Original Research Article

Application of Chitin ImprovesGrowth, Yield and Secondary Metabolite Production in Turmeric (*Curcuma longa* L.)

ABSTRACT

A field experiment was conducted in Trivandrum district during June 2021 to January 2022 to evaluate the effect of chitin on growth, yield and secondary metabolite production in turmeric. Soil application of chitin at 5g per plant was done at 60 and 180 days after transplanting, while untreated plants served as control. Observations were recorded on effect of chitin growth, defense enzymes, yield, secondary metabolitesand disease incidence. Results of the study confirmed significant enhancement in growth, yield and secondary metabolite production on chitin application compared to the untreated control. Significant increase in shootweight and rhizome weight was observed at six months after transplanting in response to chitin application. Fresh and dry rhizome yield per plant increased by 60.16% and 65.97% respectively, over the control.The defense enzymes activity and chlorophyll content were significantly higher inplants subjected to chitin application, compared to the control. Chitin treatment enhanced volatile oil and oleoresin content by 1.39 and 1.41 times and curcumin content by 21% over the control. The results confirmed that chitin is a potent biostimulant that can be used for growth, yield and quality enhancement in turmeric.

Key words: Turmeric, Chitin, Yield, Curcumin, Sobha

1.INTRODUCTION

Turmeric (*Curcuma longa* L.), a member of family Zingiberaceae is an annual spice crop contributing to the national economy of India. Turmeric has very long history of medicinal, culinary and religious use. There is agrowing demand for this spice crop due to the wide variety of uses it can be put to in the present era as spice, herbal medicine, dyeing agent and cosmetic [1]. Thus, it is inevitable to enhance the production of quality turmeric to meet the increasing market demand. Organically produced turmeric powder and its value added products have great demand in various industries, especially pharmaceutical, cosmetic and food industries. Chitin, a potential biostimulant that enhance plant growth and development, could be included in organic production system, as it is approved by IFOAM[2]. Chitin, is a biopolymer abundant in nature and is a byproduct of seafood processing industry. Chitin could be sourced from the cell wall of fungi and algae, arthropod shells, exoskeleton of crustaceans such as shrimp and crabs, and shell and cartilage of shellfish molluscs[3]. It is the second most abundant polysaccharide after cellulose [4]. It is said to be cheap, ecofriendly, biodegradable and with biocompatible properties. Global annual generation of chitinous waste is 1.5 million tonnes, 12% of which accounts to global fish industry, especially crab and shrimp shells [5]. Application of chitin in crop production would be an initiative towards alleviation of environmental pollution. Being a nutrient supplement and an enhancer of plant defense system, chitin could bring down the use of chemicals in agriculture [6]. Soil amended with chitin has been reported to decrease nematode infection [7] and to confer host resistance against fungal soil borne pathogens [8, 9].

The addition of chitin was reported to increase the population of plant growth promoting rhizobacteria (PGPR) and fungi in the rhizosphere of the plant [10]. Chitin based soil amendment has been linked to triggering plant defense mechanism and hence, considered as an alternative to chemical treatments of soil disinfection [11]. This also has been observed to reduce soil-borne diseases [12]. Hence, application of chitincould be considered as a good agricultural practice (GAP) for turmeric.Chitin amendment improves soil quality, as it depolymerizes through chitinase activity, and its decompositionis facilitated by microorganisms present in the soil [13]. According to Fathima *et al*. [14],the application of bio-fertilizers from marine waste as chitin/chitosan would be a sustainable agricultural practice, as it is rich in nitrogen and phosphorus. Jiménez-Gómez and Cecilia [15] are of the view that its applications in agriculture are limited due to its low solubility. However, its positive influence on plant growth has been established in various crops [12,16,17,18,19]. The present study was undertaken to evaluate the effect of soil application of chitin on growth, yield and secondary metabolite production in turmeric.

