

Original Research Article

Plant Growth, Yield and Secondary Metabolite Production in Turmeric (*Curcuma longa* L.) in Response to Chitin Application

ABSTRACT

Aim:The present study was undertaken to evaluate the effect of soil application of chitin on growth, yield and secondary metabolite production in turmeric.

Methodology:The field experiment was conducted in Trivandrum district during June 2021 to January 2022. 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 growth parameters, defense enzymes, yield and quality parameters.

Results: We observed that chitin application enhanced the growth parameters over the control. The defense enzymes activity and chlorophyll content were significantly higher in treated plants compared to control. Fresh and dry rhizome yield per plant increased by 60.16% and 65.97% respectively. Chitin treatment enhanced volatile oil and oleoresin content by 1.39 and 1.41 times and curcumin content by 21% over the control.

Conclusion:The results confirm 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 growing demand on this spice crop due to the wide variety of uses of turmeric in the present era as spice, herbal medicine, dyeing agent and cosmetic (Yadav and Tarun, 2017). Thus, it is of great concern to enhance the production of quality turmeric to meet the increasing market demand. Organically produced turmeric powder and its further value added products will 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 (IFOAM, 2008). Chitin, is a biopolymer abundant in nature and is a by product 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 mollusks (Jones *et al.*, 2020). It is the second most abundant polysaccharide after cellulose (Gooday, 1990). 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 (Ramirez *et al.*, 2010). 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 (Seddiqi *et al.*, 2021). Soil amended with chitin has been reported to decrease nematode infection (Radwan *et al.*, 2012) and to confer host resistance against fungal soil borne pathogens (Inderbitzin *et al.*, 2018 Postma and Schilder, 2015)

The addition of chitin was reported to increase the population of Plant Growth Promoting Rhizobacteria and Fungi in the rhizosphere of the plant (Cretoiuet *al.*, 2013). Chitin based soil amendment has been linked to triggering plant defense mechanism and hence, considered as an alternative to chemical treatments of soil disinfection (Matthiessen and Kirkegaard 2006). This also has been observed to reduce soil-borne diseases (Giotiset *al.*, 2009). Hence, it could be considered as a good agricultural practice (GAP) for turmeric. Chitin amendment improves soil quality, as it depolymerizes through chitinase activity and its decomposition is facilitated by microorganisms present in the soil (Debodeet *al.*, 2016). According to Fathima *et al.* (2018), the application of bio-fertilizers from marine waste as chitin/chitosan would a sustainable agricultural practice, being rich in nitrogen and phosphorus. Jiménez-Gómez and Cecilia (2020) 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 (Rajkumar *et al.*, 2008; Ladner *et al.*, 2008; Giotiset *al.*, 2009; Liopa-Tsakalidiet *al.*, 2010; Sharp, 2013). 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. The pro-tray 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 24 number of plants. Crop was raised organically as per package of practices recommendations (organic) (KAU, 2017). Chitin 5 g per plant was applied to the turmeric plants at 60 and 180 days after transplanting as soil application, the plants without any treatment served as control. The plant growth parameters viz., plant height (cm), number of leaves, leaf area (cm²) fresh shoot weight (g), dry shoot weight (g), fresh rhizome weight (g) and dry rhizome weight (g) were recorded at 4 and 6 MAT. Peroxidase and polyphenol oxidase activity were assayed at 6 MAT from leaf sample as per the procedure of Srivastava (1987). The chlorophyll content of leaf sample was estimated by DMSO method suggested by Arnon (1949). Plants were harvested at 7 MAT and average fresh and dry weight (g) of rhizome per plant was determined and number of fingers were counted. Curcumin content of treated sample and control plants were estimated by HPTLC method. Coarsely ground powder of dried rhizome was subjected to hydrodistillation for 3 h using Clevenger distillation apparatus for estimating volatile oil (Pruthy 1993). Oleoresin content was estimated by Soxhlet extraction for 5 h using acetone as extraction solvent (Braga *et al.*, 1998). Crop was monitored throughout the growth period and incidence of diseases and disease intensity was assessed by using 0 to 6 disease rating scale (Nambiar *et al.*, 1977). The data on growth, yield and quality attributes were subjected to analysis of variance using package KAU GRAPES (Gopinath *et al.*, 2020).

