962). 100 mg of fresh rhizome were weighed and hydrolysed with 5 ml of 2.5 N HCl in a boiling water bath. The hydrolysate was then neutralized with Na_2CO_3 until the effervescence ceased. The volume was made upto 100 ml and centrifuge the contents at 5000 rpm for 15 min. From the supernatant, 0.5 ml aliquot was taken and made up the volume to 1 ml using distilled water. Anthrone reagent (4 ml) was added to this and boiled in a water bath for 8 min. After cooling, the absorbance was measured using a spectrophotometer at 630 nm. From the standard graph amount of carbohydrate was estimated. It was expressed in mg g^{-1} on fresh weight basis.

3.2.2.7.1 Starch

Starch content in rhizome was estimated using Anthrone method (Mc Cready *et al.,* 1950). Plant sample of 0.1 g was taken and homogenized in hot 80

per cent ethanol. Centrifuged the homogenate and the residue was retained. Then it was washed repeatedly with hot 80 per cent ethanol till the washing does not give any colour with anthrone reagent. The residue was then dried well over a water bath. Added 5 ml water and 6.5 ml, 52 per cent perchloric acid to the dried residue and mixed well and it was extracted at 0°C for 20 min. The solution was centrifuged and saved the supernatant. The extraction was repeated using fresh perchloric acid. The supernatant was pooled after centrifugation and made up to 100 ml.

An aliquot of 0.1 ml of the supernatant was taken and 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 test tube using distilled water. Added 4 ml of anthrone reagent to each test tubes. These were heated in a water bath for 8 min and cooled rapidly. The intensity of colour change from green to dark green was measured at 630 nm. The glucose content was calculated using the standard curve. Starch content of the sample was obtained by multiplying the value by a factor of 0.9 and it was expressed in mg g^{-1}.

3.2.2.7.2 Reducing sugar

Reducing sugar content in plant sample was determined by using Dinitro Salicylic acid (DNS) method (Somogyi, 1952). Rhizome weighing 0.1 g was taken and the sugars were extracted with hot 80 per cent ethanol, twice. The supernatant was collected after centrifugation and boiled it on water bath at 80°C till whole alcohol was evaporated. 10 ml of water was added to it for dissolving the sugar. One ml of the sample was pipetted out in to test tubes and made up the volume to 3 ml using distilled water. 3 ml of DNS reagent was added and it was then heated in a boiling water bath for 5 min. Rochelle salt solution (40 per cent, w/v) 1 ml was added to the test tubes when the contents were hot. After cooling the intensity of dark red colour was measured using UV spectrophotometer at 510 nm. The amount of reducing sugar in the sample was calculated using a standard graph prepared from working standard glucose solution and it was expressed in mg g^{-1}.

3.2.3 Physiological parameters

The observation on dry matter production was recorded at 4 MAP, 6 MAP and at harvest. The observation on leaf area index, net assimilation rate, stomatal conductance, photosynthetic rate, proline content and cell membrane integrity were recorded at 4 and 6 MAP.

3.2.3.1 Dry matter production

The shoot, rhizomes and roots of the uprooted plants were separated and dried to a constant weight at 70°C in a hot air oven. The sum of dry weights of component parts gave the total dry matter production of the plant and expressed in g plant^{-1}.

3.2.3.2 Leaf area index

Maximum length and width of leaves from all the sample plants were recorded separately and leaf area was calculated based on length and breadth method.

$$LAI = \frac{\text{Sum of leaf area of N samples (cm}^2\text{)}}{\text{Area of land covered by N plants (cm}^2\text{)}}$$

3.2.3.3 Net assimilation rate (NAR)

Net assimilation rate was calculated as per the procedure described by Williams (1946) on plant dry weight basis. NAR was calculated using the formula given below and expressed in g m^2 day^{-1}.

$$NAR = \frac{(W_2 - W_1) \times (\log_e A_2 - \log_e A_1)}{(t_2 - t_1) \times (A_2 - A_1)}$$

Where,

W_2 – total dry weight of the plant in g at time t_2

W_1 – total dry weight of the plant in g at time t_1

$(t_2 - t_1)$ – time interval in days

A_2 – leaf area (m^2) at time t_2

A_1 – leaf area (m^2) at time t_1

3.2.3.4 Stomatal conductance

Stomatal conductance was measured at morning time between 8.30 am and 11 am using a portable photosynthetic system (Model: CIRAS-3 Ver. 1.06, Amesbury, U.S.A) and was expressed in mmoles m^{-2} s^{-1}.

3.2.3.5 Photosynthetic rate

Photosynthetic rate was measured at morning time between 8.30 am and 11 am using a portable photosynthetic system (Model: CIRAS-3 Ver. 1.06, Amesbury, U.S.A) and was expressed in µmoles CO_2 m^{-2} s^{-1}.

3.2.3.6 Proline content

Proline content was estimated as per the procedure described by Bates *et al.* (1973). A known amount (0.5g) of mid-leaf portion was homogenized with 10ml of 3 per cent aqueous sulphosalicylic acid and centrifuged at 3000 rpm for 15 min. 2ml of the supernatant was taken and mixed with an equal amount of glacial acetic acid and acid ninhydrin. The contents were allowed to react at 1000°C for one hour in water bath. The reaction was terminated by keeping it in ice bath for 10 min. The reaction mixture was mixed with 4ml toluene using vortex mixture for 15 – 20 sec. The chromophore containing toluene was aspirated from aqueous phase, warmed to room temperature and the optical density was read at 520nm with toluene as blank. A standard curve was drawn using concentration verses absorbance.

The concentration of proline was determined from graph and expressed as follows

µ moles g^{-1} tissue = {[(µg proline / ml) x ml toluene] / 115.5} x (5 / g sample), where 115.5 is the molecular weight of proline.

3.2.3.7 Cell membrane integrity

Cell membrane integrity was estimated as per the procedure described by Blum and Ebercon (1981). Leaf samples collected from both control and different treatments were washed three times in deionized water to remove electrolytes adhered on the surface. Samples were kept in a capped vial (20 ml) containing 10 ml of deionized water and incubated in the dark for 24 h 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 treated plants. Cell membrane stability index was calculated by using following formula and expressed as per cent.

$$\text{CMS (per cent)} = [\ 1 - (T_1/\ T_2)\ /\ 1 - (C_1/\ C_2)] \times 100$$

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.

3.2.4 Yield and yield components

3.2.4.1 Fresh rhizome yield

The fresh rhizome yield of observational plants from each treatment was recorded at the time of harvest and the mean was expressed in g plant^{-1}.

3.2.4.2 Dry rhizome yield

The fresh rhizomes were dried at 70°C in hot air oven till constant weight was obtained and the mean was expressed in g plant^{-1}.

3.2.4.3 Crop duration

The number of days taken from planting to harvest was recorded for each treatment.

3.2.4.4 Harvest Index

Harvest Index was calculated during harvest as the ratio of economic yield to the biological yield.

$$HI = \frac{\text{Economic yield}}{\text{Biological yield}}$$

Where,

Economic yield – total dry weight of rhizome

Biological yield – total dry weight of whole plant

3.2.5 Uptake of major nutrients (N, P and K)

The estimation of major plant nutrients such as N, P and K was done as per the procedure given by Jackson (1973). Nitrogen was estimated by Microkjeldahl method. For the analysis of P and K, diacid extracts were prepared by digesting 1 g of the sample in 15 ml of 2:1 concentrated nitric perchloric acid mixture. Aliquots of digests were taken for the analysis of total P and K. P was determined calorimetrically by Vanadomolybdo phosphoric yellow colour method. The yellow colour was read in a spectro photometer at a wavelength of 470 nm. K was estimated using flame photometer. The contents were calculated and expressed in percentage. The uptake of N, P and K contents by the plant was calculated by the nutrient contents of the plant with respective dry weight of the plant parts and expressed as g plant^{-1}.

3.3 Statistical analysis

Statistical analysis was done using OPSTAT developed by Haryana Agriculture University, a web based agriculture statistical package available at www.hau.ac.in

Results

4. RESULTS

The study entitled "Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2017-2019. The data collected from the experiment were statistically analysed and the results are presented in this chapter.

4.1 PLANT GROWTH PARAMETERS

The different concentrations of chitosan (0.5, 1, 1.5, 2, 2.5 and 3 g l^{-1}) were sprayed on kasturi turmeric plants at two different periods of crop growth *viz.*, 3 and 5 months after planting (MAP). The observations on plant growth parameters viz., plant height, number of tillers, number of leaves, leaf area, rhizome spread, rhizome thickness, number of fingers, root length, root spread, root weight and shoot weight were recorded at one month after each spraying *i.e.* 4 and 6 MAP. The plants at different stages of growth are presented in Plate 3.

4.1.1 Plant height (cm)

The effect of different concentrations of chitosan on plant height, recorded at 4 and 6 MAP are presented in Table 2. At 4 MAP, no significant variation was observed among the various chitosan treatments. However, Chitosan @ 2.5 g l^{-1} (T_5) recorded the highest plant height (101.37 cm) and the control (T_9) recorded the lowest height (87.17cm).

At 6 MAP, the plant height showed significant variation among the treatments. Chitosan @ 3 g l^{-1} (T_6) recorded a plant height of 109.91 cm, which was on par with all other treatments with chitosan (T_1, T_2, T_3, T_4 and T_5). The acetic acid spray (T_7) recorded the lowest plant height of 90.56 cm which was on par with water spray (T_8) and control (T_9). Chitosan treatments had significant influence on plant height at 6 MAP compared to those treatments devoid of it.

Plate 3. *C. aromatica* plants at different stages of growth A) 1 MAP B) 3 MAP C) 5 MAP D) at harvest

4.1.2 Number of tillers

The effect of foliar spray of chitosan on number of tillers at 4 and 6 MAP are presented in Table 2. Number of tillers did not show any significant variation at 4 and 6 MAP, among the various treatments tried. This indicated that the chitosan treatments did not have any influence on the tiller production in kasthuri turmeric

4.1.3 Number of leaves

Table 3 represents the data on the effect of chitosan spray at different concentrations on number of leaves in kasturi turmeric. The number of leaves in plant differed significantly due to chitosan treatments at plant growth stages, 4 and 6 MAP. At 4 MAP, the highest number of leaves (12.53) was recorded in the treatment with chitosan 3 g l^{-1} (T_6) which was on par with chitosan 1.5 g l^{-1} (T_3) and chitosan 2.5 g l^{-1} (T_5). The lowest number of leaves (8.63) was observed in the control treatment (T_9).

At 6 MAP, T_5 recorded highest value in number of leaves (30.89) which was on par with T_3, T_4 and T_6. T_7 recorded least value (22.78) which was found to be on par with T_1, T_8 and T_9.

4.1.4 Leaf area

The leaf area of Kasturi turmeric at 4 and 6 MAP as influenced by foliar spray of chitosan is presented in Table 4. At 4 MAP, there was significant increase in the leaf area (8593.78 cm^2, 7894.82 cm^2) in treatments chitosan @ 2.5 g l^{-1} (T_5), chitosan @ 3 g l^{-1} (T_6) respectively compared to other treatments. The lowest value was recorded in control plants T_9 (4653.30 cm^2).

At 6 MAP, maximum leaf area was recorded in T_5 (21735.79 cm^2), which was on par with all chitosan treatments except T_1. The control plants recorded the lowest leaf area (13441.22 cm^2), which was on par with T_1, T_7 and T_8. The data indicates that chitosan foliar spray, irrespective of concentration could significantly increase the leaf area in *C. aromatica*.

Table 2. Effect of foliar spray treatments on plant height (cm) and number of tillers in *C. aromatica*

Treatments	Plant height (cm)		No of Tillers	
	4 MAP	6 MAP	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	93.33±2.95	99.06±3.74	1.63±0.20	4.22±0.11
T_2 (Chitosan 1.0 g l^{-1})	97.03±3.91	103.00±3.08	2.07±0.29	5.33±0.51
T_3 (Chitosan 1.5 g l^{-1})	92.20±4.00	106.00±9.90	2.40±0.10	6.00±0.51
T_4 (Chitosan 2.0 g l^{-1})	90.83±2.95	104.57±2.59	1.97±0.20	5.56±0.68
T_5 (Chitosan 2.5 g l^{-1})	101.37±1.54	105.67±2.10	2.83±0.23	6.22±0.11
T_6 (Chitosan 3.0 g l^{-1})	97.37±1.78	109.91±0.75	2.87±0.30	6.00±0.51
T_7 (Acetic acid spray)	94.67±1.26	90.56±2.23	2.53±0.29	5.00±0.67
T_8 (Water spray)	92.60±4.80	92.78±1.75	1.97±0.52	5.11±0.29
T_9 (Control)	87.17±3.39	92.00±1.95	2.63±0.38	5.11±0.29
SEm(±)	3.17	4.08	0.30	0.46
CD (0.05)	NS	12.208	NS	NS

Table 3. Effect of foliar spray treatments on number of leaves in *C. aromatica*

Treatments	Number of leaves	
	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	8.8±0.36	24.22±1.49
T_2 (Chitosan 1.0 g l^{-1})	8.73±0.94	26.67±2.84
T_3 (Chitosan 1.5 g l^{-1})	10.30±0.51	30.33±2.22
T_4 (Chitosan 2.0 g l^{-1})	10.20±1.40	29.00±3.21
T_5 (Chitosan 2.5 g l^{-1})	12.40±0.20	30.89±0.11
T_6 (Chitosan 3.0 g l^{-1})	12.53±0.62	30.11±2.00
T_7 (Acetic acid spray)	9.23±0.31	22.78±1.42
T_8 (Water spray)	8.67±1.20	23.11±0.29
T_9 (Control)	8.63±0.20	23.11±0.59
SEm(±)	0.76	1.89
CD (0.05)	2.290	5.602

Table 4. Effect of foliar spray treatments on leaf area (cm^2) in *C. aromatica*

Treatments	Leaf area (cm^2)	
	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	5085.31±143.60	16008.45±699.39
T_2 (Chitosan 1.0 g l^{-1})	5194.35±659.75	18059.41±1832.17
T_3 (Chitosan 1.5 g l^{-1})	5559.74±392.95	20148.60±1453.94
T_4 (Chitosan 2.0 g l^{-1})	5682.54±1038.90	18811.16±1648.61
T_5 (Chitosan 2.5 g l^{-1})	8593.78±542.77	21735.79±226.01
T_6 (Chitosan 3.0 g l^{-1})	7894.82±304.67	20886.06±1683.53
T_7 (Acetic acid spray)	5379.86±47.13	14270.72±1457.38
T_8 (Water spray)	4961.70±657.30	14103.90±296.23
T_9 (Control)	4653.30±281.04	13441.22±739.27
SEm(±)	536.48	1261.84
CD (0.05)	1606.318	3778.166

4.1.5 Rhizome spread (cm)

There was no significant difference found in the rhizome spread of *C. aromatic* in response to various foliar spray treatments at 4 and 6 MAP. However, significant variation was observed at harvest among the treatments. The data is illustrated in Table 5. At harvest, the plants sprayed with chitosan 3 g l^{-1} (T_6) recorded significantly higher value (29.50 cm) among the treatments. This was on par with chitosan 2.5 g l^{-1} (T_5) and the value recorded was 29 cm. The lowest rhizome spread (23.83 cm) was noticed in acetic acid spray (T_7) and this was found on par with all other treatments except T_5 and T_6.

4.1.6 Rhizome thickness (cm)

The influence of various foliar spray treatments on rhizome thickness is illustrated in Table 5. At 4 MAP, no significant difference in rhizome thickness was observed among the treatments. However, significant variation was observed at 6 MAP and at harvest. At 6 MAP, chitosan 2.5 g l^{-1} (T_5) showed maximum rhizome thickness (2.46 cm) which was on par with T_2, T_3, T_4 and T_6. The least rhizome thickness (1.73 cm) was recorded in T_7, which was on par with T_1, T_8 and T_9. The same trend followed at harvest stage also. The highest value (3.17 cm) was recorded in T_5. This was observed to be on par with T_6 (3.09 cm). T_7 registered minimum value (1.99 cm) for rhizome thickness and was on par with T_1, T_2, T_8 and T_9.