2. MATERIALS AND METHODS

The field experiment was conducted in farmer's field in Trivandrum district during the period from June 2021 to January 2022. The variety, Sobha released from Kerala Agricultural University was used for the study. Theprotray plantlets were transplanted to the main field at a spacing of 25cm x 25cm in plots of size 1.5m x 1m. Chitin and control treatments were replicated 10 times and under each replication there are 24number of plants. The crop was raised organically as per package of practices recommendations (organic) [20].Chitin was applied at the rate of 5 g per plant,at a distance of 5 to 10 cm from the base of the plant at 60 and 180 days after transplanting. The plants grown without any chitin treatment served as control.Chitin for this experiment was procured from MatsyafedChitin and Chitosan Plant, Neendakara, Kollam, Kerala.The plant growth parameters *viz.*, plant height (cm), number of leaves, leaf area (cm^2),shoot weight (g)and rhizome weight (g) were recorded at 4 and 6 months after transplanting (MAT). Peroxidase and polyphenol oxidase activity were assayed at 6 MAT from leaf sample as per the procedureof Srivastava [21].The chlorophyll content of leaf sample obtained at 6 MATwas estimated by DMSO method assuggested by Arnon [22]. Plants were harvested at 7 MATand yield per plant was determined in terms of rhizome (fresh and dry) weight (g)and number of fingers were counted.Curcumin content of the rhizome of chitin treated and control plants were estimated by HPTLC method.Coarsely groundpowder of dried rhizomewas subjected to hydrodistillation for 3 h using Clevenger distillationapparatus for estimating volatileoil [23]. Oleoresin content was estimated using ground rhizome powderby Soxhlet extraction for 5 h using acetone as extraction solvent[24]. Crop was monitored throughout the growth period for disease incidence and intensityof leaf blotch diseasewas assessed using 0 to 6 disease rating scale[25].The mean values of growth, yield and quality parameters were compared using t-testin statistical package KAU GRAPES[26].

3. RESULT AND DISCUSSION

3.1 Effect of chitin on plant growth parameters

Soil application of chitin at the rate of 5 g per plant enhanced the plant growth parameters in turmeric over the control. The height of chitin treated plants were 97.54 cm and 108.18 cm at 4 and 6 MAT, whereas the control plants recorded92.52 cm and 95.81 cm respectively (Fig 1).Rajkumar *et al*. [16]examined the plant growth promotion effect of chitin in pepper plants and noticed the increase in growth compared to control. The shoot length in lemon balm (*Melissa officinalis* L.) was increased by 43% when grown in medium with chitincompared to control [18].