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 recorded 92.52 cm and 95.81 cm respectively (Fig 1). Rajkumar *et al.* (2008) examined the plant growth promotion effect of chitin in pepper plants and noticed the increase in the growth compared to control. The shoot length in lemon balm (*Melissa officinalis* L.) was increased by 43% when grown in medium with chitin compared to control (Liopa-Tsakalidiet *al.*, 2010).

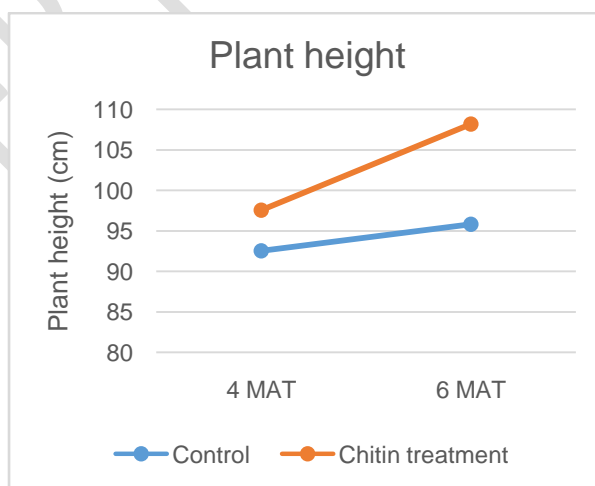


Fig 1. Effect of chitin application on plant height in turmeric during the growth period

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, number of leaves from 11.20 to 14.75 (Fig 2). At 4 MAT, the leaf area was 291.42 cm² and 272.11 cm² 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 cm² compared to control in which the leaf area was 327.71

cm² (Fig 3). Sriwichai *et al.* (2019) 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*.

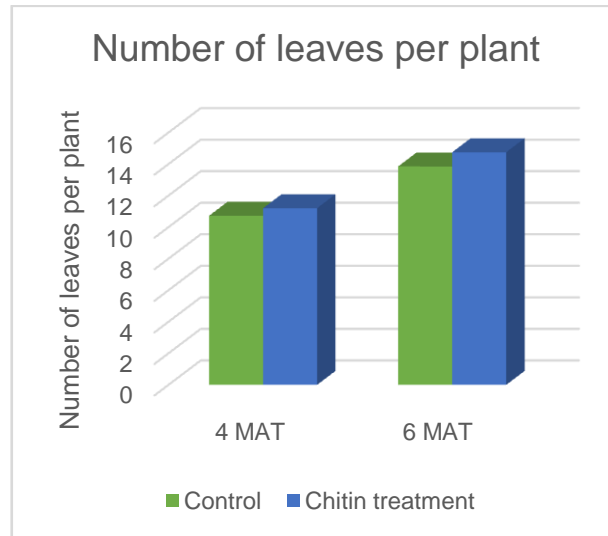


Fig 2. Effect of chitin application on number of leaves per plant in turmeric during the growth period

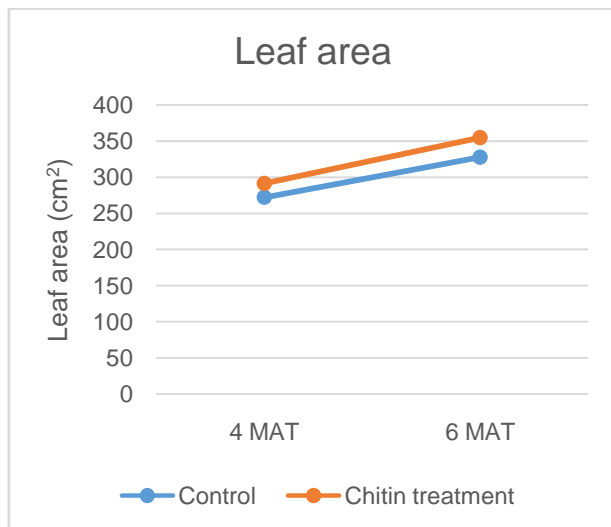


Fig 3. Effect of chitin application on leaf area in turmeric during the growth period

Fresh and dry shoot weight of plants 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). Debodee *et al.*, (2016) reported that addition of 2 % chitin significantly increased the fresh weight of lettuce plants to 213.00 ± 18.76 g per plant, compared with 172.08 ± 17.75 g per plant in the control.