4.1.7 Number of fingers

The result on the effect of various foliar spray treatments on number of fingers of *C. aromatica* is shown in Table 6. From the table it is clear that chitosan foliar spray at higher concentration had significant effect on number of fingers. At all stages of observation (4 MAP, 6 MAP and at harvest), T_6 showed maximum number of fingers (14.39, 22.05 and 22.46 respectively), which was on par with T_5 (12.81, 20.93, 21.56 respectively). The lowest values (7.62 and 13.81) was recorded in T_8 at 4 MAP and at harvest, respectively. This was on par with T_1, T_2,

Table 5. Effect of foliar spray treatments on rhizome spread (cm) and rhizome thickness (cm) in *C. aromatica*

Treatments	Rhizome spread (cm)			Rhizome thickness (cm)		
	4 MAP	6 MAP	At harvest	4 MAP	6 MAP	At harvest
T_1 (Chitosan 0.5 g l^{-1})	12.83±0.73	25.33±0.67	25.92±1.02	1.19±0.03	1.93±0.13	2.43±0.06
T_2 (Chitosan 1.0 g l^{-1})	13.50±0.76	25.4±1.55	26.18±0.35	1.20±0.05	2.11±0.15	2.40±0.41
T_3 (Chitosan 1.5 g l^{-1})	13.67±1.45	22.77±1.30	26.41±0.37	1.28±0.10	2.14±0.11	2.86±0.25
T_4 (Chitosan 2.0 g l^{-1})	14.83±0.17	23.83±2.13	26.00±1.15	1.06±0.08	2.34±0.20	2.96±0.17
T_5 (Chitosan 2.5 g l^{-1})	17.00±1.00	27.50±2.18	29.00±0.58	1.20±0.09	2.46±0.05	3.17±0.19
T_6 (Chitosan 3.0 g l^{-1})	16.33±1.30	27.43±0.31	29.50±1.32	1.15±0.04	2.27±0.15	3.09±0.18
T_7 (Acetic acid spray)	14.17±2.52	22.10±1.81	23.83±0.73	0.88±0.14	1.73±0.12	1.99±0.12
T_8 (Water spray)	16.00±1.53	23.37±2.20	24.67±0.67	1.12±0.19	1.91±0.12	2.22±0.23
T_9 (Control)	14.00±1.73	22.50±0.87	24.17±1.48	0.94±0.07	1.87±0.20	2.00±0.28
SEm(±)	1.40	1.59	0.94	0.10	0.14	0.23
CD (0.05)	NS	NS	2.803	NS	0.435	0.694

Table 6. Effect of foliar spray treatments on number of fingers in *C. aromatica*

Treatments	Number of fingers		
	4 MAP	6 MAP	At harvest
T_1 (Chitosan 0.5 g l^{-1})	7.90±0.88	13.78±0.98	15.19±0.47
T_2 (Chitosan 1.0 g l^{-1})	8.89±0.87	14.69±1.10	15.64±0.89
T_3 (Chitosan 1.5 g l^{-1})	10.79±0.97	15.92±0.44	17.03±0.18
T_4 (Chitosan 2.0 g l^{-1})	10.38±1.25	19.51±1.12	19.74±0.61
T_5 (Chitosan 2.5 g l^{-1})	12.81±1.37	20.93±0.6	21.56±0.63
T_6 (Chitosan 3.0 g l^{-1})	14.39±0.43	22.05±1.17	22.46±1.18
T_7 (Acetic acid spray)	8.22±1.02	14.34±0.53	15.30±0.53
T_8 (Water spray)	7.62±0.47	13.05±1.25	13.81±0.74
T_9 (Control)	8.57±0.59	12.77±0.81	14.40±0.75
SEm(±)	0.93	0.96	0.72
CD (0.05)	2.758	2.848	2.145

T_7 and T_9. At 6 MAP, T_9 recorded the lowest value (12.77) which was on par with T_1, T_2, T_7 and T_8.

4.1.8 Root length (cm)

Table 7 illustrates the values of root length of *C. aromatica* with response to various foliar spray treatments. At 4 MAP, there was no significant difference in root length among the treatments. Plants which were sprayed with chitosan at 2.5 g l^{-1} (T_5) recorded maximum root length (52 cm) followed by those sprayed with chitosan at 3 g l^{-1} (T_6) which gave a root length of 44 cm.

At 6 MAP and at harvest, significant variation was observed in root length among the various foliar spray treatments. At both stages, the treatments T_5 and T_6 produced significantly higher root length. At 6 MAP, the highest value (56.67 cm) was recorded in chitosan 2.5 g l^{-1} (T_5) which was on par with T_6 which recorded a root length of 55.67cm. Water sprayed treatment (T_8) recorded the lowest value (33 cm) which was on par with T_7 and T_9 with values 34.33 cm and 35.67 cm. At harvest, the highest root length (56.76 cm) recorded in T_6 which was significantly superior and found to be on par with T_5. This was followed by T_4 and T_3 with values 49.17 cm and 46.43 cm. The control plants (T_9) recorded the least value for root length (38.63 cm) and was on par with T_7 (40.55 cm) and T_8 (38.80 cm).

4.1.9 Root spread

Table 7 represents the effect of different foliar spray treatments on root spread of the *C. aromatica* plants at different periods of observation. No significant variation in root spread was observed at 4 MAP. At 6 MAP, chitosan 3 g l^{-1} (T_6) showed maximum root spread (48.33 cm) which was on par with T_5 (47 cm). T_4 recorded the lowest value (32.67 cm) which was on par with all other foliar spray treatments except T_5 and T_6.

At the time of harvest, T_5 was found to give maximum root spread (49.10 cm) among the various foliar spray treatments and was on par with T_6 (48.53 cm). The lowest value (33.72 cm) found in T_8, which was on par with T_1, T_2, T_7 and T_9.

Table 7. Effect of foliar spray treatments on root length (cm) and root spread (cm) in *C. aromatica*

Treatments	Root length (cm)			Root spread (cm)		
	4 MAP	6 MAP	At harvest	4 MAP	6 MAP	At harvest
T_1 (Chitosan 0.5 g l^{-1})	41.00±2.89	40.33±2.40	45.74±0.94	38.00±2.65	35.33±1.20	37.02±1.28
T_2 (Chitosan 1.0 g l^{-1})	42.33±7.42	42.33±2.03	43.31±1.10	45.67±5.36	33.17±1.59	37.20±1.23
T_3 (Chitosan 1.5 g l^{-1})	43.00±7.51	44.33±2.03	46.43±1.37	40.00±4.36	35.33±2.40	38.78±1.15
T_4 (Chitosan 2.0 g l^{-1})	41.50±2.36	44.00±2.89	49.17±0.44	41.33±5.24	32.67±1.20	38.72±0.81
T_5 (Chitosan 2.5 g l^{-1})	52.00±7.00	56.67±2.33	55.47±1.01	37.33±4.37	47.00±2.08	49.10±1.47
T_6 (Chitosan 3.0 g l^{-1})	44.00±7.37	55.67±2.19	56.76±0.65	37.67±4.98	48.33±0.67	48.53±1.63
T_7 (Acetic acid spray)	38.67±4.98	34.33±1.76	40.55±0.63	42.00±2.08	35.33±2.33	36.17±0.98
T_8 (Water spray)	43.83±5.85	33.00±1.53	38.80±1.69	39.67±5.81	34.00±6.43	33.72±1.90
T_9 (Control)	39.33±2.33	35.67±2.03	38.63±1.13	34.00±2.08	36.67±2.03	37.50±0.76
SEm(±)	5.71	2.16	1.06	4.33	2.73	1.29
CD (0.05)	NS	6.477	3.177	NS	8.161	3.881

4.1.10 Root weight

The data on the influence of different foliar spray treatments on root (fresh and dry) weight at 3 different periods (4 MAP, 6 MAP and at harvest) of observation is presented in Table 8. There was no significant difference in both fresh and dry weight of root at 4 MAP.

At 6 MAP there was a significant difference in root weight (fresh and dry) observed among the treatments. T_6 recorded the highest root weight (31.29 g plant^{-1} and 15 g plant^{-1}) which was statistically on par with T_2, T_3, T_4 and T_5. The control plants recorded the lowest root weight with a fresh weight of 14.99 g plant^{-1} and dry weight of 7.18 g plant^{-1}.

At harvest, no significant difference was found among the treatments for both fresh and dry weight of root.

4.1.11 Shoot weight

The results of shoot weight of the plants at 2 different growth periods, 4 and 6 MAP are presented in Table 9. Among the various foliar spray treatments, significant variation was observed in shoot weight at both the periods. At 4 MAP, chitosan 2.5 g l^{-1} (T_5) produced maximum shoot weight in both fresh (75.33 g plant^{-1}) and dry (42.69 g plant^{-1}) stages. This was found on par with chitosan 2 g l^{-1} (T_4) and 3 g l^{-1} (T6). The fresh (65.98 g plant^{-1} and 72.94 g plant^{-1}) and dry (36.03 g plant^{-1} and 40.53 g plant^{-1}) weights were recorded in T_4 and T_6, respectively. The lowest values of fresh (54.55 g plant^{-1}) and dry (26.33 g plant^{-1}) weights were obtained with acetic acid spray (T_7). This was found to be on par with all the treatments except T_4, T_5 and T_6.

At six month after planting, plants treated with chitosan at 3 g l^{-1} (T_6) recorded significantly superior fresh shoot weight (126.33 g plant^{-1}) which was observed to be on par with T_3 (113.92 g plant^{-1}) and T_5 (125.56 g plant^{-1}). The dry weight of shoots was also the highest in T_6 (63 g plant^{-1}) which was found on par with T_5 (61g plant^{-1}). The shoot weight, both fresh (89.82 g plant^{-1}) and dry (39.17 g plant^{-1}) weight was found to be the lowest in T_7.

Table 8. Effect of foliar spray treatments on root weight (g $plant^{-1}$) in *C. aromatica*

Treatments	Fresh weight (g $plant^{-1}$)			Dry weight (g $plant^{-1}$)		
	4 MAP	**6 MAP**	**At harvest**	**4 MAP**	**6 MAP**	**At harvest**
T_1 (Chitosan 0.5 g l^{-1})	15.67±3.28	20.92±0.72	26.67±2.19	6.12±0.94	7.89±0.52	13.00±0.66
T_2 (Chitosan 1.0 g l^{-1})	21.33±1.45	30.00±1.15	29.67±1.20	7.89±0.29	12.03±0.64	12.99±0.38
T_3 (Chitosan 1.5 g l^{-1})	14.50±4.01	28.22±1.54	29.00±3.21	6.31±1.19	11.77±0.96	13.49±0.78
T_4 (Chitosan 2.0 g l^{-1})	16.83±4.69	30.56±2.95	30.33±2.73	6.55±1.43	14.05±1.55	14.71±1.68
T_5 (Chitosan 2.5 g l^{-1})	15.33±1.76	27.45±1.04	33.00±1.15	6.06±0.71	13.31±0.23	15.36±1.35
T_6 (Chitosan 3.0 g l^{-1})	21.33±1.20	31.29±0.88	31.00±1.15	8.56±0.52	15.00±0.57	14.33±1.26
T_7 (Acetic acid spray)	14.17±1.01	23.67±1.45	30.00±2.31	5.27±0.42	9.99±0.32	14.47±0.75
T_8 (Water spray)	13.17±3.92	22.12±1.74	28.67±3.93	6.37±1.93	9.71±1.30	13.62±1.25
T_9 (Control)	10.00±1.53	14.99±1.52	23.67±3.18	4.70±0.38	7.18±0.47	11.98±1.09
SEm(±)	2.87	1.57	2.53	1.01	0.84	1.09
CD (0.05)	NS	4.706	NS	NS	2.521	NS

Table 9. Effect of foliar spray treatments on shoot weight (g $plant^{-1}$) in *C. aromatica*

Treatments	Fresh weight (g $plant^{-1}$)		Dry weight (g $plant^{-1}$)	
	4 MAP	6 MAP	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	63.00±2.08	102.67±2.91	32.72±2.42	44.00±1.73
T_2 (Chitosan 1.0 g l^{-1})	62.00±4.36	108.00±8.72	31.08±3.69	45.83±1.88
T_3 (Chitosan 1.5 g l^{-1})	62.67±4.05	113.92±4.95	31.97±3.58	49.67±1.20
T_4 (Chitosan 2.0 g l^{-1})	65.98±7.18	107.33±7.26	36.03±5.31	45.00±4.73
T_5 (Chitosan 2.5 g l^{-1})	75.33±2.10	125.56±5.72	42.69±1.94	61.00±2.65
T_6 (Chitosan 3.0 g l^{-1})	72.94±5.92	126.33±9.21	40.53±4.23	63.00±5.57
T_7 (Acetic acid spray)	54.55±1.82	89.82±2.15	26.33±2.19	39.17±1.30
T_8 (Water spray)	57.55±1.28	92.45±5.60	28.18±1.32	43.00±2.31
T_9 (Control)	62.22±2.70	100.00±2.65	29.62±2.07	44.00±2.89
SEm(±)	4.01	5.75	3.21	3.05
CD (0.05)	11.995	17.218	9.624	9.129

4.2 METABOLITE PRODUCTION

4.2.1 Chlorophyll content

The data presented in the Table 10 showed the results of chlorophyll a, b and total chlorophyll content of the plants at 4 and 6 MAP. The foliar spray treatments did not show any significant variation in chlorophyll content (chlorophyll a, b and total chlorophyll) at 4 MAP.

However, at 6 MAP, the treatments varied significantly with respect to chlorophyll content. Chitosan 2.5 g l^{-1} (T_5) recorded significantly higher chlorophyll a content (1.397 mg g^{-1}) compared to all other treatments while acetic acid spray treatment recorded 0.809 mg g^{-1} which was the lowest. For chlorophyll b, chitosan 3 g l^{-1} (T_6) registered significantly higher value (0.615 mg g^{-1}) which was on par with T_5 and T_4. However treatment T_7 recorded the lowest value (0.313 mg g^{-1}). Treatment T_5 gave significantly higher value of total chlorophyll content (1.959 mg g^{-1}) and was on par with T_4 and T_6. The least value (1.123 mg g^{-1}) of total chlorophyll content was observed in the T_7.

4.2.2 Total Proteins

The effect of different foliar spray treatments on *Curcuma aromatica* plants at 4 and 6 MAP is shown in Table 11. Significant difference existed in the total protein content, among the treatments at both the periods of observation. The highest content of total protein exhibited by chitosan 2.5 g l^{-1} (T_5) at 4 MAP with a mean value of 6.89 mg g^{-1} and was significantly superior compared to all other treatments. Treatment chitosan 3 g l^{-1} (T_6) held the next highest value (5.97 mgg^{-1}) and found to be on par with chitosan 2 g l^{-1} (T_4) which is having a mean value of total protein content 5.35 mg g^{-1}.The foliar spray with acetic acid (T_7) recorded the lowest value (2.93 mg g^{-1}) among the treatments which was found to be on par with T_8 (3.14 mg g^{-1}) and T_9 (3.75 mg g^{-1})

During 6 MAP, treatment T_6 recorded maximum mean value for total protein content (8.46 mg g^{-1}) and was found to be on par with T_5 (8.35 mg g^{-1}) and

Table 10. Effect of foliar spray treatments on chlorophyll content (mg g^{-1}) in *C. aromatica*

Treatments	Chlorophyll a (mg g^{-1})		Chlorophyll b (mg g^{-1})		Total chlorophyll (mg g^{-1})	
	4 MAP	6 MAP	4 MAP	6 MAP	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	0.719±0.098	0.984±0.033	0.607±0.122	0.395±0.018	1.327±0.215	1.378±0.023
T_2 (Chitosan 1.0 g l^{-1})	0.813±0.017	1.089±0.194	0.741±0.044	0.433±0.072	1.553±0.033	1.522±0.267
T_3 (Chitosan 1.5 g l^{-1})	0.886±0.119	1.036±0.062	0.661±0.078	0.437±0.065	1.547±0.173	1.472±0.117
T_4 (Chitosan 2.0 g l^{-1})	0.823±0.103	1.071±0.173	0.750±0.089	0.500±0.099	1.573±0.192	1.571±0.271
T_5 (Chitosan 2.5 g l^{-1})	1.005±0.025	1.397±0.050	0.882±0.024	0.562±0.011	1.887±0.047	1.959±0.040
T_6 (Chitosan 3.0 g l^{-1})	1.014±0.041	1.098±0.023	0.862±0.066	0.615±0.035	1.877±0.105	1.713±0.019
T_7 (Acetic acid spray)	0.650±0.043	0.809±0.038	0.517±0.030	0.313±0.009	1.167±0.073	1.123±0.031
T_8 (Water spray)	0.839±0.130	0.865±0.070	0.771±0.133	0.360±0.021	1.610±0.260	1.225±0.054
T_9 (Control)	0.806±0.084	0.819±0.038	0.701±0.056	0.316±0.048	1.507±0.140	1.135±0.086
SEm(±)	0.083	0.096	0.080	0.051	0.156	0.138
CD (0.05)	NS	0.288	NS	0.154	NS	0.414

T_4 (8.24 mg g^{-1}). Control treatment (T_9) recorded the least value (5.72 mg g^{-1}) which was on par with T_8 (6.14 mg g^{-1}) and T_7 (6.16 mg g^{-1}).

4.2.3 Defense enzymes

The results indicated that the application of chitosan had significantly influenced the enzyme activity in the crop.

4.2.3.1 Catalase

The data on the influence of different foliar spray treatments on enzyme activity in *C. aromatica*, at 4 and 6 MAP is depicted in Table 12. Catalase enzyme activity showed significant variation among the treatments tried. At 4 MAP, chitosan 3 g l^{-1} (T_6) gives highest value (685.580 U ml^{-1}) of catalase enzyme activity. This was found to be on par with T_5 (675.367 U ml^{-1}). But least enzyme activity was noticed in T_7 (451.830 U ml^{-1}), which was on par with control (458.867 U ml^{-1}). At 6 MAP also, T_6 recorded significantly superior value (883.360). This was on par with T_5 (838.690 U ml^{-1}) and T_4 (811.127 U ml^{-1}) The lowest catalase enzyme activity (669.320 U ml^{-1}) was observed in T_9, which was on par with all other treatments except T_4, T_5 and T_6. This indicated that foliar spray of chitosan at lower concentrations (0.5, 1 and 1.5 g l^{-1}) did not improve the catalase activity.