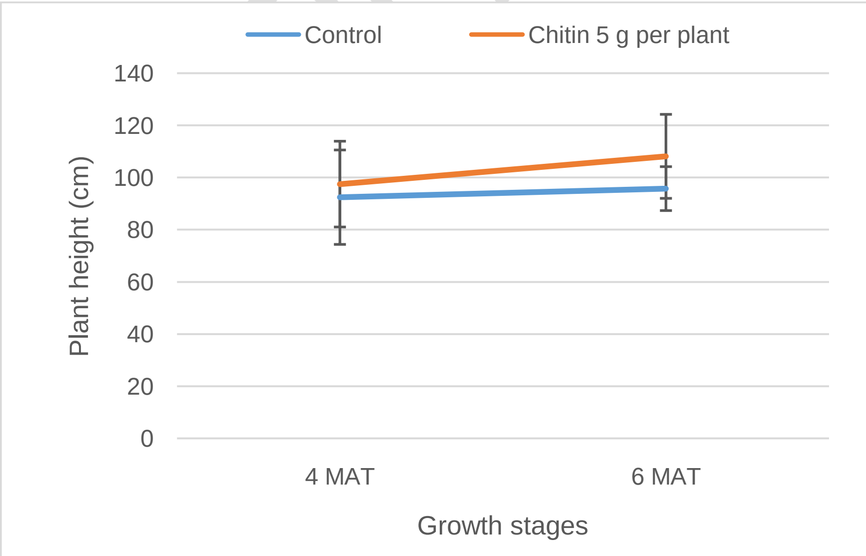

Fig 1. Effect of chitin application on plant height in turmeric at 4 and 6 MAT

In control plants, the number of leaves increased from 10.72 at 4 MAT to 13.85 at 6 MAT, whereas in chitin treated plants, itincreasedfrom 11.20 to 14.75 (Fig 2). At 4 MAT, the leaf area was 291.42 cm^2 and 272.11 cm^2 in treated and control plants respectively. At 6 MAT, the chitin treated plants recorded a significantly higher (p = 0.00) leaf area of 354.95 cm^2 compared to control in which the leaf area was327.71 cm^2 (Fig 3).Sriwichai*et al*.[27]observed a significantly higher leaf number in chitin treated plants compared to control in black sesame (*Sesamum indicum* L.). Chitin 5, 10 and 15 g recorded a leaf number of 20.47, 20.4 and 20.2 and control plants recorded 15.33 number of leaves in *S. indicum*.

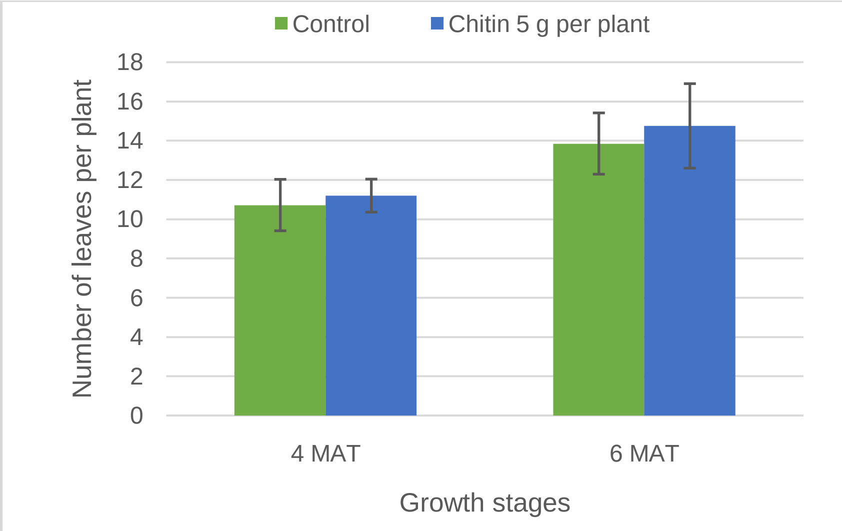

Fig 2. Effect of chitin application on number of leaves per plant in turmeric at 4 and 6 MAT