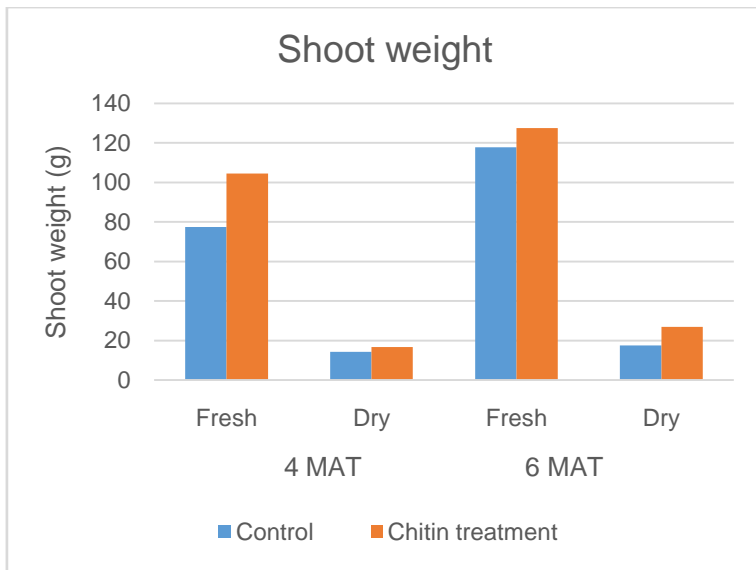


Fig 4. Effect of chitin application on shoot weight in turmeric during the growth period

A significantly higher fresh ($p = 0.00$) and dry ($p = 0.11$) rhizome weight of 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, 145.11 g fresh and 28.29 g dry rhizome weight were observed in treated plants and observed to be significantly higher ($p = 0.00$) when compared to control (88.25 g fresh and 13.06 g dry respectively) (Table 1).

Table 1. Effect of chitin application on rhizome weight in turmeric during growth and at harvest

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*
	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*
	At harvest	19.98 ± 0.95	33.16 ± 1.39*

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

A significant difference was also observed in the growth rate of tomato (*L. esculentum*) plants treated with 100 g chitin compared to the control with no chitin. The mean fresh weights of tomato plants were 78.0 ± 22.3g and 109.0 ± 25.4g at 0 and 100g chitin respectively (Lander *et al.*, 2008).

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.* (2021) conducted an experiment in strawberry and a significant increase in the chlorophyll content of leaves was observed when plants were treated with chitin.

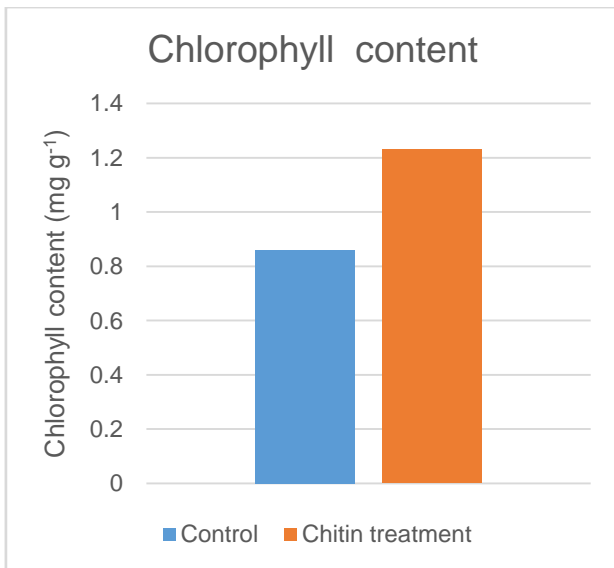


Fig 5. Effect of chitin application on chlorophyll content in turmeric leaves

Chitin as soil amendment increases the abundance of several fungal and bacterial groups involved in plant growth promotion (Muymaset *et al.*, 2015). 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.* (2002) 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 by-products such as ammonia may become a source of slow but sustained release of nitrogen, which helps in the improvement of vegetative growth (Velasquez and Pierla, 2016).

