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RESEARCH ARTICLE

Growth, yield and secondary metabolite elicitation in response to chitosan application in turmeric (*Curcuma longa* L.)

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Abstract

Curcuma longa L., is a rhizomatous, herbaceous plant belonging to Zingiberaceae family and has a wide range of pharmacological activities and cosmetic industrial value. Chitosan, extracted from fungal cell wall and crustacean shells is an emerging plant biostimulant that evokes growth promotion and metabolite elicitation. An experiment was conducted to study the effect of different concentrations and frequencies of foliar application of chitosan on plant growth, yield and secondary metabolite production in turmeric varieties, Sobha and Sona. The experiment was laid out in Randomized Block Design with three replications. The treatments included, F_1: Chitosan 1 g/L monthly, F_2: Chitosan 2 g/L monthly, F_3: Chitosan 3 g/L bimonthly, F_4: Chitosan 4 g/L bimonthly, F_5: Chitosan 4 g/L trimonthly, F_6: Chitosan 5 g/L trimonthly, C_p: Primed control and C: Unprimed control. The growth parameters were recorded at 6 months after transplanting (MAT) and yield at harvest. Curcumin content was analysed through HPTLC and expression profile of curcumin synthase gene was carried out by Quantitative Real-time PCR. Among the treatments, monthly application of Chitosan 2 g/L was observed to give better results in terms of plant height, leaf area, shoot weight and rhizome spread at 6 MAT. Monthly application of Chitosan 2 g/L, F_2 and bimonthly application of Chitosan 4 g/L, F_4 recorded significantly higher fresh rhizome yield per plant in variety Sobha (312.89 g and 322.85 g, respectively) and Sona (286.37 g and 284.06 g, respectively). Monthly application of Chitosan 2 g L^{-1} (F_2) recorded a significantly higher curcumin content. The curcumin content enhanced by 89 % in Sobha and 54 % in Sona over the unprimed control. Chitosan treatment enhanced the expression of curcumin synthase gene by 1.48 fold in Sobha and 1.77 fold in Sona over control. Thus, monthly foliar application of chitosan 2 g/L gave better growth, yield, curcumin production and regulate curcumin synthase gene expression in turmeric in comparison to other frequencies and concentrations of chitosan.

Keywords

Chitosan; *Curcuma longa*; Curcumin; Curcumin synthase; Elicitation; Gene expression

Introduction

Curcuma longa L., a rhizomatous, herbaceous plant belonging to Zingiberaceae family, is a native of Southeast Asia and widely cultivated in the tropical and subtropical regions of the world. Turmeric is cultivated extensively in Asia, mostly in India and China (1). India is the largest producer, consumer and exporter of turmeric in the world. The annual production of turmeric in India is around 11.61 lakh tonnes per annum. India contributes to more than 75 % share of world production and turmeric is grown in more than 20 states in the country. The major turmeric producing states in India are Telangana, Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh and Madhya Pradesh (2).

Rhizomes of *C. longa* is used extensively for imparting flavour and colour in foods. It is used as dietary pigment and for the treatment of various illnesses in Indian folk medicine. It is also used in textile and pharmaceutical industries (3). Curcuminoids comprising of curcumin, demethoxycurcumin and bisdemethoxycurcumin form the important bioactive constituents of turmeric. Turmeric contains 3-5% curcuminoids with major proportion of bioactive ingredients being curcumin (4). Curcumin from this 'golden spice' has a wide range of pharmacological and cosmetic value (5,6). Due to inherent qualities and high content of curcumin, Indian turmeric is considered to be the best in the world. Curcumin, the bioactive substance in turmeric is produced through secondary metabolism. The medicinal values of this plant lies with this component that have definite physiological action on the human body. Turmeric shows a wide spectrum of biological actions, which include its anti-inflammatory, antioxidant, anticarcinogenic, anti-mutagenic, anticoagulant, antifertility, anti-diabetic, anti-microbial, anti-venom, antiulcer, hypotensive and hypercholesterolemic activities (7).