4.2.3.2 Peroxidase

The results obtained for the peroxidase enzyme activity in *C. aromatica* at four and six month after planting on different foliar spray treatments are illustrated in Table 12. The data described significant enhancement in peroxidase activity at both periods of observation. Chitosan 2.5 g l^{-1} (T_5) recorded maximum peroxidase activity (4.308 activity g^{-1} min^{-1}) at 4 MAP. This was observed to be on par with T_6 (4.300 activity g^{-1} min^{-1}). This was followed by treatment chitosan 2 g l^{-1} (T_4) and chitosan 2.5 g l^{-1} (T_3) which were recorded 3.054 activity g^{-1} min^{-1} and 2.474 activity g^{-1} min^{-1} respectively. The least peroxidase activity (0.791

Table 11. Effect of foliar spray treatments on total protein content (mg g^{-1}) in *C. aromatica*

Treatments	Total protein content (mg g^{-1})	
	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	4.76±0.27	6.21±0.18
T_2 (Chitosan 1.0 g l^{-1})	4.74±0.23	6.15±0.16
T_3 (Chitosan 1.5 g l^{-1})	4.65±0.38	7.12±0.18
T_4 (Chitosan 2.0 g l^{-1})	5.35±0.40	8.24±0.11
T_5 (Chitosan 2.5 g l^{-1})	6.89±0.22	8.35±0.35
T_6 (Chitosan 3.0 g l^{-1})	5.97±0.11	8.46±0.26
T_7 (Acetic acid spray)	2.93±0.15	6.16±0.30
T_8 (Water spray)	3.14±0.23	6.14±0.39
T_9 (Control)	3.75±0.33	5.72±0.35
SEm(±)	0.27	0.27
CD (0.05)	0.822	0.813

activity g^{-1} min^{-1}) was recorded in control treatment (T_9), which was on par with T_1, T_2, T_7 and T_8.

At 6 MAP, maximum peroxidase activity (5.344 activity g^{-1} min^{-1}) was recorded in the treatment T_6, which was on par with T_5 (5.063 activity g^{-1} min^{-1}). The lowest peroxidase activity (1.503 activity g^{-1} min^{-1}) was recorded in T_8, which was on par with all other treatments except T_5 and T_6. This indicated that foliar spray of chitosan at lower concentrations (0.5, 1, 1.5 and 2.0 g l^{-1}) did not influence the peroxidase activity.

4.2.3.3 Superoxide dismutase (SOD)

The response of superoxide dismutase activity to different foliar spray treatments in *C. aromatica* were analaysed at 4 and 6 MAP and the results are presented in Table 12. At 4 MAP, Chitosan 3 g l^{-1} (T_6) was found to be significantly superior in superoxide dismutase activity, among the treatments. The activity recorded was 0.140 activity g^{-1} min^{-1} in T_6. This was followed by T_5 (0.099 activity g^{-1} min^{-1}). The lowest value was registered in T_9 (0.026 activity g^{-1} min^{-1}).

T6 recorded maximum value (0.290 activity g^{-1} min^{-1}) of superoxide dismutase activity at 6 MAP. This was found to be on par with T_5 (0.271 activity g^{-1} min^{-1}) and T4 (0.234 activity g^{-1} min^{-1}). All other treatments were on par with control plants which was recorded the lowest enzyme activity (0.123 activity g^{-1} min^{-1}). Here also, results indicated that foliar spray of chitosan at lower concentrations (0.5 to 1.5 g l^{-1}) did not influence the SOD activity.

4.2.4 Curcumin content

The curcumin content of *C. aromatica* in response to various foliar spray treatments are presented in Table 13. Significant variation was observed in curcumin content among the various treatments. Chitosan 3 g l^{-1} (T_6) holds the highest curcumin content of 2.18 per cent, which was found to be on par with treatments T_5 (2.03 per cent) and T_4 (1.95 per cent). The lowest curcumin content (1.14 per cent) observed in water spray (T_8) which was on par with T_7 and T_9.

Table 12. Effect of foliar spray treatments on defense enzymes in *C. aromatica*

Treatments	Catalase (U ml^{-1})		Peroxidase (activity g^{-1} min^{-1})		SOD (activity g^{-1} min^{-1})	
	4 MAP	**6 MAP**	**4 MAP**	**6 MAP**	**4 MAP**	**6 MAP**
T_1 (Chitosan 0.5 g l^{-1})	493.057±12.610	724.143±22.921	1.026±0.103	1.570±0.135	0.041±0.002	0.151±0.017
T_2 (Chitosan 1.0 g l^{-1})	557.833±21.630	733.297±26.493	1.223±0.090	1.838±0.041	0.044±0.002	0.195±0.021
T_3 (Chitosan 1.5 g l^{-1})	595.600±16.897	741.940±28.845	2.474±0.326	2.012±0.084	0.052±0.002	0.164±0.038
T_4 (Chitosan 2.0 g l^{-1})	619.313±13.430	811.127±13.347	3.054±0.300	2.112±0.094	0.064±0.006	0.234±0.004
T_5 (Chitosan 2.5 g l^{-1})	675.367±10.593	838.690±24.317	4.308±0.161	5.063±0.421	0.099±0.010	0.271±0.051
T_6 (Chitosan 3.0 g l^{-1})	685.580±14.295	883.360±27.489	4.300±0.278	5.344±0.066	0.140±0.019	0.290±0.075
T_7 (Acetic acid spray)	451.830±20.177	701.443±36.619	1.044±0.132	1.525±0.283	0.029±0.003	0.126±0.004
T_8 (Water spray)	468.437±23.454	686.853±13.105	1.141±0.102	1.503±0.254	0.028±0.004	0.139±0.012
T_9 (Control)	458.867±24.367	669.320±28.614	0.791±0.173	1.522±0.164	0.026±0.004	0.123±0.010
SEm(±)	18.136	25.638	0.205	0.208	0.008	0.034
CD (0.05)	54.303	76.764	0.613	0.624	0.023	0.103

174691

From the data it is evident that chitosan had influenced the curcumin production in the rhizomes. The foliar spray with chitosan enhanced curcumin content by approximately 70 to 80 per cent over the control.

4.2.5 Volatile oil content

The influence of different foliar spray treatments on volatile oil content of *C. aromatica* at harvest is illustrated in Table 13. The maximum volatile oil content (4.50 per cent) was obtained in the treatment chitosan 2.5 g l^{-1} (T_5), which was on par with chitosan 2 g l^{-1} (T_4) and chitosan 3 g l^{-1} (T_6) with 4.08 and 4.25 per cent oil, respectively. Treatment 8 recorded the lowest oil content with 2.25 per cent followed by T_9 (2.43 per cent) and T_7 (2.83 per cent). The higher concentration of chitosan (2 to 3 g L^{-1}) gave 68 to 85 per cent increase in the volatile oil over the control.

4.2.6 Oleoresin content

The oleoresin content varied significantly in *C. aromatica* among the different foliar spray treatments. The data on oleoresin content is presented in Table 13. *C. aromatica* plants subjected to foliar spray with Chitosan 3 g l^{-1} (T_6) produced higher quantity of oleoresin (8.83 per cent) which was observed to be on par with T_4 (7.92 per cent) and T_5 (7.83 per cent). Treatment acetic acid spray (T_7) recorded the lowest mean value content of 5.25 per cent which was found to be on par with T_9 (5.58 per cent). The foliar spray at higher concentration of chitosan (2 to 3 g L^{-1}) was found to enhance the oleoresin content by 42 to 58 per cent.

4.2.7 Carbohydrate

The data pertaining to the effect of foliar spray treatments on carbohydrate content of *C. aromatica* rhizome is presented in Table 14. The carbohydrate content (18.39 mg g^{-1}) was significantly higher in chitosan T_6. This was observed to be on par with T_5 with a carbohydrate content of 17.76 mg g^{-1}. The lowest carbohydrate content recorded in T_7 (10.90 mg g^{-1}) however it was on par with control plants (11.69 mg g^{-1}).

Table 13. Effect of foliar spray treatments on curcumin, volatile oil and oleoresin (per cent) in *C. aromatica*

Treatments	**Curcumin (%)**	**Volatile oil (%)**	**Oleoresin (%)**
T_1 (Chitosan 0.5 g l^{-1})	1.65±0.09	3.67±0.17	6.50±0.25
T_2 (Chitosan 1.0 g l^{-1})	1.50±0.14	3.58±0.36	6.67±0.22
T_3 (Chitosan 1.5 g l^{-1})	1.71±0.18	3.17±0.30	7.17±0.60
T_4 (Chitosan 2.0 g l^{-1})	1.95±0.12	4.08±0.36	7.92±0.36
T_5 (Chitosan 2.5 g l^{-1})	2.03±0.10	4.50±0.29	7.83±0.44
T_6 (Chitosan 3.0 g l^{-1})	2.18±0.13	4.25±0.25	8.83±0.60
T_7 (Acetic acid spray)	1.26±0.27	2.83±0.17	5.25±0.38
T_8 (Water spray)	1.14±0.06	2.25±0.14	6.00±0.76
T_9 (Control)	1.17±0.04	2.43±0.16	5.58±0.46
SEm(±)	0.14	0.26	0.48
CD (0.05)	0.428	0.774	1.448

4.2.7.1 Starch content

Table 14 represents the data on starch content of *C. aromatica* recorded at harvest, in response to different treatments. The highest starch content is observed in treatment T_6 (199.33 mg g^{-1}) which was significantly superior and was on par with treatment T_5 (193.67 mg g^{-1}) and T_4 (190.67 mg g^{-1}). Treatment T_7 recorded the lowest starch content (92.67 mg g^{-1}), which was found to be on par with T_9 (98.33 mg g^{-1}) and T_8 (102.67 mg g^{-1}).

4.2.7.2 Reducing sugar content

The results of reducing sugar content of *C. aromatica* at harvest, due to different treatments, are depicted in table 14. There was significant difference in reducing sugar content, among the treatments. Out of all the treatments T_5 recorded the highest sugar content of 28.67 mg g^{-1} which was found on par with T_6 (28 mg g^{-1}). The least sugar content was noticed in T_9 (17.73 mg g^{-1}) and on par with T_7 (17.80 mg g^{-1}) and T_8 (18.43 mg g^{-1}).

4.3 PHYSIOLOGICAL PARAMETERS

4.3.1 Dry matter production

The data pertaining to dry matter production of *C. aromatica* plants on different foliar spray treatments is depicted in Table 15. Significant difference was observed in dry matter production among the various treatments tried. Maximum dry matter production was observed in T_6 (129.67 g $plant^{-1}$) followed by T_5 (116.44 g $plant^{-1}$) which was on par. The least value was noticed in T_8 (69.57 g $plant^{-1}$) which was on par with T_9 (70.74 g $plant^{-1}$) and T_7 (72.28 g $plant^{-1}$).

At 6 MAP, also treatment T_6 scored a dry matter production of 201 g $plant^{-1}$, which was significantly higher, among the various treatments. T_5 was found to be on par with T_6 with a mean value of 194.83 g $plant^{-1}$. This was followed by T_4 and T_3 with a mean value of 157.67 g $plant^{-1}$ and 147.64 g $plant^{-1}$, respectively. The treatment T_7 recorded the lowest dry matter production of 123.89 g $plant^{-1}$.

Table 14. Effect of foliar spray treatments on carbohydrate, starch and reducing sugar content in *C. aromatica*

Treatments	Carbohydrate ($mg\ g^{-1}$)	Starch ($mg\ g^{-1}$)	Reducing Sugar ($mg\ g^{-1}$)
T_1 (Chitosan 0.5 $g\ l^{-1}$)	12.85±0.40	158.67±10.48	22.47±0.55
T_2 (Chitosan 1.0 $g\ l^{-1}$)	14.02±0.30	162.33±6.94	23.10±0.17
T_3 (Chitosan 1.5 $g\ l^{-1}$)	14.11±0.33	177.00±11.50	23.90±0.61
T_4 (Chitosan 2.0 $g\ l^{-1}$)	15.87±0.69	190.67±10.17	25.40±0.60
T_5 (Chitosan 2.5 $g\ l^{-1}$)	17.76±0.15	193.67±10.40	28.67±0.47
T_6 (Chitosan 3.0 $g\ l^{-1}$)	18.39±0.32	199.33±7.69	28.00±0.26
T_7 (Acetic acid spray)	10.90±0.19	92.67±8.45	17.80±0.40
T_8 (Water spray)	12.08±0.15	102.67±5.17	18.43±0.61
T_9 (Control)	11.69±0.39	98.33±7.75	17.73±0.33
SEm(±)	0.36	8.94	0.47
CD (0.05)	1.080	26.766	1.415

At harvest, T_6 was found significantly higher in dry matter production (239.02 g $plant^{-1}$) and was on par with T_5. The lowest dry matter production (154.97 g $plant^{-1}$) observed in T_7 and was found to be on par with T_1, T_8 and T_9.

4.3.2 Leaf Area Index

Table 16 gives the leaf area index of the crop at four and six month after planting in response to various foliar spray treatments. T_5 was found significantly superior at four MAP with a mean value of 6.51 which was on par with T_6 with a mean value of 5.98. The control plants recorded the least value (3.53) and found on par with all other treatments, except T_5 and T_6.

At 6 MAP also T5 showed maximum leaf area index (16.47) and this was found on par with T_2 (13.84), T_3 (14.64), T_4 (14.26) and T_6 (15.84). Treatment T_1 (12.13) was found on par with the control which recorded the lowest value in leaf area index (10.19).

4.3.3 Net assimilation rate (NAR)

The influence of various foliar spray treatments on NAR in *C. aromatica* is depicted in Table17. There was no significant variation observed among the treatments.

4.3.4 Stomatal conductance

The data on stomatal conductance of *C. aromatica* in response to various treatments is depicted in Table 18.

The data indicates that chitosan treatments had profound influence on stomatal conductance. At both growth periods (4 and 6 MAP) of observation, T_6 showed maximum stomatal conductance (162.67 and 164.33 mmol m^{-2} s^{-1}, respectively) among the treatments and it was found to be on par with T_5 with values of 154.33 and 155 mmol m^{-2} s^{-1}, respectively. The lowest values, 94 and 96.67 mmol m^{-2} s^{-1} were observed in the control treatment, at 4 and 6 MAP.

71

Table 15. Effect of foliar spray treatments on dry matter production (g plant^{-1}) in *C. aromatica*

Treatments	Dry matter production (g plant^{-1})		
	4 MAP	6 MAP	At harvest
T_1 (Chitosan 0.5 g l^{-1})	77.96±4.35	135.47±2.98	177.92±8.04
T_2 (Chitosan 1.0 g l^{-1})	74.68±2.86	136.53±2.13	200.01±9.17
T_3 (Chitosan 1.5 g l^{-1})	93.53±5.08	147.64±3.70	198.28±4.27
T_4 (Chitosan 2.0 g l^{-1})	89.97±2.69	157.67±2.77	208.86±7.71
T_5 (Chitosan 2.5 g l^{-1})	116.44±12.18	194.83±4.38	237.65±8.51
T_6 (Chitosan 3.0 g l^{-1})	129.67±7.19	201.00±8.45	239.02±12.06
T_7 (Acetic acid spray)	72.28±7.01	123.89±3.92	154.97±8.16
T_8 (Water spray)	69.57±2.69	138.10±5.86	171.92±11.50
T_9 (Control)	70.74±4.26	127.50±5.01	175.15±5.93
SEm(±)	6.10	4.72	8.68
CD (0.05)	18.258	14.121	25.99

Table 16. Effect of foliar spray treatments on leaf area index in *C. aromatica*

Treatments	Leaf Area Imdex	
	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	2.12±0.06	6.67±0.29
T_2 (Chitosan 1.0 g l^{-1})	2.16±0.27	7.52±0.76
T_3 (Chitosan 1.5 g l^{-1})	2.32±0.16	8.40±0.61
T_4 (Chitosan 2.0 g l^{-1})	2.37±0.43	7.84±0.69
T_5 (Chitosan 2.5 g l^{-1})	3.58±0.23	9.06±0.09
T_6 (Chitosan 3.0 g l^{-1})	3.29±0.13	8.70±0.70
T_7 (Acetic acid spray)	2.24±0.02	5.95±0.61
T_8 (Water spray)	2.07±0.27	5.88±0.12
T_9 (Control)	1.94±0.12	5.60±0.31
SEm(±)	0.22	0.53
CD (0.05)	0.672	1.574

4.3.5 Photosynthetic rate

Table 19 shows the result of photosynthetic rate of *C. aromatica* at 2 different growth periods (4 and 6 MAP). The highest value was obtained in T_6 at both stages, which was significantly higher than all other treatments. At 4 MAP, chitosan 3 g l^{-1} recorded significantly higher photosynthetic rate (10.53 μ mol CO2 m^{-2} s^{-1}). This was followed by T_4 and T_5 (9.63 and 9.60 μ mol CO2 m^{-2} s^{-1}, respectively) which were found to be on par. The minimum photosynthetic rate (6.47 μ mol CO2 m^{-2} s^{-1}) observed in T_7 which was on par with T_8 and T_9 and the values were 6.80 and 6.93 μ mol CO2 m^{-2} s^{-1}, respectively.

At 6 MAP also, T_6 recorded significantly higher photosynthetic rate with value of 10.50 μ mol CO2 m^{-2} s^{-1}. The lowest value (6.73 μ mol CO2 m^{-2} s^{-1}) recorded in T_7 which was found to be on par with T_8 and T_9 (6.87 and 6.77 μ mol CO2 m^{-2} s^{-1}, respectively).