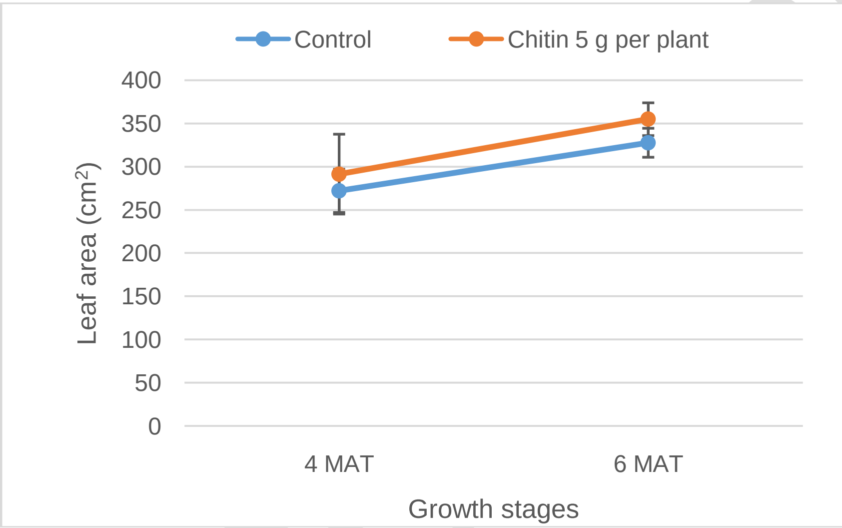

Fig 3. Effect of chitin application on leaf area in turmeric at 4 and 6 MAT

Fresh and dry shoot weight per plant were recorded at 4 and 6 MAT. Fresh shoot weight at 4 MAT recorded a significantly higher (p = 0.00) value in treated plants (104.52 g) when compared to control (77.38 g). The dry shoot weight was 16.76 g in treated plants and 14.22 g in control. At 6 MAT, a significantly higher (p = 0.02) fresh shoot weight of 127.51 g was recorded in chitin treated plants compared to control (117.88 g). The dry shoot weight also recorded a significantly higher (p = 0.00) value in treated plants (26.85 g) and the control plants recorded a dry shoot weight of 17.44 g (Fig 4). Debodeet *al*.[13]reported that addition of chitin 2 % significantly increased the fresh weight (213.00 ± 18.76 g per plant) of lettuce plants, compared with 172.08 ± 17.75 g per plant in the control.

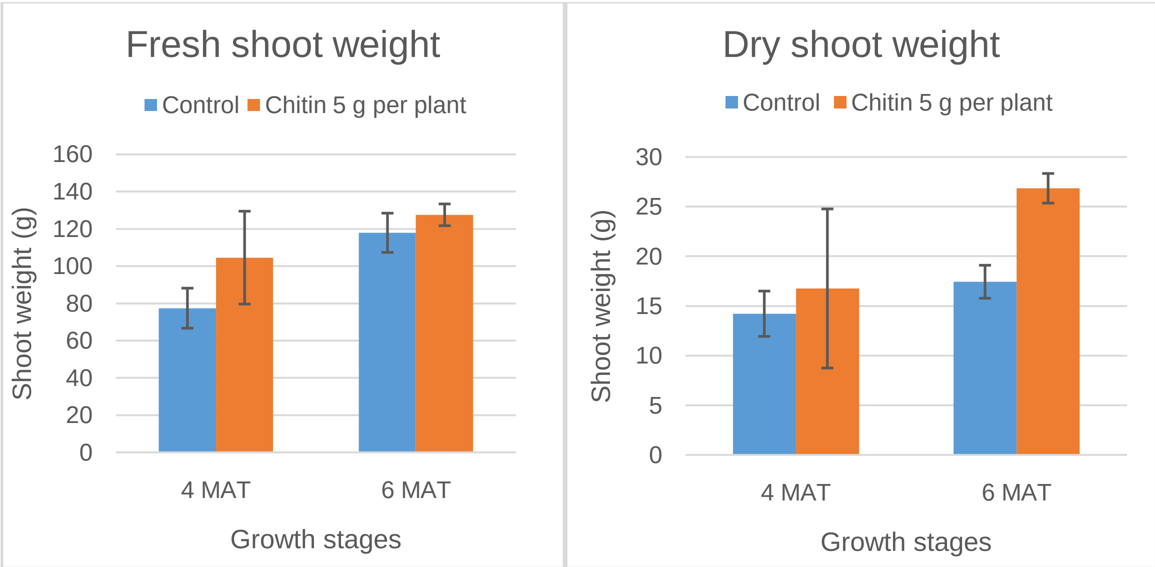

Fig 4. Effect of chitin application on shoot weight in turmeric at 4 and 6 MAT

A significantly higher fresh (*p* = 0.00) and dry (*p* = 0.11) rhizome weight per plant at 4 MAT (58.20 g fresh and 7.13 g dry) was recorded in plants from chitin treated plot when compared to control (44.24 g fresh and 5.98 g dry). At 6 MAT, fresh (145.11 g) and dry (28.29 g) rhizome weight per plantwere observed in treated plants and were significantly higher (*p* = 0.00) when compared to thecontrol (88.25 g fresh and 13.06 g dry respectively) (Table 1).