Chitosan, extracted from fungal cell wall and crustacean shells is an emerging plant biostimulant that evokes growth promotion and metabolite elicitation. It is a linear polymer, composed of two sub-units such as D-glucosamine and N-acetyl-D-glucosamine, linked with each other through 1,4-glycosidic bonds. It is non-toxic, bio-compatible and bio-degradable (8). Chitosan can adopt several conformations or structures and a wide range of functional groups could modify its composition, for specific applications (9). This enables the utilization of chitosan in various fields like cosmetology, food technology, biotechnology, pharmacology and medical science (10). Chitosan acts as a molecule for eliciting plant metabolism (11).

Chitosan is observed to enhance photosynthetic activity, vegetative growth, antioxidant activities, fruit quality attributes and overall growth and yield of plants. Chitosan application has also been reported to enhance biomass production and secondary metabolite synthesis in several crops including spices, medicinal and aromatic plants (12). Preliminary studies showed that chitosan foliar spray increased plant growth parameters such as plant height, number of leaves, leaf area and shoot weight in Kasthuri turmeric (13). In turmeric, chitosan 0.1 % foliar spray increased the concentration of curcumin in the rhizomes by 56 % and overall curcumin production in the plant was doubled (14). Aswini (15) also reported that foliar application of chitosan was effective in enhancing the curcumin content and curcumin yield in turmeric. As turmeric is an export oriented crop, its yield has to be enhanced without compromising on its quality. Chitosan being a biodegradable biopolymer, it can be effectively incorporated into the organic production system of turmeric. In this contest the experiment has been formulated to study the effect of different concentrations and frequencies of foliar application of chitosan on growth, yield and secondary metabolite production in turmeric.

Materials and Methods

The experiment was conducted during the period from June 2021 to January 2022 in the farmer's field at Thiruvananthapuram, Kerala located at 8°57'16'' N latitude and 76°89'10'' E longitude. The study was conducted using two medium duration (240-270 days) varieties of turmeric, Sobha and Sona. Single bud rhizome bits (5-7 g) from healthy, disease and pest free rhizomes of the two varieties were primed with chitosan 1 g/L for 1 h. The rhizome bits were then shade dried and sown in portrays, filled with potting mixture containing soil, coirpith and cowdung in the ratio 1:1:1. The protrays were maintained under shade and was given need based irrigation for 45 days. These plantlets were then transplanted to the main field at a spacing of 25 cm x 25 cm in plots of size 3 m x 1 m with a spacing of 40 cm between the beds. Farm yard manure was applied as basal dose at 35 t/ha at the time of land preparation and 3 t/ha each at 1 month after transplanting (MAT) and 2 MAT by spreading over the beds. Ash at the rate of 125 kg/ha was applied twice at 1 and 2 MAT. Mulching was done immediately after planting with green leaves at the rate of 15 t/ha. The mulching was repeated with green leaves twice at the 7.5 t/ha rate at 1.5 and 3 MAT. Intercultural operations, weeding and earthing up were carried out as and when required (16).

The biostimulant chitosan was given as foliar spray at different concentrations and frequencies from transplanting to five months after transplanting (MAT) in the main field. The experiment was laid out in Randomised Block Design (RBD) with eight treatments and three replications. The treatments included, F_1: Chitosan 1 g/L monthly, F_2: Chitosan 2 g/L monthly, F_3: Chitosan 3 g/L bimonthly, F_4: Chitosan 4 g/L bimonthly, F_5: Chitosan 4 g/L trimonthly, F_6: Chitosan 5 g/L trimonthly, C_p: Primed control and C: Unprimed control.

The observations on plant growth parameters *viz*. plant height, number of tillers, number of leaves per plant, leaf area (Eqn. 1), shoot weight, rhizome spread, rhizome thickness, number of fingers, shoot and rhizome weight were recorded at 6 MAT.

$$Y = 4.09 + 0.564 (L \times B) \quad \text{(Eqn. 1)}$$

Where, Y = Leaf area

Length = Length of the reference leaf in cm

Breadth = Breadth of the reference leaf in cm (17)

The biochemical parameters viz., total chlorophyll content, peroxidase and polyphenol oxidase activities were recorded at 6 MAT. The leaf sample's chlorophyll content (Eqn. 2) was estimated by the DMSO method suggested by Arnon (18).

$$\text{Total chlorophyll} = [\{20.2\ (A_{645}) + 8.01\ (A_{663})\} \times \text{volume}/\ (\text{weight} \times 1000)] \quad \text{(Eqn. 2)}$$

Peroxidase and polyphenol oxidase activity were assayed from the leaf sample as per the procedure of Srivastava (19).