4.3.6 Proline Content

The result of proline content of the crop at 4 and 6 MAP in response to various foliar spray treatments are presented in Table 20. The data confirmed that treatment with chitosan significantly influenced proline content. T6 showed significantly superior value in proline content (0.259 μmoles g^{-1}) which was on par with all other treatments except T7, T8 and T9. The minimum value was recorded in T8 (0.103 μmoles g^{-1}), which was on par with T9 (0.124 μmoles g^{-1}).

Similarly, at 6 MAP, maximum value of proline content was obtained in treatment T_6 (1.188 μmoles g^{-1}) which was significantly higher and was on par with T_4 and T_5 (1.140 and 1.166 μmoles g^{-1}). The lowest proline content (0.325 μmoles g^{-1}) recorded in treatment T_8.

4.3.7 Cell membrane integrity

The result of cell membrane integrity is illustrated in Table 20. CMI recorded the highest value in treatments T_5 (100.72 per cent) at 4 MAP and was comparable with the T_3, T_4 and T_6. While at 6 MAP, there was no significant variation in cell membrane integrity among the treatments.

Table 17. Effect of foliar spray treatments on net assimilation rate (g m^{-2} day^{-1}) in *C. aromatica*

Treatments	NAR (g m^{-2} day^{-1})
	120 - 180 DAP
T_1 (Chitosan 0.5 g l^{-1})	0.0044±0.001
T_2 (Chitosan 1.0 g l^{-1})	0.0044±0.000
T_3 (Chitosan 1.5 g l^{-1})	0.0035±0.000
T_4 (Chitosan 2.0 g l^{-1})	0.0046±0.001
T_5 (Chitosan 2.5 g l^{-1})	0.0040±0.001
T_6 (Chitosan 3.0 g l^{-1})	0.0038±0.001
T_7 (Acetic acid spray)	0.0042±0.001
T_8 (Water spray)	0.0057±0.000
T_9 (Control)	0.0050±0.001
SEm(±)	0.001
CD (0.05)	NS

Table 18. Effect of foliar spray treatments on stomatal conductance (mmol m^{-2} s^{-1}) in *C. aromatica*

Treatments	Stomatal conductance (mmol m^{-2} s^{-1})	
	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	120.00±6.66	124.00±4.51
T_2 (Chitosan 1.0 g l^{-1})	111.33±8.11	114.00±4.73
T_3 (Chitosan 1.5 g l^{-1})	123.00±4.58	125.67±7.05
T_4 (Chitosan 2.0 g l^{-1})	149.33±11.05	150.00±9.24
T_5 (Chitosan 2.5 g l^{-1})	154.33±13.17	155.00±11.59
T_6 (Chitosan 3.0 g l^{-1})	162.67±13.30	164.33±9.02
T_7 (Acetic acid spray)	102.33±5.81	105.33±3.48
T_8 (Water spray)	100.33±8.11	104.00±8.14
T_9 (Control)	94.00±9.61	96.67±5.61
SEm(±)	9.40	7.48
CD (0.05)	28.147	22.394

Table 19. Effect of foliar spray treatments on photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) in *C. aromatica*

Treatments	Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1})	
	4 MAP	**6 MAP**
T_1 (Chitosan 0.5 g l^{-1})	7.47±0.19	7.53±0.20
T_2 (Chitosan 1.0 g l^{-1})	8.03±0.19	8.13±0.20
T_3 (Chitosan 1.5 g l^{-1})	8.07±0.20	8.03±0.32
T_4 (Chitosan 2.0 g l^{-1})	9.63±0.26	9.73±0.14
T_5 (Chitosan 2.5 g l^{-1})	9.60±0.21	9.77±0.09
T_6 (Chitosan 3.0 g l^{-1})	10.53±0.52	10.50±0.35
T_7 (Acetic acid spray)	6.47±0.27	6.73±0.12
T_8 (Water spray)	6.80±0.10	6.87±0.12
T_9 (Control)	6.93±0.14	6.77±0.18
SEm(±)	0.26	0.21
CD (0.05)	0.774	0.628

Table 20. Effect of foliar spray treatments on proline content (μmoles g^{-1}) in *C. aromatica*

Treatments	**Proline content (μmoles g^{-1})**	
	4 MAP	**6 MAP**
T_1 (Chitosan 0.5 g l^{-1})	0.228±0.006	0.784±0.062
T_2 (Chitosan 1.0 g l^{-1})	0.238±0.007	1.034±0.026
T_3 (Chitosan 1.5 g l^{-1})	0.232±0.007	1.028±0.016
T_4 (Chitosan 2.0 g l^{-1})	0.243±0.003	1.140±0.008
T_5 (Chitosan 2.5 g l^{-1})	0.251±0.005	1.166±0.008
T_6 (Chitosan 3.0 g l^{-1})	0.259±0.003	1.188±0.010
T_7 (Acetic acid spray)	0.196±0.014	0.718±0.067
T_8 (Water spray)	0.103±0.021	0.325±0.090
T_9 (Control)	0.124±0.013	0.744±0.051
SEm(±)	0.010	0.047
CD (0.05)	0.031	0.142

Table 21. Effect of foliar spray treatments on cell membrane integrity (per cent) in *C. aromatica*

Treatments	CMI (%)	
	4 MAP	6 MAP
T_1 (Chitosan 0.5 g l^{-1})	64.34±3.03	84.54±10.75
T_2 (Chitosan 1.0 g l^{-1})	76.26±4.06	87.43±2.51
T_3 (Chitosan 1.5 g l^{-1})	89.14±10.01	95.72±4.37
T_4 (Chitosan 2.0 g l^{-1})	96.13±5.46	96.37±6.19
T_5 (Chitosan 2.5 g l^{-1})	100.72±10.60	107.20±10.60
T_6 (Chitosan 3.0 g l^{-1})	97.12±2.93	102.18±6.61
T_7 (Acetic acid spray)	69.70±1.30	83.39±4.49
T_8 (Water spray)	63.26±9.28	85.18±3.28
SEm(±)	6.75	6.77
CD (0.05)	20.413	NS

4.4 YIELD AND YIELD COMPONENTS

4.4.1 Rhizome yield – Fresh and Dry

The data on rhizome fresh and dry weight of rhizome at harvest of *C. aromatica* is presented in Table 22. The rhizome yield varied significantly among the treatments with respect to both fresh and dry weight.

Maximum fresh weight was obtained from the plants treated with chitosan at 3.0 g l^{-1} (Plate 4A) and the mean value recorded were 696.67 g $plant^{-1}$. T_6 was found to be on par with T_5 which was recorded a value of 692.33 g $plant^{-1}$ for fresh rhizome (Plate 4B). The least rhizome yield was observed in treatment T_7 (acetic acid spray) and the value recorded was 395.33 g $plant^{-1}$. Control treatment (Plate 4C) recorded 491.67 g $plant^{-1}$. This was on par with treatment T_8 (water spray) and mean value recorded was 478.33 g $plant^{-1}$.

The dry yield of *C. aromatica* found to be higher in T_6 with a value of 173.27 g $plant^{-1}$ and this was comparable with T_5 (170.95 g $plant^{-1}$). The lowest value for dry yield observed in T_7 and the value recorded was 101.55 g $plant^{-1}$. However T_9 recorded a yield of 118.08 g $plant^{-1}$ and was comparable with T_8 (115.55 g $plant^{-1}$).

The chitosan at higher concentration enhanced rhizome yield by approximately 45 per cent over the control.

4.4.2 Crop Duration

The effect of various foliar spray treatments on crop duration of Kasturi turmeric plants is presented in the table 23. The minimum duration for crop upto harvest recorded in both T_5 and T_6 (224 days). This was found to be on par with all other chitosan treatments *viz.*, T_1 (225), T_2 (225.33) and T_3 (226) and T_4 (224.67). The highest crop duration was recorded in T_8 (230.33) followed by T_9 (229.33) and T_7 (227.33) which were found to be on par with T_8.

Table 22. Effect of foliar spray treatments on fresh and dry rhizome yield (g $plant^{-1}$) in *C. aromatica*

Treatments	Fresh rhizome yield (g $plant^{-1}$)	Dry rhizome yield (g $plant^{-1}$)
T_1 (Chitosan 0.5 g l^{-1})	524.00±38.43	125.48±6.58
T_2 (Chitosan 1.0 g l^{-1})	578.33±20.99	139.97±3.46
T_3 (Chitosan 1.5 g l^{-1})	575.33±13.37	138.80±6.20
T_4 (Chitosan 2.0 g l^{-1})	612.33±23.14	147.67±3.25
T_5 (Chitosan 2.5 g l^{-1})	692.33±17.25	170.95±2.6
T_6 (Chitosan 3.0 g l^{-1})	696.67±35.51	173.27±9.20
T_7 (Acetic acid spray)	395.33±39.94	101.97±5.84
T_8 (Water spray)	478.33±29.45	115.55±7.48
T_9 (Control)	491.67±7.17	118.08±1.47
SEm(±)	27.29	5.66
CD (0.05)	81.718	16.947

Plate 4. Harvested rhizome from *C. aromatica* plants exposed to foliar spray with A) Chitosan 3 g l^{-1} B) Chitosan 2.5 g l^{-1} C) Control

Table 23. Effect of foliar spray treatments on crop duration (days) and harvest index in *C. aromatica*

Treatments	Crop duration	Harvest Index
T_1 (Chitosan 0.5 g l^{-1})	225.00±1.00	0.705±0.013
T_2 (Chitosan 1.0 g l^{-1})	225.33±0.88	0.702±0.020
T_3 (Chitosan 1.5 g l^{-1})	226.00±1.15	0.699±0.017
T_4 (Chitosan 2.0 g l^{-1})	224.67±1.45	0.708±0.010
T_5 (Chitosan 2.5 g l^{-1})	224.00±1.53	0.720±0.015
T_6 (Chitosan 3.0 g l^{-1})	224.00±0.58	0.725±0.017
T_7 (Acetic acid spray)	227.33±0.88	0.658±0.006
T_8 (Water spray)	230.33±0.67	0.672±0.009
T_9 (Control)	229.33±0.67	0.675±0.014
SEm(±)	1.03	0.014
CD (0.05)	3.085	0.042

4.4.3 Harvest Index

Table 23 represents the data on harvest index of the crop. There was significant variation in harvest index among the treatments. The highest value for harvest index noticed in treatment T_6 (0.725) and found on par with T_1 (0.705), T_2 (0.702), T_3 (0.699), T_4 (0.708) and T_5 (0.720). Treatment T_7 recorded the least value (0.658) and it was on par with T_8 (0.672) and T_9 (0.675).

4.5 UPTAKE OF PLANT NUTRIENTS (N, P AND K)

The plant analysis for the uptake of major plant nutrients (N, P and K) by *C. aromatica* in response to various foliar spray treatments is illustrated in Table 24. The various treatments showed a significant difference in the uptake of nutrients. For N uptake, treatment T_5 recorded maximum uptake (3.29 g plant^{-1}) and found to be on par with treatments T_6 (3.22 g plant^{-1}), T_3 (2.84 g plant^{-1}) and T_4 (2.82 g plant^{-1}). Minimum value for N uptake was recorded in treatment T_7 (1.79 g plant^{-1}) which was on par with T_8 (1.82 g plant^{-1}) and T_9 (2.00 g plant^{-1}).

The uptake of P was found to be higher in T_6 (0.76 g plant^{-1}), which was on par with T_5 (0.75 g plant^{-1}), T_4 (0.72 g plant^{-1}), T_3 (0.66 g plant^{-1}) and T_2 (0.64 g plant^{-1}). Treatment T_7 was recorded the lowest of all treatments with a P uptake of 0.42 g plant^{-1}. This was on par with T_1 (0.44 g plant^{-1}), T_9 (0.46 g plant^{-1}) and T_8 (0.47 g plant^{-1}).

The data confirmed the profound influence of chitosan foliar spray on K uptake in *C. aromatica*. T6 recorded the highest value (4.27 g plant^{-1}) and was on par with T_5 which recorded an uptake of 4.03 g plant^{-1}. T_7 recorded the lowest uptake of K with a value of 2.38 g plant^{-1}. This was followed by T_8 (2.56 gplant^{-1}), which was on par with the control T_9 (2.95 g plant^{-1}).

Table 24. Effect of foliar spray treatments on nutrient uptake (N, P and K) in *C. aromatica*

Treatments	**Uptake of plant nutrients (g plant^{-1})**		
	N	**P**	**K**
T_1 (Chitosan 0.5 g l^{-1})	2.08±0.17	0.44±0.05	3.44±0.09
T_2 (Chitosan 1.0 g l^{-1})	2.54±0.27	0.64±0.05	3.54±0.02
T_3 (Chitosan 1.5 g l^{-1})	2.84±0.21	0.66±0.05	3.37±0.16
T_4 (Chitosan 2.0 g l^{-1})	2.82±0.08	0.72±0.02	3.67±0.26
T_5 (Chitosan 2.5 g l^{-1})	3.29±0.22	0.75±0.02	4.03±0.08
T_6 (Chitosan 3.0 g l^{-1})	3.22±0.13	0.76±0.03	4.27±0.10
T_7 (Acetic acid spray)	1.79±0.16	0.42±0.04	2.38±0.30
T_8 (Water spray)	1.82±0.15	0.47±0.05	2.56±0.14
T_9 (Control)	2.00±0.07	0.46±0.03	2.95±0.07
SEm(±)	0.17	0.04	0.16
CD (0.05)	0.522	0.126	0.482

Discussion

5. DISCUSSION

The present study entitled "Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)" was carried out in Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvanathapuram during 2017-2019 to study the effect of different concentrations of chitosan on plant growth, yield and metabolite production in *Curcuma aromatica*. The results obtained in the study are discussed in this chapter.

5.1 EFFECT OF CHITOSAN ON PLANT GROWTH PARAMETERS

The chitosan foliar spray has significantly influenced various plant growth parameters *viz.*, plant height, number of leaves, leaf area, rhizome thickness, number of fingers, root length, root spread, root weight and shoot weight. These parameters showed an increasing trend with increasing concentration of chitosan. The higher values with respect to these plant growth parameters were recorded in plants subjected to foliar spray treatments with chitosan 2.5 and 3 g l^{-1}. Sheikha and Al-Malki (2011) observed improvement in shoot and root length, fresh and dry weight of shoot and root as well as leaf area by the application of chitosan in bean plants. According to Mondal *et al.* (2012) plant height and leaf number per plant increased with the increasing concentration of chitosan from 50 ppm to 125 ppm in okra. The report of Abu-Muriefah (2013) in common bean (*Phaseolus vulgaris* L.) also corroborates with the results of our study in kasturi turmeric. He observed that plant growth parameters *viz.,* plant height, number of leaves, leaf area, shoot fresh and dry weights were enhanced significantly compared to the control plants, with the foliar spray of chitosan. Ibraheim and Mohsen (2015) reported an increase in number of leaves in summer squash by the application of chitosan. Malekpoor *et al.* (2016) also observed significant enhancement of plant growth characters in *Ocimum basilicum*, when chitosan was applied as foliar spray. The study conducted by Mondal *et al.* (2016) in summer tomato (*Solanum lycopersicum*) also reported similar results. The morphological parameters *viz.*, plant height, number of fingers

and leaf area enhanced significantly with the chitosan application compared to control treatment. The improvement in plant growth characters could be attributed to increased enzyme activities of the nitrogen metabolism by the application of chitosan (Ke *et al.*, 2001).

The root and shoot biomass were also enhanced significantly by the application of chitosan in the study. But root weight did not show any significant variation at harvest, this could be due to the degeneration of the roots at harvest. According to Khan *et al.* (2002), the improvement in plant biomass might be due to the increased photosynthetic activity. Asghari-Zakaria *et al.* (2009) observed that chitosan 500 mg l^{-1} significantly enhanced the shoot biomass while root biomass was significantly increased at lower concentrations of chitosan (5 and 15 mg l^{-1}) in *Solanum tuberosum.* Kra *et al.* (2019) reported that the growth enhancement of the plants could be the result of cell division and/or their extension.

5.2 EFFECT OF CHITOSAN ON METABOLITE PRODUCTION

5.2.1 Chlorophyll content

The content of chlorophyll a, b and total chlorophyll at 6 MAP were found to be significantly influenced by the foliar spray with chitosan. The foliar spray treatments with chitosan 2, 2.5 and 3 g l^{-1} showed higher chlorophyll content. Application of chitosan increased chlorophyll content in robusta coffee due to the increase in leaf magnesium and nitrogen, the key elements in the chemical structure of chlorophyll (Van *et al.*, 2013). The amino compounds present in the chitosan also contributes to enhanced synthesis of chlorophyll (Chibu and Shibayama, 2001). Naderi *et al.* (2015) explained that increase in chlorophyll content on chitosan application might be due to the activation of genes in the biosynthesis of photosynthetic pigments.

5.2.2 Total proteins

The protein content of *C. aromatica* is significantly influenced by foliar spray with higher concentrations of chitosan, 2, 2.5 and 3 g l^{-1}. Sultana *et al.* (2017)

found that the protein content was significantly enhanced by the application of chitosan in egg plant. The increase in plant nitrogen might be a reason for enhanced protein content in the plant.