Table 1. Effect of chitin application on rhizome weight in turmeric at 4, 6 and 7 MAT

Rhizome weight	Growth stages	Control	Chitin treatment
Fresh rhizome weight	4 MAT	44.24 ± 6.04	58.20 ± 9.79*
	6 MAT	88.25 ± 6.49	145.11 ± 8.55*
	7 MAT (at harvest)	114.18 ± 4.07	182.87 ± 1.29*
Dry rhizome weight	4 MAT	5.98 ± 2.11	7.13 ± 0.53
	6 MAT	13.06 ± 2.14	28.29 ± 2.14*
	7 MAT (at harvest)	19.98 ± 0.95	33.16 ± 1.39*

*Asterisk indicates a significant difference over the control (*P*< 0.05) by analysis of variance at 5% level of significance.

A significant difference was also observed in the growthof tomato (*Lycopersiconesculentum*) plants treated with chitin 100 gcompared to the control plants with no chitin treatment. The mean fresh weight of tomato plants were 78.0 ± 22.3g and 109.0 ± 25.4g at 0 and 100g chitin, respectively[17].

Significantly higher (*p* = 0.00) leaf chlorophyll content of 1.23mg g^{-1} was recorded in treated plants and 0.86mg g^{-1} in control (Fig 5). De Tender *et al*. [28]conducted an experiment in strawberry and a significant increase in the chlorophyll content of leaves was observed when plants were treated with chitin.

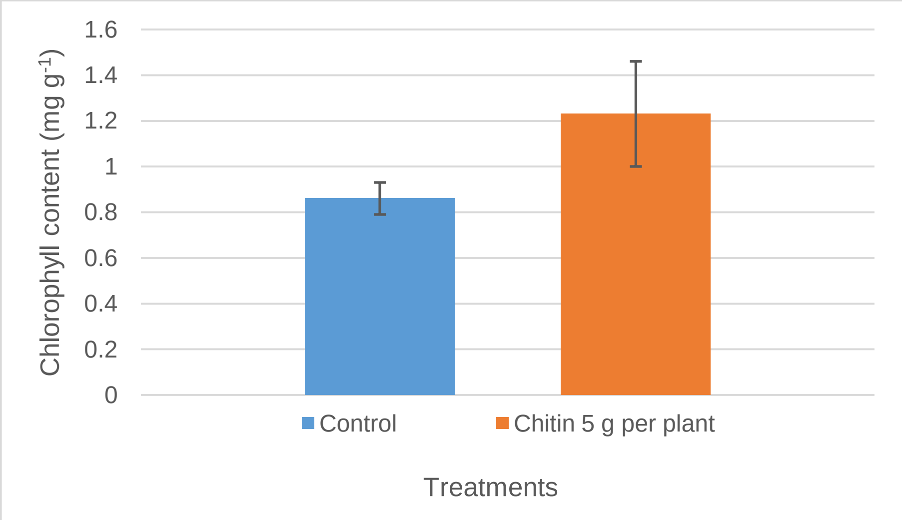

Fig 5. Effect of chitin application on chlorophyllcontent in turmeric leaves

Chitin as soil amendment increases the abundance of several fungal and bacterial groups involved in plant growth promotion [29].The decomposition of chitin by microbial population alters soil physical properties that in turn favours the plant growth. The result of the study conducted by El-Sayed *et al*. [30]showed that the increasing chitinolytic microbial activities in the soil by chitin application might be a major factor in the increase of plant length, weight and leaf chlorophyll content. Chitin decomposition byproducts such as ammonia may become a source of slow but sustained release of nitrogen, which helps in the improvement of vegetative growth[31].