The observations on yield parameters *viz*. rhizome spread, rhizome thickness, weight of mother, primary and secondary fingers and rhizome yield per plant were recorded at the time of harvest (7 MAT).

Curcumin content in the rhizomes of all the treatments was analysed through HPTLC method. The rhizome samples from all the treatments were collected at 2 weeks after final application of chitosan at 5 MAT and kept under -80°C. The rhizomes from the treatment that yielded the highest curcumin content and the rhizomes from untreated control treatment were used for expression profile analysis in both varieties. RNA was isolated from the rhizomes of both the samples. RNA isolation from the rhizome samples was performed using the TRIzol reagent method, and the extracted RNA samples were stored at -80°C. The purity of the isolated RNA was determined by OD_{260}/OD_{280} value. This was then quantified and used for cDNA synthesis using the cDNA preparation kit (G biosciences). Expression profile of *CURS 1* (curcumin synthase) gene was carried out in a Quantitative Real-time PCR (qPCR) with *actin* as reference gene.

Results

Plant growth parameters

In the study, the plant above ground parameters *viz.* plant height, number of tillers, number of leaves per plant, leaf area (Table 1) and shoot weight (Fig. 1) were recorded at 6 MAT in both the varieties, Sobha and Sona. The plant height showed significant variation among the treatments in both varieties. In both the varieties, Sobha and Sona monthly application of chitosan 2 g/L (F_2) recorded significantly higher plant height of 149.06 cm and 155.28 cm respectively. No significant variation was observed in the number of tillers among the various treatments tried in both the varieties. Tillers ranged from 1.17 to 1.83 in Sobha and 1 to 1.56 in Sona. The number of leaves per plant showed significant differences among the various treatments tried in both varieties. In variety Sobha, F_2 recorded a significantly higher number (17.17) of leaves per plant which was on par with F_4 and F_6. In Sona, the highest number of leaves per plant was recorded in F_4 (18.50), followed by F_2 and F_6. In case of leaf area also F_2 recorded significantly higher values (509.40 cm^2 and 661.68 cm^2) in variety Sobha and Sona respectively. Significantly lower leaf area was observed in unprimed control, which was 326.47 cm^2 in Sobha and 378.55 cm^2 in Sona. F_2 recorded higher fresh (275.93 g 284.16 g) and and dry shoot weight (77.54 g and 51.01 g) in variety Sobha and Sona respectively (Fig. 1). Significantly lower value has been observed in unprimed control with respect to all the above ground parameters.

Table 1. Above ground morphological parameters in response to chitosan foliar application in turmeric varieties Sobha and Sona at 6 MAT

Treatments	Plant height (cm)		Number of tillers per plant		Number of leaves per plant		Leaf area (cm^2)	
	Sobha	Sona	Sobha	Sona	Sobha	Sona	Sobha	Sona
F_1: CTS 1 g/L monthly	123.50 ± 7.72^{b}	120.50 ± 1.50^{cd}	1.67 ± 0.29	1.17 ± 0.29	14.75 ± 0.25^{bc}	15.1±0.10^{c}	361.68 ± 4.58^{cd}	417.92 ± 21.04^{de}
F_2: CTS 2 g/L monthly	149.06 ± 10.96^{a}	155.28 ± 3.71^{a}	1.83 ± 0.29	1.50 ± 0.00	17.17 ± 0.83^{a}	17.0±1.00^{b}	509.40 ± 86.60^{a}	661.68 ± 24.26^{a}
F_3: CTS 3 g/L bimonthly	133.83 ± 11.86^{ab}	128.56 ± 14.62^{bc}	1.28 ± 0.26	1.33 ± 0.29	14.42 ± 1.08^{c}	15.1±0.10^{c}	429.45 ± 32.06^{b}	500.90 ± 29.05^{cd}
F_4: CTS 4 g/L bimonthly	148.44 ± 2.69^{a}	139.00 ± 16.02^{b}	1.61 ± 0.35	1.56 ± 0.51	16.42 ± 3.08^{ab}	18.5±0.50^{a}	492.81 ± 7.78^{a}	610.04 ± 46.33^{ab}
F_5: CTS 4 g/L trimonthly	120.33 ± 2.08^{b}	128.23 ± 8.99^{bcd}	1.17 ± 0.29	1.33 ± 0.29	14.83 ± 0.17^{bc}	16.5±0.50^{b}	406.77 ± 48.05^{bc}	427.86 ± 45.56^{cde}
F_6: CTS 5 g/L trimonthly	118.61 ± 17.85^{bc}	130.61 ± 8.42^{bc}	1.33 ± 0.29	1.33 ± 0.58	15.42 ± 0.08^{abc}	17.0±1.00^{b}	433.12 ± 18.04^{b}	529.49 ± 48.71^{bc}
C_p: Primed control	100.44 ± 15.22^{cd}	113.76 ± 15.15^{de}	1.17 ± 0.29	1.17 ± 0.29	14.08 ± 0.42^{c}	15.0±0.00^{c}	327.45 ± 7.53^{d}	367.22 ± 6.38^{e}
C : Unprimed control	96.06 ± 9.75^{d}	105.39 ± 3.23^{e}	1.17 ± 0.29	1.00 ± 0.00	14.00 ± 1.00^{c}	14.5±0.50^{c}	326.47 ± 18.36^{d}	378.55 ± 127.55^{e}
SEm (±)	6.088	4.803	0.18	0.188	0.598	0.349	18.824	33.661
CD (0.05)	**18.467**	**14.569**	**NS**	**NS**	**1.813**	**1.059**	**57.097**	**102.099**
CV	**8.519**	**6.517**	**22.171**	**25.108**	**6.839**	**3.758**	**7.935**	**11.979**