5.2.3 Defense enzymes

The application of higher concentrations of chitosan had significantly influenced the activity of defense enzymes in *C. aromatica*. The higher activity of catalase and superoxide dismutase was observed in plants exposed to foliar spray with chitosan 2, 2.5 and 3 g l^{-1}. The enzyme activity of peroxidase was observed to be higher in 2.5 and 3 g l^{-1}. The results from the study conducted by Song *et al.* (2006) revealed that the application of chitosan in cucumber seedlings which were grown under salt stress could significantly increase the activities of enzymes *viz.,* catalase, peroxidase and SOD in the leaves. This indicated that chitosan has the ability to remove the reactive oxygen species and thereby protecting the functions of bio-membrane and also raising the physiological activities. According to Zong *et al.* (2017), the activities of defense enzymes such as catalase, peroxidase and SOD were enhanced with the chitosan foliar spray in edible rape (*Brassica rapa*). Similar results were also reported in *Hordeum vulgare* by Behboudi *et al.* (2018) wherein chitosan treatments showed maximum activity of enzymes such as catalase and superoxide dismutase.

Kra *et al.* (2019) observed that, in *Manihot esculenta* the peroxidase activity increased with increase in chitosan concentration and maximum peroxidase activity was recorded in plants treated with chitosan at 75 and 100 mg l^{-1}. Chitosan application activates photosynthesis and as a result hydrogen peroxide produced (Mondal *et al.*, 2012). Hydrogen peroxide is the key substrate for the peroxidases, hence its activity is increased.

5.2.4 Curcumin content

The curcumin content of *C. aromatica* increased significantly by the foliar application of Chitosan (Fig.1). About 70-80 per cent increase in curcumin content

was observed in plants treated with higher concentrations of chitosan (2.5 and 3 g l^{-1}) compared to control. In confirmation with our results, the ability of chitosan to elicit the production of secondary metabolites has been reported in several plant species (Zhao *et al.,* 2005). According to Namdeo (2007) and Ionkova (2007), chitosan enhances the production of secondary metabolites in plants. The chitosan activates the genes which are responsible for plant defense responses that in turn result in enhanced production of secondary metabolites (Loschke *et al.,* 1983; Gorelick and Bernstein, 2014). The major secondary metabolites of *Atropa belladonna i.e.* scopolamine and hyoscyamine enhanced considerably with the chitosan application (Hashimoto *et al.,* 1993). Gorelick *et al.* (2015) reported that the biosynthesis of secondary metabolite, withaferin A was improved significantly in Ashwagandha, by the biotic stimuli chitosan. The study conducted by Sathiyabama *et al.* (2016) revealed that foliar spray with chitosan resulted in four fold increase in the curcumin production in *Curcuma longa* plants and established chitosan elicited curcumin production in the plant. In contrast to our study, Lei *et al.*, (2011) reported that foliar application of chitosan did not have any effect on plant growth in *Artemisia annua.* However, they observed substantial increase in artemisinin content of the plant. Chitosan at 100 mg l^{-1} enhanced the biosynthesis of artemisinin by 53 per cent over the control plants.

5.2.5 Volatile oil

In the study, the volatile oil present in the rhizomes of *C. aromatica* was significantly influenced by the foliar spray treatment of chitosan (Fig. 1). Chitosan at higher concentrations (2, 2.5 and 3 g l^{-1}) gave 68 to 85 per cent increase in the volatile oil over the control. This result was in conformity with the findings of Salehi *et al.* (2017) in savory and Bistgani *et al.* (2017a) in *Thymus daenensis*. They observed that the essential oil content and oil yield of the crop increased considerably by the application of chitosan. In sweet basil, Kim *et al.* (2005) also reported the positive effect of chitosan at 0.4 per cent on essential oil content. Zhang *et al.* (2006) reported that chitosan has the ability to enhance the activity of enzymes by changing the function of genes thereby activating certain biosynthetic pathways

in plants. Malekpoor *et al*. (2016) also observed enhancement in the oil content by the chitosan foliar spray in basil plants.

5.2.6 Oleoresin content

The foliar application of chitosan influenced the oleoresin content of *C. aromatic* in the study (Fig.1). It was observed that at higher concentrations of chitosan (2.5 and 3 g l^{-1}) oleoresin content was improved by 42 to 58 percent over the control.

5.2.7 Carbohydrate (Starch, Sugar)

The biochemical parameters *viz.*, carbohydrate, starch and reducing sugar content of *C. aromatica* were found to be significantly higher in treatments sprayed with chitosan 2.5 g l^{-1} and 3 g l^{-1}. Chitosan 3 g l^{-1} found to be higher in carbohydrate and starch content while chitosan 2.5 g l^{-1} recorded higher value for reducing sugar content. Abdel-Mawgoud *et al.* (2010) observed that total carbohydrate and sugar content in strawberry improved significantly with the application of chitosan solution. The results of a study in common bean by Abu-Muriefah (2013) is also in agreement with the findings of present investigation. It was found that carbohydrate concentrations improved significantly as a result of chitosan 200 mg l^{-1} foliar spray. Farouk *et al.* (2008, 2011, 2012) also reported the positive influence of chitosan 200 mg l^{-1} on carbohydrate production in cucumber, radish and cowpea.

5.3 EFFECT OF CHITOSAN ON PHYSIOLOGICAL PARAMETERS

5.3.1 Dry matter production

The results indicated that the dry mater production of *C. aromatica* was significantly influenced by the foliar application of chitosan. The highest dry matter production was recorded in plants subjected to chitosan spray at 3 g l^{-1} which was found to be on par with the treatment, chitosan at 2.5 g l^{-1}. In the study, chitosan application significantly enhanced the plant growth parameters *viz.,* plant height, number of leaves, leaf area and shoot weight. The increased vegetative growth

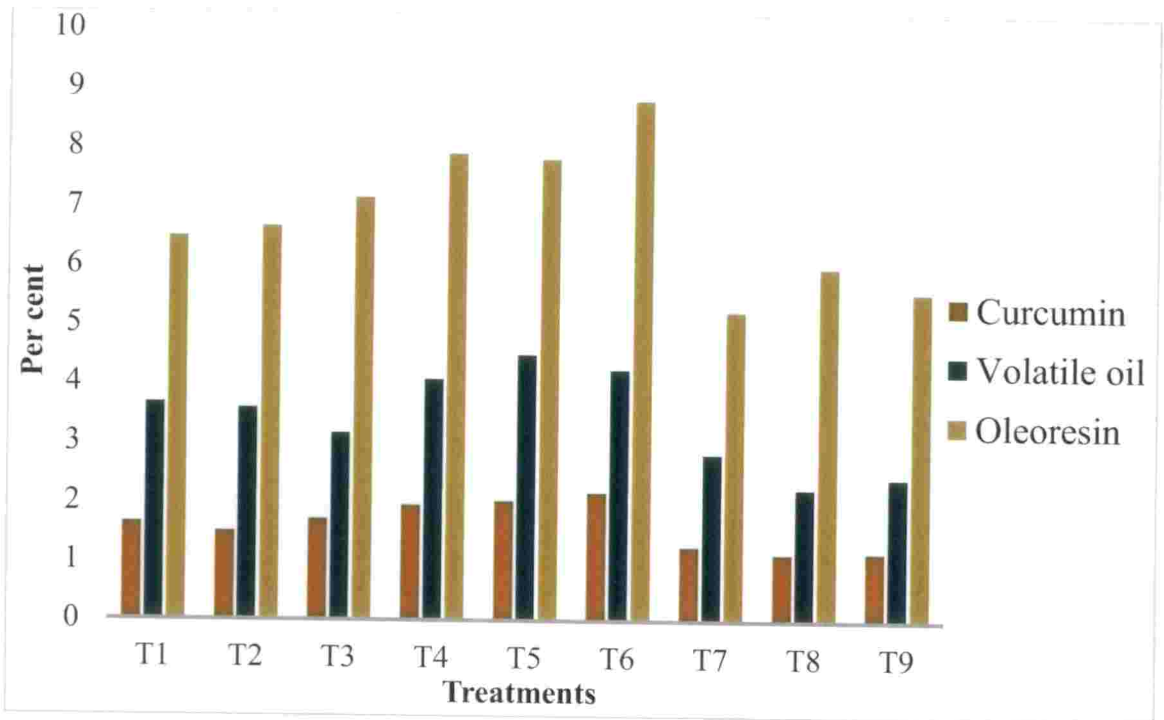

Fig 1: Effect of foliar spray treatments on curcumin content, volatile oil and oleoresin

might have contributed to the increased dry matter production. In consensus with our findings, El-Tantawy (2009) reported the positive effect of chitosan application on dry matter production in tomato. He observed that plant growth and development increased significantly with the application of chitosan. Mondal *et al.* (2016) reported increased dry matter production in summer tomato, when exposed to foliar spray application of chitosan 75 mg l^{-1}. According to Abdel-Mawgoud *et al.* (2010) higher number of leaves and chlorophyll content contributed to higher dry matter production.

5.3.2 Leaf area index

The leaf area index of *C. aromatica* was found to be significantly influenced by the chitosan application at all stages of observation (4 and 6 MAP). Higher LAI might have resulted in capturing more sunlight which leads to the production of more photosynthates and their translocation to rhizomes. This might have resulted in increased rhizome yield. The higher leaf area index due to chitosan application has been reported in plants like strawberry (Abdel-Mawgoud *et al.*, 2010) and highbush blueberry, *Vaccinium corymbosum* (Cabrera *et al.*, 2010).

5.3.3 Stomatal conductance and Photosynthetic rate

The stomatal conductance and photosynthetic rate were found to be significantly increased with higher concentrations of chitosan foliar spray. Maximum stomatal conductance and photosynthetic rate were recorded in plants exposed to foliar spray of chitosan 3g l^{-1}. The positive influence of chitosan foliar spray on stomatal conductance and photosynthetic rate were reported by Khan *et al.* (2002). They explained that increase in photosynthetic rate and stomatal conductance might be due to the increased uptake of CO_2. Similar effect of chitosan application on photosynthetic rate and stomatal conductance was reported by Phothi and Theerakarunwong (2017) in rice. In opposition to our finding, Iriti *et al.* (2009) observed a significant decrease in stomatal conductance with foliar spray treatment of chitosan in bean plants. However, the photosynthetic activity was found to enhance due to chitosan foliar spray.

5.3.4 Proline content

The results of the present study indicated that chitosan had significantly influenced the proline content of *C. aromatica*. The highest proline content was recorded in treatment with chitosan 3 g l^{-1}. These results are in accordance with the findings of Song *et al.* (2006) in cucumber. They found that application with chitosan resulted in enhanced proline content. Proline is described as an osmoprotectant and it is usually accumulated under different stress conditions especially drought (Moradshahi *et al.*, 2004). The study conducted by Bistgani *et al* (2017b) in *Thymus daenensis* also showed the positive effect of exogenous application of chitosan on proline content. In contrast with the result of our study, Behboudi *et al.* (2018), significant variation was not observed in proline content on chitosan treatment in barley plants. Similarly, Mahdavi *et al.* (2011) noticed a decrease in proline content of safflower, at lower concentrations of chitosan decreased. However, at higher concentrations, the proline level was found to be enhanced. According to Karimi et al. (2012), the chitosan treatment did not influence the proline content in *Ricinus communis*. These reports explain that the effect of chitosan on proline production may follow different mechanism in different plant species.

5.3.5 Cell membrane integrity

As per the data obtained in the study, cell membrane integrity of the crop varied significantly at four months after planting, but no variation was observed at six months after planting, though the values of cell membrane integrity was found to be enhanced. At four months after planting, the cell membrane stability index was recorded maximum in plants exposed to chitosan 2.5 g l^{-1}. Song *et al.* (2006) reported that the application of chitosan reduced electrolyte permeability thereby increasing the cell membrane stability of the cucumber seedlings. The cell membrane stability was found to increase in apple seedlings which were sprayed with chitosan (Yang *et al.*, 2009). According to them, the chitosan might have lessened the adverse reactions of reactive oxygen species (ROS) towards the

membrane and reduced the amount of superoxide anion radicals, hydroxyl radicals and hydrogen peroxide by the activation of ROS scavenging enzymes.

5.4 EFFECT OF CHITOSAN ON YIELD AND YIELD COMPONENTS

5.4.1 Rhizome yield (Fresh and Dry)

The rhizome yield (fresh and dry) obtained in the study revealed that application of different concentrations of chitosan had influenced the yield significantly (Fig.2 and 3). Significantly higher fresh and dry rhizome yield was registered in treatment chitosan 3 g l^{-1}. The chitosan at 3g l^{-1} enhanced the rhizome yield by 41 per cent (fresh weight) and 46 per cent (dry weight) over the control. Similar increase in yield was reported in *Curcuma longa* by (Anusuya and Sathiyabama, 2016. According to them, the rhizome yield of *C. longa* increased by 60 percent (fresh weight) and 50 per cent (dry weight) with the foliar spray of chitosan 0.1 per cent (w/v) at 30 days intervals upto seven months. Mondal *et al.* (2012) observed 28 per cent yield increase in okra over the control, on the foliar application of chitosan 75 ppm. Salachna and Zawadzinska (2014) reported the positive influence of chitosan on yield in fressia plant. Dzung *et al.* (2017) in also observed yield increment in chilli with the application of chitosan. Rahman *et al.* (2018) found that foliar spray with chitosan 1000 ppm gave a 42 per cent hike in yield over the control in strawberry plants.

The increase in rhizome yield might be due to the increase in the uptake of major plant nutrients (N, P and K) and also due to the increase in chlorophyll content, that in turn increase the photosynthetic activity of the plant (Farouk and Amany, 2012). The increase in rhizome yield may also be due to the effect of chitosan on physiological processes that improve vegetative growth with resultant active translocation of photoassimilates from source to sink tissues i.e. translocation of assimilates towards the economic part (Kumar *et al.,* 1994). Abdel-Mawgoud *et*

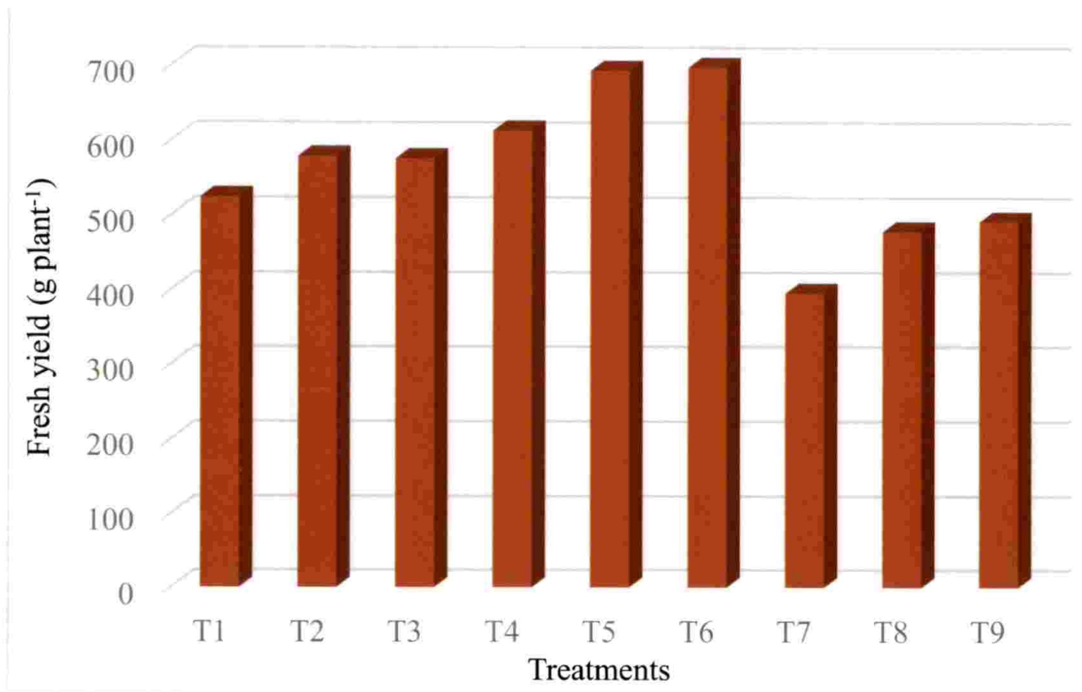

Fig 2: Effect of foliar spray treatments on fresh rhizome yield

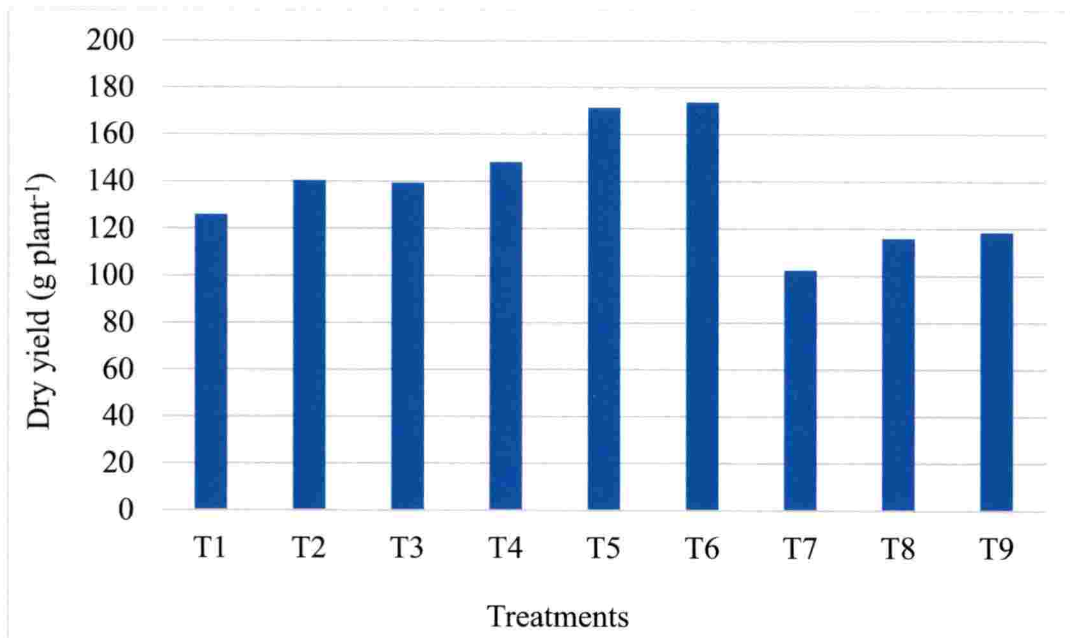

Fig 3: Effect of foliar spray treatments on dry rhizome yield

al. (2010) opined that the increased crop yield reflected by the higher dry matter production.