3.2 Effect of chitin on defense enzymes

The activity of defense enzymes, peroxidase and polyphenol oxidase were increased in the plants treated with chitin 5 g per plant (Fig 6). In treated plants, peroxidase enzyme activity of 3.11 activity g^{-1} min^{-1} was recorded and was found significantly higher (p = 0.00) when compared to control (1.58 activity g^{-1} min^{-1}). Polyphenol enzyme activity wasalso significantly higher (p = 0.00) in treated plants (1.62activity g^{-1} min^{-1})when compared to control plants (0.88 activity g^{-1} min^{-1}). The increased activity of peroxidase enzyme was also reported in tomato [32] and *Cannabis sativa* L. [33] plants by chitin treatment. Defense enzymes are important protective enzymes thateliminate reactive oxygen in plants and thereby protect the cell membrane stability and reduces the cell damage [34]. The enhanced activity of plant defense enzymes helps in better establishment and survival of plants in the field and thereby attribute to the improvement in growth and yield characters.

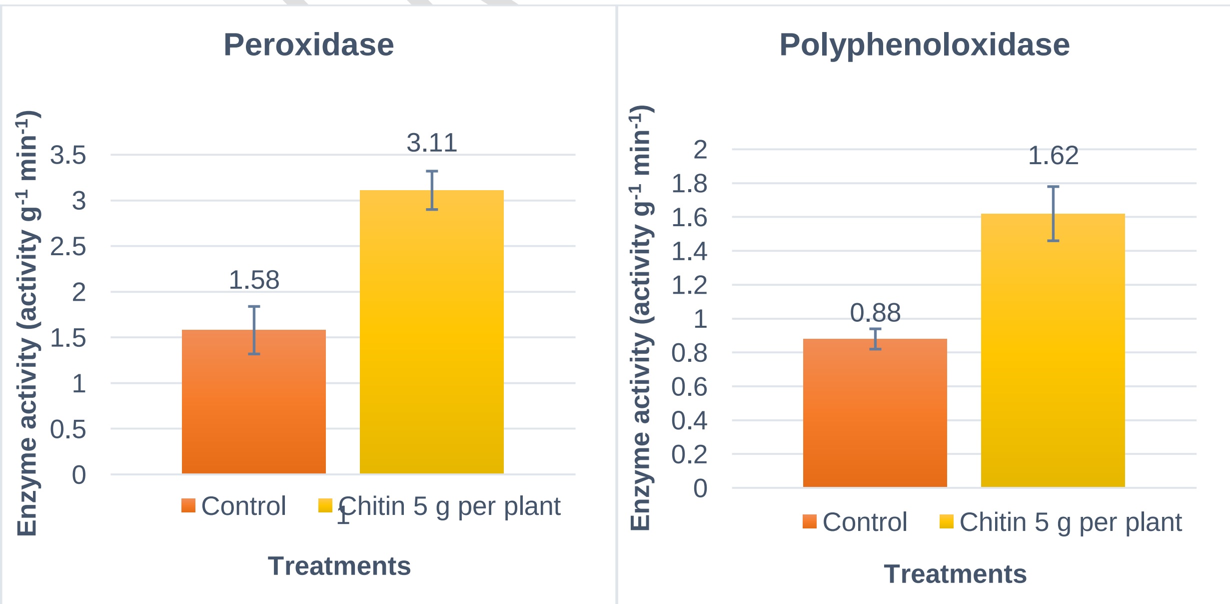

Fig 6. Effect of chitin application on defense enzyme activity in turmeric leaves

3.3 Effect of chitin on yield parameters

A significant (*p* = 0.00) enhancement in rhizome yield over the control was noticed in plants treated with chitin at harvest (Plate 1). The rhizome yield per plant, fresh (182.87 g)and dry (33.16 g)was recorded in treated plants. In control plants the rhizome yield per plant was 114.18 g and 19.98 g, fresh and dry respectively (Table 1). Fresh and dry rhizome yield per plant increased by 60.16% and 65.97% respectivelyover the control.

Plate 1. Rhizome of A) chitin treated plant; B)control plant

A significantly higher (*p* = 0.00) number of fingers of 45.06 was recorded in treated plants at the time of harvest, whereas in control plants it was 35.34. The increased number of fingers by chitin treatment attributes to the improvement in rhizome yield per plant. Thus chitin is a potent biostimulant that can be used for yield enhancement in turmeric.