The data represents mean of 3 replications. Values that are followed by the same letter in a column do not differ significantly between them; CTS : Chitosan

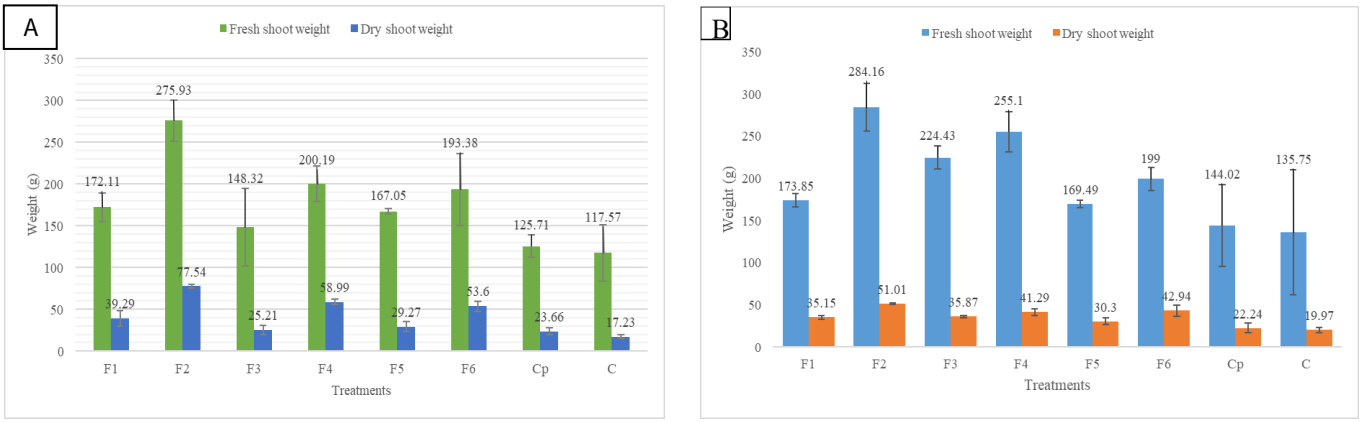

Fig. 1. Shoot weight (at 6 MAT) in response to chitosan foliar application in turmeric varieties (A) Sobha and (B) Sona

F_1: CTS 1 g L^{-1} monthly, F_2: CTS 2 g L^{-1} monthly, F_3: CTS 3 g L^{-1} bimonthly, F_4: CTS 4 g L^{-1} bimonthly, F_5: CTS 4 g L^{-1} trimonthly, F_6: CTS 5 g L^{-1} trimonthly, C_p: Primed control and C: Unprimed control.