5.4.2 Crop duration

The various concentrations of chitosan spray significantly influenced the crop duration of *C. aromatica*. The number of days varied from 224 to 230 days. The minimum days of crop duration was observed in plants subjected to foliar spray of chitosan 2.5 and 3 g l^{-1}. The foliar spray with chitosan might have enabled the rapid mobilization of assimilates from source to sink so that the crop attained maturity at an early date.

5.4.3 Harvest index

The chitosan treatments significantly enhanced the harvest index of *C. aromatica* over the control. The plants treated with chitosan 3 g l^{-1} recorded the highest harvest index. This recorded the highest rhizome yield among the treatments. Hence, a higher harvest index is an indicator of higher rhizome yield in *C. aromatica*. According to Behboudi *et al.* (2018), the yield and yield components of barley plants increased considerably with the application of chitosan. The harvest index of the crop was also enhanced, accordingly.

5.5 EFFECT OF CHITOSAN ON UPTAKE OF MAJOR PLANT NUTRIENTS

The foliar application of chitosan enhanced the uptake of plant nutrients *viz.*, N, P and K (Fig.4). The uptake of N was found significantly higher in treatments with foliar spray of chitosan 2.5 g l^{-1} and 3 g l^{-1}. This may be attributed to higher root length and root spread of the plants subjected to these treatments.

Van *et al.* (2013) reported that there was significant increase in the uptake of plant nutrients (N, P and K) with the application of chitosan in robusta coffee. According to them increase in nutrient uptake might have due to the increase in chlorophyll content and net photosynthetic rate. The positive effect of exogenous

application of chitosan on uptake of major plant nutrients such as nitrogen, phosphorus and potassium has also been reported in common bean (Abu-Muriefah, 2013) and in black gram (Bakiyalakshmi *et al.*, 2016).

According to Guan *et al.* (2009), the application of chitosan improves the availability and uptake of water and plant nutrients, by adjusting the cell osmotic pressure. As observed in our study, the positive effect of chitosan on plant growth and development could be due to the increased uptake of plant nutrients such as N, P and K. According to Possingham (1980), increase in N and K uptake enables the production of more chloroplast per cell and increase in the production of chlorophyll. P and K plays an important role in stimulating cell division and in the biosynthesis and translocation of carbohydrates and thereby increasing the vegetative growth and yield (Farouk and Amany, 2012).

In the present study, chitosan application as foliar spray at 3 and 5 MAP elicited plant growth, production of curcumin, volatile oil, oleoresin and yield. The chitosan concentration of 2.5 and 3 g l^{-1} gave maximum enhancement in the yield and metabolite production.

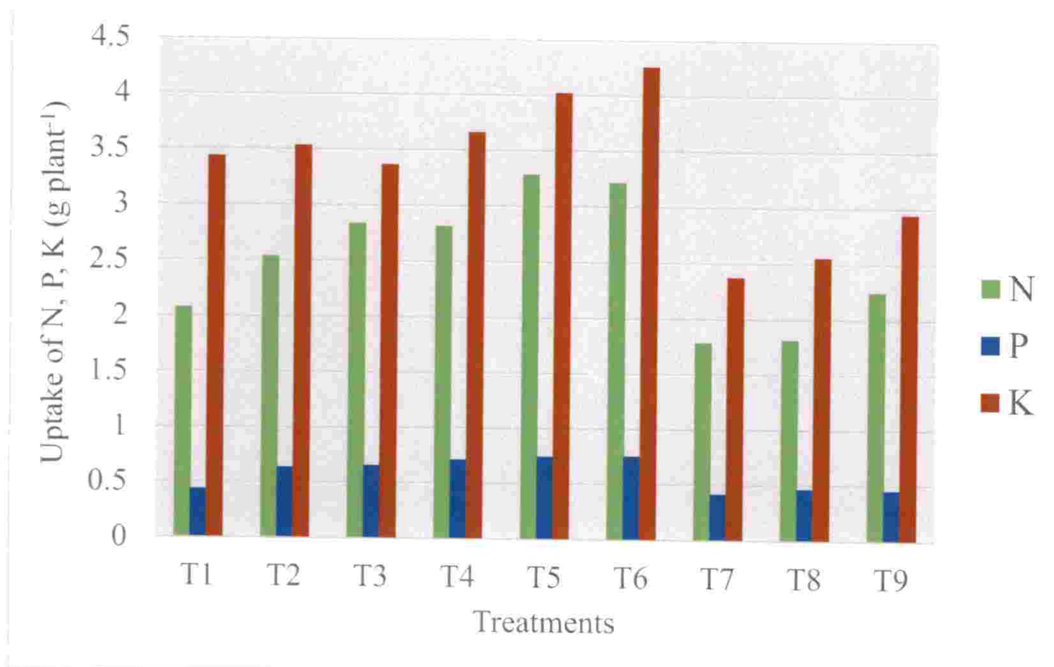

Fig 4: Effect of foliar spray treatments on uptake of N, P and K

Summary

6. SUMMARY

The present study entitled, "Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)" was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram. The experiment was carried out during the period, 2017- 2019. The main objective was to study the effect of different concentrations of chitosan on plant growth, yield and metabolite production in *Curcuma aromatica*.

The planting material for the study was obtained from Instructional Farm, College of Agriculture, Vellayani. The experiment was laid out in completely randomized block design (CRD) with nine treatments and three replications. The treatments consisted of foliar spray of chitosan at different concentrations and control treatments *viz.*, chitosan 0.5 g l^{-1} (T_1), chitosan 1 g l^{-1} (T_2), chitosan 1.5 g l^{-1} (T_3), chitosan 2 g l^{-1} (T_4), chitosan 2.5 g l^{-1} (T_5), chitosan 3 g l^{-1} (T_6), acetic acid (0.25 per cent) spray (T_7), water spray (T_8) and control (T_9). The treatments were given at 3 and 5 months after planting (MAP). The study was designed to evaluate the effect of foliar spray of chitosan at different concentrations on plant growth parameters, metabolite production, physiological parameters, yield parameters and nutrient uptake.

The salient findings of the study are summerized in this chapter.

The plant growth parameters *viz.*, plant height, number of tillers, number of leaves, leaf area and shoot weight were recorded at 4 and 6 MAP. All chitosan foliar spray treatments resulted in significantly taller plants compared to treatments devoid of chitosan at 6 MAP, with the highest value (109.91 cm) recorded in treatment chitosan 3 g l^{-1} (T_6). The plant height at 4 MAP did not show any variation among the treatments. The plants subjected to foliar spray treatments with chitosan 2.5 and 3 g l^{-1} (T_5 and T_6) recorded significantly higher number of leaves at 4 MAP while at 6 MAP, it was recorded in treatments, T_2 to T_6, with the highest

number of leaves (30.11) in T_6. Similar trend was observed with leaf area also, with the highest leaf area (8593.78 cm^2, 21735.79 cm^2, respectively) being recorded in chitosan 2.5 g l^{-1} at 4 MAP and 6 MAP. The treatments T_4, T_5 and T_6 recorded significantly higher shoot weight (fresh) at 4 MAP, and maximum fresh weight was registered in the treatment with chitosan 2.5 g l^{-1} with a value of 75.33 g $plant^{-1}$, while at 6 MAP, chitosan 3 g l^{-1} recorded higher shoot (fresh) weight (126.33 g $plant^{-1}$) and was comparable with the treatments T_3 and T_5. The highest dry weight, 42.69 g $plant^{-1}$ was recorded in T_5 and 63.0 g $plant^{-1}$ in T_6 at 4 MAP and 6 MAP, respectively. The number of tillers did not show any variation among treatments at both stages of observation.

The plant growth parameters *viz.*, rhizome spread, rhizome thickness, number of fingers, root length, root spread and root weight were recorded at 4 MAP, 6 MAP and at harvest. Significant enhancement in rhizome spread (29.50 cm) was observed only at harvest, in chitosan 3 g l^{-1} (T_6), which was on par with that in chitosan 2.5 g l^{-1} (T_5). The higher concentrations of chitosan foliar spray significantly influenced rhizome thickness at 6 MAP and at harvest; significantly higher values were observed in treatments, T_2 to T_6 at 6 MAP and T_3 to T_6 at harvest. Among these, T_5 recorded maximum values (2.46 cm and 3.17 cm, respectively) at 6 MAP and at harvest. At all stages of observation, number of fingers was found to be significantly higher in T_5 and T_6. The maximum numbers of fingers (22.46) was recorded in the plants exposed to chitosan 3 g l^{-1} (T_6) at harvest. Root length and root spread were significantly higher with the treatments T_5 and T_6 at 6 MAP and at harvest. The highest root length (56.76 cm) and root spread (49.10 cm) was recorded in T_6 and T_5 respectively at harvest. Significant variation in root weight was observed in *C. aromatica* only at 6 MAP, with the highest values (fresh-31.29 g $plant^{-1}$, dry-15 g $plant^{-1}$) was recorded in the treatment with chitosan 3 g l^{-1} (T_6).

The observations on plant metabolites *viz.*, chlorophyll content, total proteins and defence enzymes were recorded at 4 and 6 MAP. T_4, T_5 and T_6 were observed to have significantly higher chlorophyll content among the treatments, at

6 MAP. The higher chlorophyll a (1.397 mg g^{-1}) and total chlorophyll (1.959 mg g^{-1}) was observed in the treatment with chitosan 2.5 g l^{-1} and chlorophyll b (0.615 mg g^{-1}) in that with chitosan 3 g l^{-1} at 6 MAP. The foliar spray treatments did not show any significant variation in chlorophyll content at 4 MAP. Total proteins and defense enzymes were observed to give significant variation at both stages of observation. Protein content was found to be significantly higher (6.89 mg g^{-1}) in the plants subjected to foliar spray with chitosan 2.5 g l^{-1} at 4 MAP and in treatments T_4 to T_6 at 6 MAP, the highest value (8.46 mg g^{-1}) being recorded in the treatment with chitosan 3 g l^{-1}. Catalase and peroxidase activity were found significantly higher in T_5 and T_6 at both stages of observation. The higher catalase activity of 685.580 U ml^{-1} and 883.360 U ml^{-1} was recorded in T_6 at 4 MAP and 6 MAP, respectively. The higher peroxidase activity of 4.308 activity g^{-1} min^{-1} was recorded in T_5 at 4 MAP and 5.344 activity g^{-1} min^{-1} in T_6 at 6 MAP. SOD activity was found to be significantly higher (0.140 activity g^{-1} min^{-1}) in T_6 at 4 MAP and T_4 to T_6 at 6 MAP, with highest value (0.290 activity g^{-1} min^{-1}) being recorded in T_6.

The observations on curcumin content, volatile oil and oleoresin were recorded at harvest. Curcumin, volatile oil and oleoresin was observed to be significantly higher in treatments T_4, T_5 and T_6. The higher curcumin (2.18 per cent) and oleoresin (8.83 per cent) content were recorded in the treatment with chitosan 3 g l^{-1} and volatile oil (4.50 per cent) in that with chitosan 2.5 g l^{-1}. The foliar spray with higher concentrations of chitosan (2, 2.5 and 3 g l^{-1}) at 3 and 5 MAP enhanced curcumin content by 70 to 80 per cent, volatile oil 68 to 85 per cent and oleoresin by 42 to 58 per cent over the control.

The observations on carbohydrate, starch and reducing sugar were recorded at harvest. The treatments with chitosan 2.5 g l^{-1} and 3 g l^{-1} recorded significantly higher carbohydrate content of 17.76 mg g^{-1} and 18.39 mg g^{-1}, respectively. The significantly higher values for starch content were recorded in treatments, T_3 to T_6. The maximum value of 199.33 mg g^{-1} was observed in the treatment with chitosan 3 g l^{-1}. The plants exposed to chitosan foliar spray with 2.5 g l^{-1} and 3 g l^{-1} registered higher values of 28.67 mg g^{-1} and 28 mg g^{-1}, respectively.

The physiological parameters *viz.*, leaf area index (LAI), stomatal conductance, photosynthetic rate, proline content and cell membrane integrity (CMI) were recorded at 4 and 6 MAP. LAI was found to be significantly higher, 3.58 and 3.29, respectively in treatments T_5 and T_6 at 4 MAP and T_2, T_3, T_4, T_5 and T_6 at 6 MAP, with maximum value (9.06) being recorded in T_5. With respect to stomatal conductance, T_4, T_5 and T_6 gave significantly higher values at both, 4 and 6 MAP. The maximum value of 162.67 and 164.33 mmol m^{-2} s^{-1} being registered in T6 at 4 and 6 MAP, respectively. T_6 recorded significantly higher photosynthetic rate among the treatments tried at both stages of observation and the values were 10.53 and 10.50 μ mol CO_2 m^{-2} s^{-1}. All foliar spray treatments with chitosan recorded significantly higher proline content at 4 MAP and higher value was observed in T_6 (0.259 μmoles g^{-1}). At 6 MAP, maximum proline content recorded in T_6 (1.188 μmoles g^{-1}) and was found to be on par with T_4 and T_5. CMI was found to be significantly superior in T_3, T_4, T_5 and T_6 at 4 MAP, but did not show any variation at 6 MAP. The dry matter production was recorded at 4 MAP, 6 MAP and at harvest. The highest dry matter production was obtained in T_6 at all stages of observation and the values being recorded were 129.67, 201.00 and 239.02 g $plant^{-1}$, respectively and was comparable with T_5. The foliar spray treatments did not show any significant variation with respect to net assimilation rate during the period between 4 and 6 MAP.

The chitosan foliar spray significantly influenced the rhizome yield, crop duration and harvest index of *C. aromatica*. The treatments with chitosan 2.5 g l^{-1} and 3 g l^{-1} recorded significantly higher rhizome yield for both fresh and dry yield. The values recorded were 692.33 and 696.67 g $plant^{-1}$ for fresh yield and 170.95 and 173.27 g $plant^{-1}$ for dry yield. Crop duration and harvest index of plants exposed to chitosan foliar spray were found to be significantly superior to those devoid of chitosan. The minimum duration for crop upto harvest was observed in treatments T_5 and T_6 with 224 days and maximum duration of 229.33 days was observed in the control treatment. The highest value (0.725) for harvest index registered in the treatment with chitosan 3 g l^{-1}.

The uptake of major plant nutrients (N, P and K) were found to be influenced by the chitosan application. The maximum N uptake was recorded in treatment T_5 (3.29 g plant^{-1}) and was comparable with T_3, T_4 and T_6. The higher value for P uptake was noticed in T_6 (0.76 g plant^{-1}) and found to be on par with T_2, T_3, T_4 and T_5. Significantly higher P uptake was observed in treatments T_5 and T_6 and the maximum value was recorded in T_6 (4.27 g plant^{-1}).

In the present study, chitosan application at different concentrations as foliar spray at 3 and 5 MAP elicited plant growth, production of curcumin, volatile oil, oleoresin and yield. The chitosan concentration of 2.5 and 3 g l^{-1} gave maximum enhancement in the yield and metabolite production.

Future line of work

- The alternate methods of chitosan application *viz.,* soil application, seed priming need to be investigated in different spice, medicinal and aromatic crops.
- Effect of chitosan derivatives on growth, physiological attributes, metabolite production and yield of crops need to be investigated.
- Preparation of chitosan nanoparticles and its effect on yield and secondary metabolite production in economically important spice, medicinal and aromatic plants need to investigated.
- Further investigations need to be taken up to study the effect of chitosan on tissue culture of medicinal plants.

References

7. REFERENCES

Abdel-Mawgoud, A. M. R., Tantawy, A. S., El-Nemr, M. A., and Sassine, Y. N. 2010. Growth and yield responses of strawberry plants to chitosan application. *Eur. J. Sci. Res*. 39(1): 161-168.

Abu-Muriefah, S. 2013. Effect of chitosan on common bean (*Phaseolus vulgaris* L.) plants grown under water stress conditions. *Int. Res. J. Agric. Sci. Soil. Sci.* 3(6): 192-199.

Agrawal, G. K., Rakwal, R., Tamogami, S., Yonekura, M., Kubo, A., and Saji, H. 2002. Chitosan activates defense/stress responses in the leaves of *Oryza sativa* seedlings. *Plant Physiol. Biochem*. 40: 1061–1069.

Ahmed, M. E. M. 2015. Response of garlic plants (*Allium sativum* L.) to foliar application of some bio-stimulants. *Egypt. J. Hortic*. 42 (1): 613- 625.