In a study,Lin *et al*. [35]reported that 2 g/kg chitin significantly increased the total fresh weight by 75.00 % in comparison with control.Fatima *et al*.[14] demonstrated the effect of biofertilizer, chitin in soft wheat, Arrehane variety and observed that a maximum yield of 30 q/ha was obtained by biofertilizers application against 16.18 q/ha for commercial fertilizer treatment. Chitin 5,10 and 15g application at every two weeks recorded a significantly higher seed weight of 3, 3 and 4.5 g respectively in black sesame compared to control (2 g) [27].Chitin 1 %as soil amendment significantly increased the fruit yield (1.2 kg plant^{-1}) and number of fruits (35) per plant in tomatoplants compared to control (0.7 kg plant^{-1} and 25 respectively) [12].

3.4 Effect of chitin on secondary metabolite production

Significantly higher (*p* = 0.00) volatile oil, oleoresin and curcumin content was recorded in rhizomes of treated plants compared to thecontrol (Fig 7). The volatile oil content recorded in the rhizome of the chitin treated plants was 2.88 %and in control was 2.07 %. Oleoresin content of 11.68 % was recorded in the rhizome of the treated plant and 8.24 % in control. Chitin treatment enhanced the volatile oil and oleoresin content by 1.39 times and 1.41 times, respectivelyover the control. The curcumin content of4.23 % was observed in rhizomes of chitin treated plants and 3.50 % in those of control plants. Chitin treatment enhanced curcumin content by 21% over the control. Orlita *et al.*[36]studied the effect of chitin on theproduction of secondary metabolites in *Ruta graveolens* and reported that application of chitin0.01 % substantially increased the production of secondarymetabolites such as coumarins, furanocoumarins, acridone and quinolone alkaloids and flavonoids.Chitosan, a derivative of chitin has been observed to enhance the essential oil yield in turmeric. Chitosan 200 ppm enhanced the essential oil in turmeric by 11% [37].