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 the peroxidase enzyme activity of 3.11 activity g⁻¹ min⁻¹ was recorded and found significantly higher ($p = 0.00$) when compared to control (1.58 activity g⁻¹ min⁻¹). The polyphenol enzyme activity was also significantly higher ($p = 0.00$) in treated plants (1.62 activity 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 (Sun *et al.*, 2018) and *Cannabis sativa* L. (Suwanchaikasem *et al.*, 2023) plants by chitin treatment. Defense enzymes are important protective enzymes that eliminate reactive oxygen in plants and thereby protect the cell membrane stability and reduces the cell damage (Jiao *et al.*, 2012). The enhanced activity of plant defense enzymes helps in better establishment and survival of plants in the field and thereby attributes to the improvement in growth and yield characters.

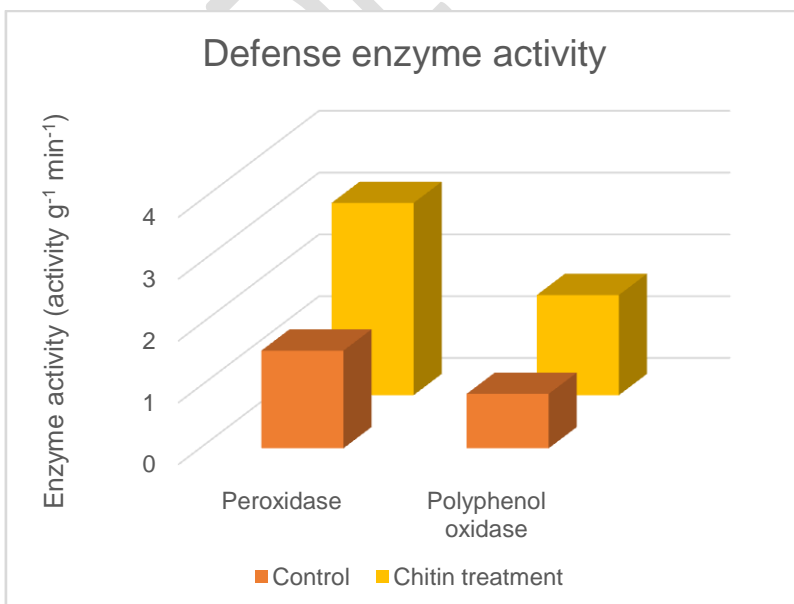


Fig 6. Effect of chitin application on defense enzyme activity in turmeric leaves

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 of 182.87 g fresh and 33.16 g dry 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% respectively over the control.



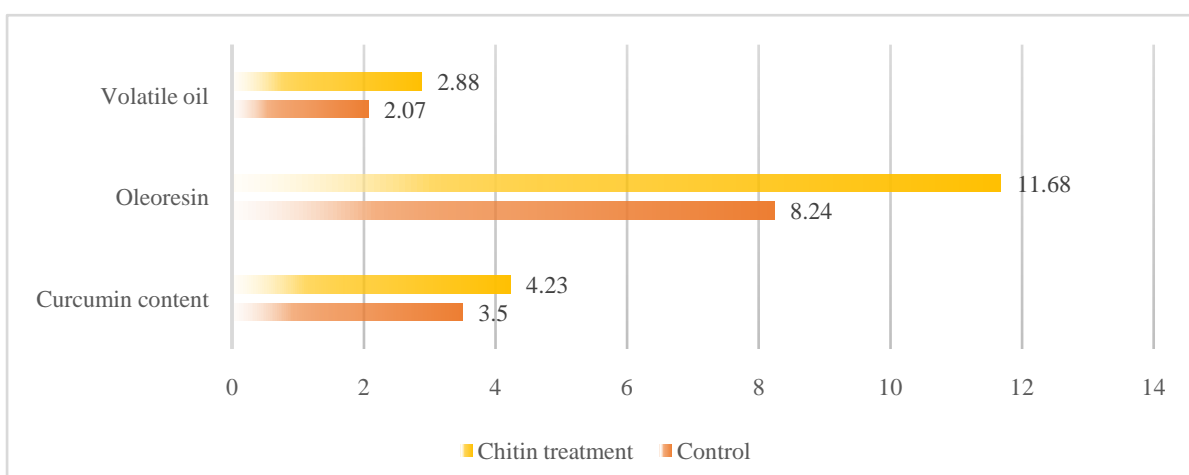
Plate 1. Rhizome of A) chitin treated plant; B) control plant