Al- Reza, S. M., Rahman, A., Sattar, M. A., Rahman, M. O., and Fida, H. M. 2010. Essential oil composition and antioxidant activities of *Curcuma aromatic* Salisb. *Food. Chem. Toxicol.* 48:1757-1763.

Ampasavate, C., Warinthip, N., and Yotsawimonwat, S. 2009. Bioencapsulation of *Curcuma aromatica* extract in solid lipid nanoparticles (SLN). In: *Abstracts, XVIIth International Conference on Bioencapsulation,* 24-26, September, 2009, Groningen, Netherlands. Abstract No. 06-2.

Anjusha, S. and Gangaprasad, A. 2014. Phytochemical and antibacterial analysis of two important Curcuma species, *Curcuma aromatica* Salisb. and *Curcuma xanthorrhiza* Roxb. (Zingiberaceae*). J. Pharmacogn. Phytochem.* 3 (3): 50-53

Anoop, K. 2015. *Curcuma aromatica* salisb: a multifaceted spice. *Int. J. Phytopharma. Res.* 6(1): 10-15.

Anusuya, S. and Sathiyabama, M. 2016. Effect of chitosan on growth, yield and curcumin content in turmeric under field condition. *Biocatal. Agric. Biotechnol.* 6: 102–106.

Arnon, D. L. 1949. Copper enzymes in isolated chloroplasts, polyphenols oxidase in *Beta vulgaris. Plant Physiol.* 24: 1-15.

Asghari-zakaria, R., Maleki-zanjani, B. and Sedghi, E. 2009. Effect of *in vitro* chitosan application on growth and minituber yield of *Solanum tuberosum* L. *Plant Soil Environ.* 55(6): 252-256.

Bakiyalakshmi, S.V., Valli, V., and Swarnila, R. D. L. 2016. Isolation and application of chitin and chitosan from crab shell. *Int. J. Curr. Microbiol. Appl. Sci.* 3: 91–99.

Baque, A., Shiragi, H. K., Lee, E. J., and Paek, K. Y. 2012. Elicitor effect of chitosan and pectin on the biosynthesis of anthraquinones, phenolics and flavonoids in adventitious root suspension cultures of *Morinda citrifolia* (L.). *Aust. J. Crop Sci.* 6(9): 1349-1355.

Barka, E. A., Eullaffroy, P., Clement, C., and Vernet, G. 2004. Chitosan improve development, and protects *Vitis vinifera* L. against *Botrytis cinerea. Plant Cell Rep.* 22: 608-614.

Bates, L. S., Waldren, R. P., and Teare, I. D. 1973. Rapid determination of free proline for water-stress studies. *Plant soil* 39(1): 205-207.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Annu. Biochem.* 44: 276-287.

Behboudi, F., Sarvestani, Z. T., Kassaee, M. Z., Sanavi, S. A. M., Sorooshzadeh, A., Ahmadi, S. B. 2018. Evaluation of chitosan nanoparticles effects on yield and yield components of barley (*Hordeum vulgare* L.) under late season drought stress. *J. Water Environ. Nanotechnol.* 3(1): 22-39.

Ben-Shalom, N., Ardi, R., Pinto, R., Aki, C., and Fallik, E. 2003. Controlling gray mould caused by *Botrytis cinerea* in cucumber plants by means of chitosan. *Crop Prot*. 22: 285–290.

Bistgani, Z. E., Siadata, S. A., Bakhshandeha, A., Pirbaloutib, A. G., and Hashemic, M. 2017a. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. *Crop J*. 5(5): 407-415.

Bistgani, Z. E., Siadata, S. A., Bakhshandeha, A., Pirbaloutib, A. G., and Hashemic, M. 2017b. Morpho-physiological and phytochemical traits of (*Thymus daenensis* Celak.) in response to deficit irrigation and chitosan application. *Acta Physiol. Plant*. 39(10): 231.

Bittelli, M., Flury, M., Campbell, G. S., and Nichols, E. J. 2001. Reduction of transpiration through foliar application of chitosan. *Agric. For. Meteorol*. 107: 167–175.

Blum, A. and Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop. Sci.* 21(1): 43-47.

Boonlertnirun, S., Suvannasara, R., Promsomboon, P., and Boonlertnirun, K. 2011. Application of chitosan for reducing chemical fertilizer uses in waxy corn growing. *Thai J. Agric. Sci*. 44: 22–28.

Bradford, M. M. 1976. A rapid and sensitive method for quantification of microgram of protein utilizing the principle of protein dye binding. *Annu. Biochem*. 72: 248.

Cabrera, G., González, D., Silva, C., Bernardo, Y., Gidekel, M., Osorio, J., Taboada, E., Gutiérrez, A., Cabrera, J. C., and Paix, D. 2010. Effect of chitosan on growth and development of highbush blueberry plants. *Adv. Chitin Sci.* 8:9.

Chandrkrachang, S., Sompongchaikul, P., and Sangtain, S. 2005. Profitable spin off from using chitosan in orchid farming in Thailand. *J. of Metals, Mater. Minerals.* 15:45-48.

Chang, J. H., Shin, J. H., Chung, I. S., and Lee, H. J. 1998. Improved menthol production from chitosan-elicited suspension culture of *Mentha piperita. Biotechnol. Lett.* 20 (12): 1097–1099.

Chibu, H. and Shibayama, H. 2001. Effects of chitosan applications on the growth of several crops. In: Uragami, T., Kurita, K., and Fukamizo, T. (eds) *Chitin and chitosan in life science*. Yamaguchi pp 235–239.

Choi, C., Nam, J. P., and Nah, J. W. 2016. Application of chitosan and chitosan derivatives as biomaterials. *J. Ind. Eng. Chem.* 33: 1–10.

Chookhongkha, N., Miyagawa, S., Jirakiattikul, Y., and Photchanachai, S. 2012. Chili growth and seed productivity as affected by chitosan. In:Abstracts, International Conference on Agriculture Technology and Food Sciences; 17–18, Nov. 146–149.

Domard, A. and Domard, M. 2002. Chitosan: structure- properties relationship and biomedical applications. *Polym. Biomater.* 10: 187–212.

Dzung, N. A., Khanh, V. T. P., and Dzung, T. T. 2011. Research on impact of chitosan oligomers on biophysical characteristics, growth development and drought resistance of coffee. *Carbohyd. Polym*. 84: 751–755.

Dzung, P. D., Phu, D. V., Du, B. D., Ngoc, L. S., Duy, N. N., Hiet, H. D., Nghia, D. H., Thang, N. T., Le, B. V., and Hien, N. Q. 2017. Effect of foliar application of oligochitosan with different molecular weight on growth promotion and fruit yield enhancement of chili plant. *Plant Prod. Sci*. 20(4): 389–395.

El-Sawy, N. M., El-Rehim, H. A. A., Elbarbary, A. M., and Hegazy, E. A. 2010. Radiation induced degradation of chitosan for possible use as a growth promoter in agricultural purposes. *Carbohyd. Polym.*79: 555–562.

El-Tantawy, E. M. 2009. Behaviour of tomato plants as affected by spraying with chitosan and aminofort as natural stimulator substances under application of soil organic amendments. *Pak. J. Biol. Sci.* 12: 1164-1173.

Escudero, N., Lopez-Moya, F., Ghahremani, Z., Zavala-Gonzalez, E. A., Alaguero-Cordovilla, A., Ros-Ibañez, C., Lacasa, A., Sorribas, F. J., and Lopez-Llorca, L. V. 2017. Chitosan increases tomato root colonization by *Pochonia chlamydosporia* and their combination reduces root-knot nematode damage. *Front. Plant Sci.* 8:11-12.

Falcon-Rodriguez, A. B., Costales, D., Gonzalez-Pena, D., Morales, D., Mederos, Y., Jerez, E., and Cabrera, J. C. 2017. Chitosans of different molecular weight enhance potato (*Solanum tuberosum* L.) yield in a field trial. *Span. J. Agric. Res.* 15: e0902.

Farouk, S. and Amany, A. R. 2012. Improving growth and yield of cowpea by foliar application of chitosan under water stress. *Egyptian J. Biol.* 14: 14-26.

Farouk, S., Ghoneem, K. M., and Abeer. A. A. 2008. Induction and expression of systematic resistance to downy mildew disease in cucumber plant by elicitors. *Egyptian J. Phytopathol.* 1-2: 95-111.

Farouk, S., Mosa, A. A., Taha, A. A, Ibrahim, H. M., and EL-Gahmery, A. M. 2011. Protective effect of humic acid and chitosan on radish (*Raphanus sativus* L. *var. sativus*) plants subjected to cadmium stress. *J. Stress Physiol. Biochem.* 7(2): 99-116.

Gopichand, M., Singh, R. D., Meena, R. L., Singh, M. K., Kaul, V. K., Lal, B., Acharya, R., and Prasad, R. 2006. Effect of manure and plant spacing on crop growth, yield and oil-quality of *Curcuma aromatica* Salisb. in mid hill of western Himalaya. *Ind. Crop Prod.* 24: 105–112.

Gorelick, J. and Bernstein, N. 2014. Elicitation: An underutilized tool in the development of medicinal plants as a source of therapeutic secondary metabolites. *Adv. Agron.* 124: 201-230.

Gorelick, J., Rosenberg, R., Smotrich, A., Hanus, L., and Bernstein, N. 2015. Hypoglycemic activity of withanolides and elicitated *Withania somnifera*. *Phytochem.* 116: 283–289.

Gornik, K., Grzesik, M., and Romanowska-Duda, B. 2008. The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. *J. Fruit Ornamental Plant Res*. 16: 333–343.

Guan, Y. J., Hu, J., Wang, X. J., and Shao, C. X. 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univ. Sci.* 10: 427–433.

Guo, H. L., Du, Y. G., Bai, X. F., and Zhao, X. M. 2003. Effects of active oxygen on suspended cotton cell culture by oligochitosan. *Chin. J. Mar. Drugs* 1:11–12.

Hadwiger, L. A., Klosterman, S. J., and Choi, J. J. 2002. The mode of action of chitosan and its oligomers in inducing plant promoters and developing disease resistance in plants, In: Suchiva, K., Chandrkrachang, S., Methacanon, P., and Peter, M. G. (Eds.), *Advances in Chitin Science,* vol. 5, Bangkok, pp. 452–457.

Hashimoto, T., Yun, D. J., and Yamada, Y. 1993. Production of tropane alkaloids in genetically engineered root cultures. *Phytochem*. 32: 713–718.

Hedge, J. E. and Hofreiter, B. T. 1962. In: Whistler, R. L. and Be Miller, J. N. (eds), *Methods in Carbohydrate Chemistry*. Vol. 17, Academic Press, New York, 420 p.

Ibraheim, S. K. A. and Mohsen, A. A. M. 2015. Effect of chitosan and nitrogen rates on growth and productivity of summer squash plants. *Middle East J.Agric. Res.* 4 (4): 673-681.

Ionkova, I. 2007. Biotechnological approaches for the production of lignans. *Pharmacogn. Rev*. 1: 427-438.

Iriti, M., Picchi, V., Rossoni, M., Gomarasca, S., Ludwig, N., Gargano, M., and Faoro, F. 2009. Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. *Environ. Exp. Bot.* 66:493–500.

Jackson, M. L. 1973. *Soil chemistry analysis*. Prentice Hall of India Pvt. Ltd., New Delhi, 498p.

Jalgaonkar, S. P. 2016. Effect of manures and fertilizers on growth, yield and medicinal parameters of turmeric (*Curcuma aromatica* Salisb.) as an intercrop under coconut plantation in konkan region of Maharashtra. M. Sc. (Forestry) thesis, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, 81p.

Janmohammadi, M., Mostafavi, H., Kazemi, H., Mahdavinia, G., and Sabaghnia, N. 2014. Effect of chitosan application on the performance of lentil genotypes under rainfed conditions. *Acta Technologica Agric.* 4:86–90.

Kananont, N., Pichyangkura, R., Chanprame, S., Chadchawan, S., and Limpanavech, P. 2010. Chitosan specificity for the in vitro seed germination of two Dendrobium orchids (Asparagales: Orchidaceae). *Sci. Hortic*. 124: 239–247.

Karimi, S., Abbaspour, H., Sinaki, J. M., and Makarian, H. 2012. Effects of water deficit and chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean (*Ricinus communis* L.). *J. Physiol. Biochem.* 8:160–169.

Katiyar D., Hemantaranjan, A., Bharti, S., and Bhanu, A. N., 2014. A Future perspective in crop protection: chitosan and its oligosaccharides. *Adv. Plants Agric. Res.* 1(1): 04-06.

KAU (Kerala Agricultural University) 2013. *Package of Practices Recommendations (Adhoc) for Organic Farming: Crops*. Kerala Agricultural University, Thrissur, 200p.

Ke, L., Yang, L. X., and LiSha, P. 2001. Effects of carboxymethyl chitosan on key enzymes activities of nitrogen metabolism and grain protein contents in rice. *J. Hunan Agric. Univ*. 27:421-424.

Khan, M. H., Singha, K. L. B., and Panda, S. K. 2002. Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl salinity stress. *Acta Physiologia Plant*. 24:145–148.

Khan, W. M., Prithiviraj, B., and Smith, D. L. 2002. Effect of foliar application of chitin and chitosan oligosaccharides on photosynthesis of maize and soyabean. *Photosynth*. 40 (4): 621–624.

Kim, H. J., Chen, F., Wang, X., and Rajapakse, N. C. 2005. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* 53: 3696-3701.

Kowalski, B., Terry, J. F., Herrera, L., and Penalver, A. D. 2006. Application of soluble chitosan in vitro and in the greenhouse to increase yield and seed quality of potato minitubers. *Potato Res*. 49: 167–176.

Kra, K. D., Gogbeu, S. J., Kafana Soro, K., Kouakou, K. J., Kouassi, K. N., and Dogbo, D. O. 2019. Effects of chitosan on vegetative organs growth and peroxidases activities in cassava (*Manihot esculenta* Crantz) cultivars YACE, 9620A, TMS4(2)1425 and TMS30572. *Trop. Plant Res*. 6(1): 08–14.

Kumar, A., Singh, D. P., and Singh, P. 1994. Influence of water stress on photosynthesis, transpiration, water use efficiency and yield of *Brassica juncea* L. *Field Crops Res.* 37: 95-101.

Lee, S., Choi, H., Suh, S., Doo, I. S., Oh, K. Y., Choi, E. J., Taylor, A. T. S., Low, P. S., and Lee, Y. 1999. Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from

guard cells of tomato and *Commelina communis*. *Plant physiol.* 121(1): 147-152.

Lei, C., Maa, D., Pua, G., Qiua, X., Dua, Z., Wanga, H., Li, G., Yea, H., and Liua, B. 2011. Foliar application of chitosan activates artemisinin biosynthesis in *Artemisia annua* L. *Ind. Crop Prod.* 33: 176–182.

Li, Y., Wo, J. M., Liu, Q., Li, X., and Martin, R. C. G. 2009. Chemoprotective effects of *Curcuma aromatica* on esophageal carcinogenesis. *An. Surg. Oncol.* 16(2): 515–523.

Loschke, D. C., Hadwiger, L. A., and Wagoner, W. 1983. Comparison of mRNA populations coding for phenylalanine ammonia lyase and other peptides from pea tissue treated with biotic and abiotic phytoalexin inducers. *Physiol. Plant Pathol.* 23: 163–173.

Luck, H. 1974. *Methods of enzymology*. Academic Press, New York, 885p.

Ma, L. J., Li, Y. Y., Yu, C. M., Wang, Y., Li, X. M., Li, N., Chen, Q., and Bu, N. 2014. Germination and physiological response of wheat (*Triticum aestivum*) to pre-soaking with oligochitosan. *Int. J. Agric. Biol.* 16: 766–770.

Mahdavi, B. 2013. Seed germination and growth responses of Isabgol (*Plantago ovata* Forsk) to chitosan and salinity. *Int. J. Agric. Crop Sci.* 5:1084–1088.

Mahdavi, B., Sanavy, M., Aghaalikhani, M., Sharifi, M., and Dolatabadian, A. 2011. Chitosan improves osmotic potential tolerance in safflower (*Carthamus tinctorius* L.) seedlings. *J. Crop Improv.* 4:728–741.

Mahmood, N., Abbasi, N. A., Hafiz, I. A., Ali, I., and Zakia, S. 2017. Effect of biostimulants on growth, yield and quality of bell pepper cv. Yolo wonder. *Pak. J. Agric. Sci.* 54(2): 311–317.

Malayamana, V., Sisubalan, N., Senthilkumar, R. P., Sheik Mohamed, S., Ranjithkumar, R., and Ghouse Basha, M. 2017. Chitosan mediated

enhancement of hydrolysable tannin in *Phyllanthus debilis* Klein exWilld via plant cell suspension culture. *Int. J. Biol. Macromol.* 104: 1656–1663.

Malekpoor, F., Pirbalouti, A. G., and salami, A. 2016. Effect of foliar application of chitosan on morphological and physiological characteristics of basil under reduced irrigation. *Res. Crops* 17(2): 354-359.

Malerba, M. and Cerana, R. 2017. Recent advances of chitosan applications in plants. *Ploym.* 10: 118.

Mandal, S. 2010. Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. *Afr. J. Biotechnol.* 9 (47): 8038–8047.