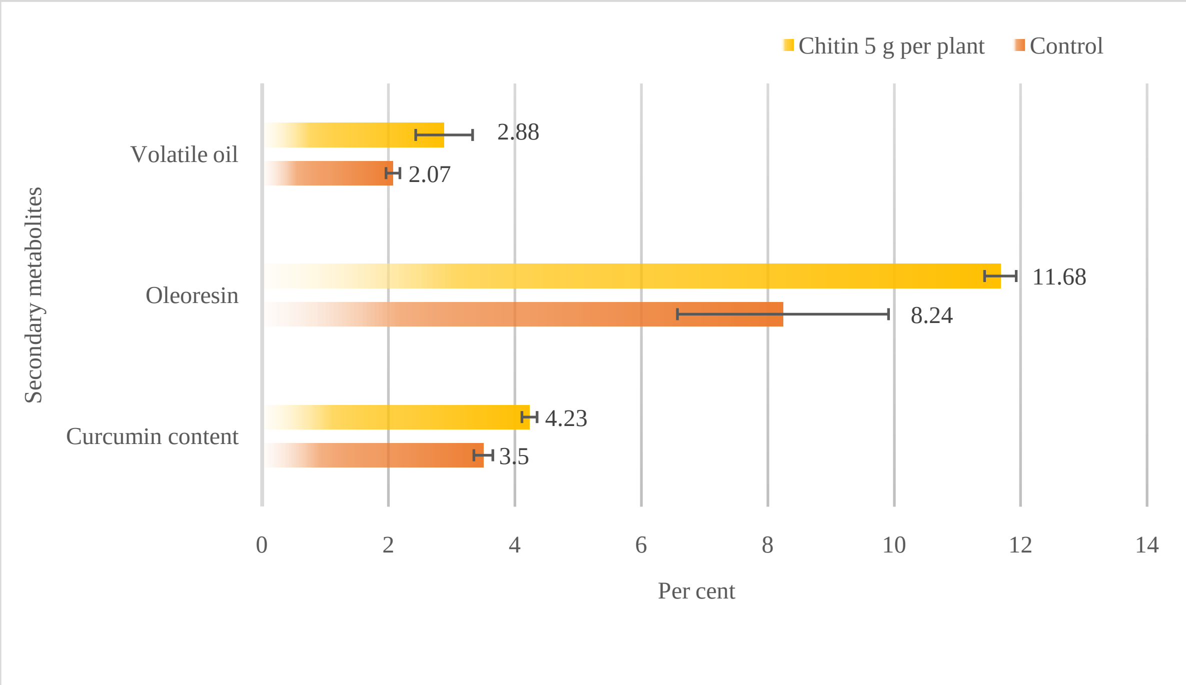

Fig 7. Effect of chitin application on volatile oil, oleoresin and curcumin content in turmeric rhizomes at harvest

3.5Effect of chitin on disease incidence

Incidence of leaf blotch disease caused by *Taphrina maculans*was noticed in the experimental field at 6 MAT and showed significant (p = 0.00) difference in per cent disease incidence. Plants treated with chitin showed a lower per cent disease incidence (42.00 %) compared to control (48.50 %). The lower incidence of leaf blotch disease observed in the present experiment could be attributed to the enhanced activity of plant defence enzymes due to chitin application. In a study by Rajkumar *et al*. [16]it was reported that there was reduction in disease incidence and severity of damping off disease in black pepper by chitin soil amendment.The amendment with chitin increasesdisease tolerance in plants by enhancing the activity of antagonists and defense capacity of plants.Chitin application stimulates the growth of microorganisms that produce chitinolytic enzymes. Thus, chitinases help in degrading cell wall of pathogens in the soil [31].

Patino *et al*. [38]have found that the fungus *Mycosphaerellafijiensis*, causing Black Sigatoka disease in banana (*Musa paradisiaca* L.) and plantain (*Musa acuminata* Colla)could be controlled with the application of foliar substrates containing colloidal chitin. The conventional fungicide applicationdecreased by about 43 - 46%, when chitin is sprayed in rotationwith the fungicide. Chitin amendment to the soil enhances native bacterial population with lytic activity on plant pathogens, therebyfacilitating the reducedusage of chemical fungicides.Sharp [19]stated that a range of beneficial responses could occur when chitin is added to the growing medium. This resulted in the enhancement of beneficial microbes, responsible for both plant defense and growth.

The present study confirmed the improvement in plant growth parameters in response to chitin application to the soil. The degradation of chitin in soil into an absorbable form of nitrogen might have influenced the vegetative growth of plants that in turn had a positive effect on the yield and quality parameters. The enhanced activity of plant defence enzymes and improvement in systemic resistance of plants by chitin application could be the reason for improved disease suppression in chitin treated plants.

4. CONCLUSION

The major byproduct of seafood processing industry, chitin, thus represents a potential source of plant biofertilizer. In the current study,it was demonstrated that chitin application enhanced the growth parametersin turmeric, which in turn reflected inthe rhizomeyield. Soil application of chitin increased the activity of defense enzymes, peroxidase and polyphenol oxidaseover the control and enhanced the content of secondary metabolites*viz,* volatile oil, oleoresin and curcumin content.The effect of chitin on enhancement in specific secondary metabolites has been established in other crops as well.However, The effect of chitin on volatile oil and oleoresin content has not yet been reported in any of the spice or aromatic crops. This is the pioneer report of enhancement in volatile oil and oleoresin content in turmeric on soil application of chitin. It also reduced the incidence of leafblotch disease. Thus, the role of chitin as an eco-friendly and potential biostimulant for improving the growth, yield and quality of turmerichas been established through the experiment.The promotion of its use in crop production system could significantly contribute to reduce theuse of environmentally harmful chemicals and maintainthe soil fertility.

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