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.* (2020) reported that 2 g/kg chitin significantly increased the total fresh weight by 75.00 % in comparison with control. Fatima *et al.*, (2018) demonstrated the effect of biofertilizer, chitin in soft wheat, Arrehane variety and observed that a maximum yield of 30 q/ha was obtained by bio-fertilizers 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) (Sriwichai *et al.*, 2019). 1 % chitin as soil amendment significantly increased the fruit yield (1.2 kg plant⁻¹) and number of fruits per plant (35) in tomato plants compared to control (0.7 kg plant⁻¹ and 25 respectively) (Giotis *et al.*, 2009).

3.4 Effect of chitin on quality parameters

Significantly higher ($p = 0.00$) volatile oil, oleoresin and curcumin content was recorded in rhizomes of treated plants when compared to control (Fig 7). The volatile oil content recorded in treatment was 2.88 % and 2.07 % in control. Oleoresin content of 11.68 % was recorded in treatment and 8.24 % in control. Chitin treatment enhanced the volatile oil and oleoresin content by 1.39 times and 1.41 times over the control. In case of curcumin content, 4.23 % curcumin was observed in rhizomes of chitin treated plants and 3.50 % in control plants. Chitin treatment enhanced curcumin content by 21% over the control. Orlita *et al.* (2008) studied the effect of chitin on production of secondary metabolites in *Ruta graveolens* and reported that application of chitin at 0.01 per cent substantially increased the production of secondary metabolites such as coumarins,



furanocoumarins, acridone and quinolone alkaloids and flavonoids.

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

Effect 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 by chitin application. In a study by Rajkumar *et al.*, (2008) it was reported that there was reduction in disease incidence and severity of damping off disease in black pepper by chitin soil amendment application. The amendment with chitin increases the plant protection by enhancing the activity of antagonists and induce defense capacity of plants. Chitin application stimulates the growth of microorganisms that produce chitinolytic enzymes is one of the most proposed modes of action. Thus, chitinases help in degrading cell wall of pathogens in the soil (Velasquez and Pirela, 2016).

Patino *et al.* (2007) have found that the fungus *Mycosphaerella fijiensis*, causing Black Sigatoka disease in banana (*Musa paradisiaca* L.) and plantain (*Musa acuminata* Colla) can be controlled with the application of foliar substrates containing colloidal chitin. The conventional fungicide applications decreased by about 43 - 46%, when it is sprayed in rotation with fungicide. Thus, chitin is effective in reducing the usage of chemical fungicides that encourage native bacterial population with lytic activity on plant pathogens.

Sharp (2013) from his study concluded that a range of beneficial responses can occur when chitin is added to the growing medium *i.e.*, enhancement of beneficial microbes, both in plant defense and growth; and up-regulation of plant growth and development. The results from the present experiment showed that the chitin application improved the plant growth parameters. The degradation of chitin in soil to an absorbable form of nitrogen might influence the vegetative growth of plants that also attributes to the yield and quality characters. 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 by product of seafood processing industry, chitin represent an interesting source of plant biofertilizer. In the current study, it was demonstrated that chitin application enhanced the growth parameters which in turn reflected in rhizome yield. Soil application of chitin increased the activity of defence enzymes, peroxidase and polyphenol oxidase over the control and enhanced the content of secondary metabolites *viz.*, volatile oil, oleoresin and curcumin content. It also reduced the incidence of leaf blotch disease. Thus, the use of chitin as an eco-friendly and potential biostimulant for improving the growth, yield and quality characters in turmeric is established through the experiments. Promotion of its use can significantly contribute to reduce the use of environmentally harmful chemicals, and maintain the soil fertility.

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