The below ground parameters of the plant *viz.,* rhizome spread, rhizome thickness, number of fingers and rhizome weight were recorded at 6 MAT in both the varieties. Significant variation was observed among the treatments in all these parameters (Table 2). F_2 recorded the highest rhizome spread (25.60 cm and 23.75 cm), rhizome thickness (2.10 cm and 2.10 cm), number of fingers (42.38 and 39.90) in both the varieties Sobha and Sona respectively. The fresh and dry rhizome weight was significantly higher in F_2 in variety Sobha (268.36 g and 35.78 g respectively) and F_4 in Sona (223.33 g and 38.45 g respectively). In case of below ground parameters also the lowest value was observed in untreated control plants in both the varieties, Sobha and Sona.

Physiological parameters

The total chlorophyll content was estimated at 6 MAT and showed significant difference among the various treatments tried (Fig. 2). The total chlorophyll content was significantly higher in F_2 invariety Sobha (1.68 mg/g) and Sona (1.78 mg/g). Significantly lower total chlorophyll content was noticed in unprimed control treatment.

In both the varieties significant variation was observed among the treatments in the activity of defence enzymes, peroxidases and polyphenol oxidase (Fig. 3). In varieties, Sobha and Sona, F_2 recorded significantly higher peroxidase activity of 3.84 activity/g/min and 5.22 activity/g/min respectively. Polyphenol oxidase activity was also found to be significantly higher in F_2 in variety Sobha (2.30 activity/g/min) and Sona (2.74 activity/g/min). Significantly lower activity of defence enzymes was recorded in unprimed control treatment.

Yield parameters

The observations on yield parameters *viz.* rhizome spread, rhizome thickness, weight of mother, primary and secondary fingers and rhizome yield per plant were taken at the time of harvest in both the varieties. In all these parameters, significant variation was observed among the different chitosan treatments in both varieties. In varieties Sobha and Sona, F_2 recorded significantly higher rhizome spread of 26.11 cm and 25.57 cm respectively. The rhizome thickness was significantly higher in F_6 in variety Sobha (2.42 cm) and F_4 in variety Sona (2.63 cm). Significantly lower rhizome spread and thickness were noticed in unprimed control (Table 3).

Table 2. Below ground morphological parameters in response to chitosan foliar application in turmeric varieties Sobha and Sona at 6 MAT

Treatments	Rhizome spread (cm)		Rhizome thickness (cm)		Number of fingers per plant		Rhizome weight per plant (g)			
							Fresh		Dry	
	Sobha	Sona	Sobha	Sona	Sobha	Sona	Sobha	Sona	Sobha	Sona
F_1: CTS 1 g/L monthly	20.60 ± 2.60^{bc}	18.75 ± 0.25^{cde}	1.95 ± 0.15^{a}	1.50 ± 0.50^{bc}	32.75 ± 1.25^{d}	28.70 ± 0.70^{bc}	157.04 ± 16.46^{d}	145.65 ± 16.41^{c}	30.79 ± 4.22^{ab}	26.72 ± 2.72^{d}
F_2: CTS 2 g/L monthly	25.60 ± 0.60^{a}	23.75 ± 0.25^{a}	2.10 ± 0.20^{a}	2.10 ± 0.10^{a}	42.38 ± 1.13^{a}	39.90 ± 2.10^{a}	268.36 ± 7.35^{a}	215.00 ± 31.23^{ab}	35.78 ± 2.55^{a}	38.06 ± 2.06^{a}
F_3: CTS 3 g/L bimonthly	21.60 ± 0.40^{b}	16.75 ± 2.75^{de}	1.95 ± 0.05^{a}	1.31 ± 0.31^{c}	37.38 ± 1.38^{bc}	29.75 ± 2.85^{bc}	188.73 ± 1.23^{bc}	203.60 ± 25.60^{ab}	31.05 ± 4.95^{ab}	32.82 ± 3.17^{bc}
F_4: CTS 4 g/L bimonthly	22.10 ± 2.90^{b}	22.00 ± 3.00^{abc}	2.05 ± 0.05^{a}	1.90 ± 0.20^{ab}	39.75 ± 1.50^{ab}	37.75 ± 7.55^{a}	254.67 ± 12.94^{a}	223.33 ± 20.82^{a}	35.56 ± 2.50^{a}	38.45 ± 1.87^{a}
F_5: CTS 4 g/L trimonthly	19.00 ± 2.00^{cd}	20.00 ± 3.00^{bcd}	1.60 ± 0.10^{b}	1.75 ± 0.05^{ab}	36.00 ± 0.00^{c}	28.50 ± 0.50^{bc}	174.40 ± 12.58^{cd}	179.52 ± 26.20^{bc}	29.50 ± 4.50^{b}	30.90 ± 0.25^{c}
F_6: CTS 5 g/L trimonthly	19.25 ± 0.25^{cd}	23.00 ± 1.00^{ab}	1.95 ± 0.05^{a}	1.83 ± 0.03^{ab}	36.75 ± 2.25^{c}	32.55 ± 0.85^{b}	208.33 ± 24.66^{b}	201.67 ± 22.55^{ab}	34.50 ± 1.50^{ab}	34.92 ± 1.48^{ab}
C_p: Primed control	17.50 ± 1.50^{de}	17.85 ± 1.15^{de}	1.50 ± 0.30^{b}	1.30 ± 0.30^{c}	30.50 ± 1.50^{d}	27.60 ± 2.40^{c}	110.00 ± 10.00^{e}	93.33 ± 25.17^{d}	17.50 ± 1.50^{c}	20.53 ± 1.95^{e}
C : Unprimed control	15.50 ± 0.50^{e}	15.75 ± 0.75^{e}	1.40 ± 0.10^{b}	1.25 ± 0.05^{c}	20.63 ± 1.88^{e}	22.75 ± 3.85^{d}	87.67 ± 8.62^{e}	73.00 ± 11.27^{d}	12.00 ± 2.00^{c}	14.95 ± 1.60^{f}
SEm (±)	0.763	1.141	0.09	0.142	0.914	1.58	7.909	13.495	1.82	1.247
CD (0.05)	2.316	3.46	0.272	0.431	2.774	4.792	23.99	40.933	5.519	3.782
CV	6.564	10.015	8.566	15.225	4.589	8.845	7.562	14.006	11.123	7.279