Mc Cready, R. M., Guggolz, J., Silviera, V., and Owens, H. S. 1950. Determination of starch and amylose in vegetables. *Anal. Chem.* 22: 1156.

Mehregan, M., Mehrafarin, A., Labbafi, M. R., and Naghdibadi, H. 2017. Effect of different concentrations of chitosan biostimulant on biochemical and morphophysiological traits of stevia plant (*Stevia rebaudiana* Bertoni). *J. Med. Plants.* 16 (62): 169–181.

Meng, X., Li, B., Liu, J., and Tian, S. 2008. Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. *Food Chem.* 106: 501–508.

Mondal, M. A., Puteh, A. B., and Dafader, N. C. 2016. Foliar application of chitosan improved morpho-physiological attributes and yield in summer tomato (*Solanum lycopersicum*). *Pak. J. Agric. Sci.* 53(2): 339-344.

Mondal, M. M. A., Malek, M. A., Puteh, A. B., Ismail, M. R., Ashrafuzzaman, M., and Naher, L. 2012. Effect of foliar application of chitosan on growth and yield in okra. *Aust. J. Crop Sci.* 6: 918–921.

Mondal, M. M. A., Rana, M. I. K., Dafader, N. C., and Haque, M. E. 2011. Effect of foliar application of chitosan on growth and yield in Indian spinach. *J. Agrofor. Environ*. 5: 99–102.

Moradshahi, A., Andari, B. S. E., and Kholdebarin, B. 2004. Some physiological responses of canola (*Brassica napus* l.) to water deficit stress under laboratory conditions. *Iranian J. Sci. Technol.* 28(1):43-50.

Mutka, J. A., Rahman, M., Sabir, A. A., Gupta, D. R., Surovy, M. Z., Rahman, M., and Islam, M. T. 2017. Chitosan and plant probiotics application enhance growth and yield of strawberry. *Biocatal. Agric. Biotechnol.* 11: 9–18.

Naderi, S., Khajeh, H., and Ahmadi, H. 2015. The effect of chitosan on some morphological indices in *Lepidium sativum* L. In: *Abstracts of conference in medicinal herbs and herbal medicines,* Tehran, Center for the sustainable development of science and technology, Farzin.

Namdeo, A. 2007. Plant cell elicitation for production of secondary metabolites. *Pharmacogn. Rev*. 1: 320-345.

Nge, K. L., Nwe, N., Chandrkrachang, S., and Stevens, W. F. 2006. Chitosan as a growth stimulator in orchid tissue culture. *Plant Sci*. 170: 1185–1190.

O'Herlihy, E. A., Duffy, E. M., and Cassells, A. C. 2003. The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from microplants. *Folia Geobot.* 38: 201–207.

Ohta, K., Morishita, S., Suda, K., Kobayashi, N., and Hosoki, T. 2004. Effects of chitosan soil mixture treatment in the seedling stage on the growth and flowering of several ornamental plants. *J. Jpn. Soc. Hortic. Sci*. 73(1): 66-68.

Orlita, A., Sidwa-Gorycka, M., Paszkiewicz, M., Malinski, E., Kumirska, J., Siedlecka, E. M., Łojkowska, E., and Stepnowski, P. 2008. Application of

chitin and chitosan as elicitors of coumarins and furoquinolone alkaloids in *Ruta graveolen s*L.(common rue). *Biotechnol. Appl. Biochem.* 51(2): 91-96.

Pant, N., Misra, H., and Jain, D. C. 2013. Phytochemical investigation of ethyl acetate extract from *Curcuma aromatica* Salisb rhizomes. *Arab. J. Chem.* 6: 279-283.

Park, B. K. and Kim, M. M. 2010. Applications of chitin and its derivatives in biological medicine. *Int. J. Mol. Sci.* 11: 5152–5164.

Peru, N. G. 1962. Measurement of peroxidase activity in plant tissues. *Curr. Sci.* 31: 71-81.

Phothi, R. and Theerakarunwong, C. D. 2017. Effect of chitosan on physiology, photosynthesis and biomass of rice (*Oryza sativa* L.) under elevated ozone. *Aust. J. Crop Sci.* 11: 624–630.

Pichyangkuraa, R. and Chadchawanb, S. 2015. Biostimulant activity of chitosan in horticulture. *Sci. Hortic.* 196: 49-65.

Pliankong, P., Suksa-Ard, P., and Wannakrairoj, S. 2018. Chitosan elicitation for enhancing of vincristine and vinblastine accumulation in cell culture of *Catharanthus roseus* (L.). *J. Agric. Sci.* 10(12): 287-293.

Pornpienpakdee, P., Singhasurasak, R., Chaiyasap, P., Pichyangkura, R., Bunjongrat, R., Chadchawan, S., and Limpanavech, P. 2010. Improving the micropropagation efficiency of hybrid Dendrobium orchids with chitosan. *Sci. Hortic.* 124: 490–499.

Possingham, J. V. 1980. Plastid replication and development in the life cycle of higher plants. *Annu. Rev. Plant Physiol.* 31: 113–129.

Rahman, M., Mukta, J. A., Sabir, A. A., Gupta, D. R., Mohi-Ud-Din, M., Hasanuzzaman, M., Miah, M. G., Rahman, M., and Islam, M. T. 2018. Chitosan biopolymer promotes yield and stimulates accumulation of

antioxidants in strawberry fruit. PLoS ONE [e-journal]. Available: https://doi.org/10.1371/journal.pone.0203769. [10 Sept. 2018].

Randhawa, G. S., Mahey, R. K., and Gill, S. R. S. 1985. Leaf area measurements in turmeric. *J. Res. Punjab Agric. Univ.* 22: 163-166.

Ray, S. R., Bhuiyan, M. J. H., Hossain, M. A., Hasan, A. K., and Sharmin, S. 2016. Chitosan ameliorates growth and biochemical attributes in mung bean varieties under saline condition. *Res. Agric. Livest Fish* 3(1):45–51.

Revathy, S. and Malathy, N. S. 2013. Antibacterial activity of rhizome of *Curcuma aromatica* and partial purification of active compounds. *Ind. J. pharma. Sci.* 75(6): 732-735.

Sadasivam, S. and Manickam, A. 2008. *Biochemical methods*. New Age International Publishers, New Delhi, 256 p.

Salachna, P. and Zawadzinska, A. 2014. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. *J. Ecol. Eng*. 15 (3): 97–102.

Salehi, S., Rezayatmand, Z., and Pirbalouti, A. G. 2017. The effect of foliar application of chitosan on yield and essential oil of savory (*Satureja isophylla* L.) under salt stress. *J. Herb. Drugs*. 8(2): 101-108.

Sathiyabama, M., Akila, G., and Charles, R. E. 2014. Chitosan-induced defence responses in tomato plants against early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer. *Arch. Phytopathol. Plant Prot.* 47(16): 1963-1973.

Sathiyabama, M., Bernsteinb, N., and Anusuyaa, S. 2016. Chitosan elicitation for increased curcumin production and stimulation of defence response in turmeric (*Curcuma longa* L.). *Ind. Crop Prod.* 89: 87–94.

Shamim, A., Mohammed, A., Ansari, S. H., and Ahmed, F. 2011. Phytoconstituents from the rhizomes of *Curcuma aromatica* Salisb. *J. Saudi Chem. Soc*. 15: 287-290.

Sharif, R., Mujtaba, M., Rahman, M., Shalmani, A., Ahmad, H., Anwar, T., Tianchan, D., and Wang, X. 2018. The multifunctional role of chitosan in horticultural crops: a review. *Mol.* 23(4): 872.

Sheheta, S. A., Fawzy, Z. F., and El-Ramady, H. R. 2012. Response of cucumber plants to foliar application of Chitosan and yeast under greenhouse conditions. *Aust. J. Basic Appl. Sci.* 6: 63–71.

Sheikha, S. A. and Al-Malki, F. M. 2011. Growth and chlorophyll responses of bean plants to chitosan applications. *Eur. J. Sci. Res.* 50(1): 124-134.

Sikha, A., Harini, A., and Prakash, H. L. 2015. Pharmacological activities of wild turmeric (*Curcuma aromatica* Salisb): a review. *J. Pharmacog. Phytochem.* 3(5): 01-04.

Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.* 195: 19-23.

Song, S. Q., Sang, Q. M., and Guo, S. R. 2006. Physiological synergisms of chitosan on salt resistance of cucumber seedlings. *Acta Botanica Boreali-OccidentaliaSinica.* 26: 435–441.

Srisornkompon, P., Pichyangkura, R., and Chadchawan, S. 2014. Chitosan increased phenolic compound contents in tea (*Camellia sinensis*) leaves by pre-and post-treatments. *J. Chitin Chitosan Sci.* 2(2): 93–98.

Sultana, S., Islam, M., Khatun, M. A., Hassain, M. A., and Huque, R. 2017. Effect of foliar application of oligo-chitosan on growth, yield and quality of tomato and eggplant. *Asian J. Agric. Res.* 11: 36-42.

Tsugita, T., Takahashi, K., Muraoka, T., and Fukui, H. 1993. The application of chitin/chitosan for agriculture. In: Proceedings of the Special Session of the 7th Symposium on Chitin and Chitosan; Japanese Society for Chitin and Chitosan: Fukui, Japan, 1993; pp. 21–22. (In Japanese).

Van, S. N., Minh, H. D., and Anh, D. N. 2013. Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. *Biocatal. Agric. Biotechnol.* 2: 289–294.

Wanichpongpan, P., Suriyachan, K., and Chandrkrachang, S. 2001. Effect of chitosan on the growth of Gerbera flower plant (*Gerbera jamesonii*). In: *Chitin and chitosan: Chitin and Chitosan in Life Science*, Yamaguchi, Japan, 198-201 pp.

Williams, R. F. 1946. The physiology of plant growth with special reference to the concept of net assimilation rate. *Ann. Bot.* 10: 41-72.

Yadav, M., Goswami, P., Paritosh, K., Kumar, M., Pareek, N., and Vivekanand, V. 2019. Seafood waste: a source for preparation of commercially employable chitin/chitosan materials. *Bioresour. Bioprocess.* 6: 8.

Yahyaabadi, H. M., Asgharipour, M. R., and Basiri, M. 2016. Role of chitosan in improving salinity resistance through some morphological and physiological characteristics in fenugreek (*Trigonella foenum graecum* L.). *J. Sci. Technol. Greenhouse. Cult.* 7(25):165–174.

Yang, F., Hu, J., Li, J., Wu, X., and Qian, y. 2009. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. *Plant Grow. Regul.* 58:131–136.

Yin, H., Bai, X. F., and Du, Y. G. 2008. The primary study of oligochitosan inducing resistance to *Sclerotinia scleraotiorum* on *Brassica napus*. *J. Biotechnol.* 136: 600–601.

Yu, J., Zhou, X., He, X., Dai, M., and Zhang, Q. 2011. Curcumin induces apoptosis involving bax /bcl-2 in human hepatoma SMMC-7721 cells. *Asian Pac. J. Cancer Prev.* 12(8): 1925-1929

174691

Zagzog, O. A., Gad, M. M., and Hafez, N. K. 2017. Effect of nano-chitosan on vegetative growth, fruiting and resistance of malformation of mango. *Trends Hortic. Res.* 6: 673- 681.

Zeng, D. and Luo, X. 2012. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. *Open J. Soil Sci.* 2(03): 282-288.

Zhang, Y., Mian, M. R., and Bouton, J. H. 2006. Recent molecular and genomic studies on stress tolerance of forage and turf grasses. *Crop Sci.* 46: 497–51.

Zhao, J., Davis, L. C., and Verpoorte, R. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.* 23:283–333.

Zong, H., Liu, S., Xing, R., Chen, X., and Li, P. 2017. Protective effect of chitosan on photosynthesis and antioxidative defense system in edible rape (*Brassica rapa* L.) in the presence of cadmium. *Ecotoxicol. Environ. Saf.* 138: 271-278.

CHITOSAN MEDIATED METABOLITE ELICITATION AND GROWTH RESPONSES IN KASTHURI TURMERIC (*Curcuma aromatica* Salisb.)

By

NIVYA J THENGUMPALLY
(2017-12-013)

ABSTRACT

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN HORTICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF AGRICULTURE, VELLAYANI
THIRUVANANTHAPURAM-695 522
KERALA, INDIA

2019

ABSTRACT

The present investigation entitled "Chitosan mediated metabolite elicitation and growth responses in kasthuri turmeric (*Curcuma aromatica* Salisb.)" was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani during 2017-2019 with the objective to study the effect of different concentrations of chitosan on plant growth, yield and metabolite production in *Curcuma aromatica.*

The planting material for the study was obtained from Instructional Farm, College of Agriculture, Vellayani. The experiment was laid out in completely randomized block design (CRD) with nine treatments and three replications. The treatments consisted of foliar spray with different concentrations of chitosan and control treatments *viz.*, chitosan 0.5 g l^{-1} (T_1), chitosan 1 g l^{-1} (T_2), chitosan 1.5 g l^{-1} (T_3), chitosan 2 g l^{-1} (T_4), chitosan 2.5 g l^{-1} (T_5), chitosan 3 g l^{-1} (T_6), acetic acid (0.25 per cent) spray (T_7), water spray (T_8) and control (T_9). The treatments were given at 3 and 5 months after planting (MAP).

The plant growth parameters *viz.*, plant height, number of tillers, number of leaves, leaf area and shoot weight were recorded at 4 and 6 MAP. All chitosan foliar spray treatments resulted in significantly taller plants compared to treatments devoid of chitosan at 6 MAP, with the highest value (109.91 cm) in T_6. T_5 and T_6 recorded significantly higher number of leaves at 4 MAP while at 6 MAP, it was recorded in treatments, T_2 to T_6. Similar trend was observed with leaf area also. T_4 to T_6 recorded significantly higher shoot weight at 4 MAP and T_5 to T_6 at 6 MAP. The highest dry weight (42.69 g) was recorded in T_5 and 63.0 g in T_6 at 4 MAP and 6 MAP, respectively. Number of tillers did not show any variation among treatments at both stages of observation. The plant growth parameters *viz.*, rhizome spread, rhizome thickness, number of fingers, root

length, root spread and root weight were recorded at 4 MAP, 6 MAP and at harvest. Significant enhancement in rhizome spread was observed only at harvest, in T_6, which was on par with T_5. The higher concentrations of chitosan foliar spray significantly influenced rhizome thickness at 6 MAP and at harvest; significantly higher values were observed in treatments, T_2 to T_6 at 6 MAP and T_3 to T_6 at harvest. At all stages of observation, number of fingers was found to be significantly higher in T_5 and T_6. Root length and root spread were significantly higher with the treatments T_5 and T_6 at 6 MAP and at harvest. Significant variation in root weight was observed in *C. aromatica* only at 6 MAP.

The observations on plant metabolites *viz.*, chlorophyll content, total proteins and defence enzymes were recorded at 4 and 6 MAP. T_4, T_5 and T_6 were observed to have significantly higher chlorophyll content among the treatments, at 6 MAP. Total proteins and defense enzymes were observed to give significant variation at both stages of observation. Protein content was found to be significantly higher (6.89 mg g^{-1}) in T_5 at 4 MAP and in treatments T_4 to T_6 at 6 MAP, the highest value being recorded in T_6 (8.46 mg g^{-1}). Catalase and peroxidase activity were found significantly higher in T_5 and T_6 at both stages of observation. SOD activity was found to be significantly higher in T_6 at 4 MAP and T_4 to T_6 at 6 MAP. The observations on curcumin content, volatile oil, oleoresin and carbohydrate content were recorded at harvest. Curcumin, volatile oil and oleoresin was observed to be significantly higher in treatments T_4, T_5 and T_6. T_5 and T_6 recorded significantly higher carbohydrate content among the various treatments tried.

The physiological parameters *viz.,* leaf area index (LAI), stomatal conductance, photosynthetic rate, proline content and cell membrane integrity (CMI) were recorded at 4 and 6 MAP. LAI was found to be significantly higher in treatments T_5 and T_6 at 4 MAP and T2 to T6 at 6 MAP. With respect to stomatal conductance, T_4, T_5 and T_6 gave significantly higher values at both, 4 and 6 MAP. T_6 recorded significantly higher photosynthetic rate among the treatments tried at

both stages of observation. All foliar spray treatments with chitosan recorded significantly higher proline content at 4 MAP and T_4 to T_6 at 6 MAP. CMI was found to be significantly superior in T_3 to T_6 at 4 MAP, but did not show any variation at 6 MAP. The dry matter production was recorded at 4 MAP, 6 MAP and at harvest. The highest dry matter production was obtained in T_6 at all stages of observation and was comparable with T_5. The foliar spray treatments did not show any significant variation with respect to net assimilation rate during the period between 4 and 6 MAP.

The chitosan foliar spray significantly influenced the rhizome yield, crop duration and harvest index of *C. aromatica*. T_5 (170.95 g plant^{-1}) and T_6 (173.27 g plant^{-1}) were found significantly superior to all other treatments with regard to rhizome yield. Crop duration and harvest index of plants exposed to chitosan foliar spray were found to be significantly superior to those devoid of chitosan. Uptake of major plant nutrients (N, P and K) were found to be maximum in T_5 and T_6.

In the present study, chitosan application at different concentrations as foliar spray at 3 and 5 MAP elicited plant growth, production of curcumin, volatile oil, oleoresin and yield. The chitosan concentration of 2.5 and 3 g l^{-1} gave maximum enhancement in the yield and metabolite production.

174691