The data represents mean of 3 replications. Values that are followed by the same letter in a column do not differ significantly between them; CTS : Chitosan

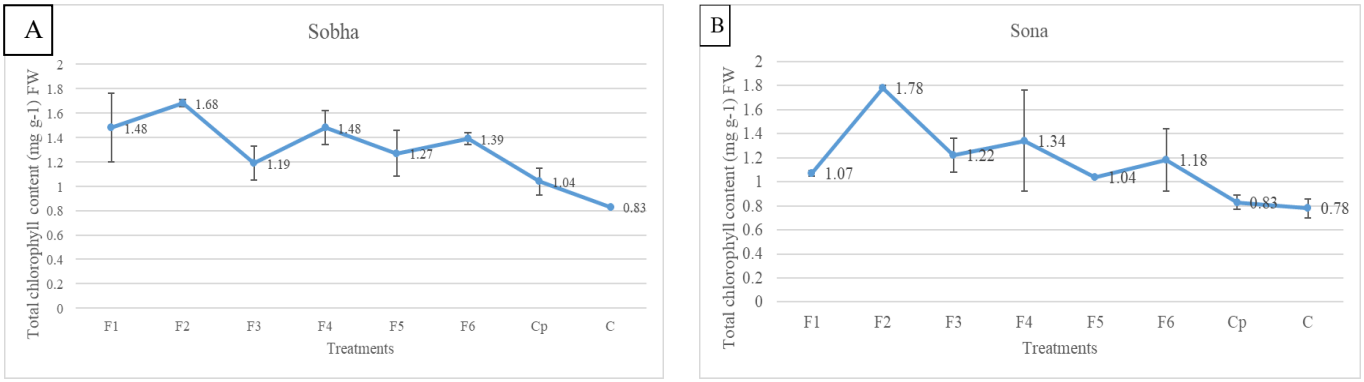

Fig. 2. Total chlorophyll content (at 6 MAT) in response to chitosan foliar application in turmeric varieties (A) Sobha and (B) Sona

F_1: CTS 1 g L^{-1} monthly, F_2: CTS 2 g L^{-1} monthly, F_3: CTS 3 g L^{-1} bimonthly, F_4: CTS 4 g L^{-1} bimonthly, F_5: CTS 4 g L^{-1} trimonthly, F_6: CTS 5 g L^{-1} trimonthly, C_P: Primed control and C: Unprimed control.

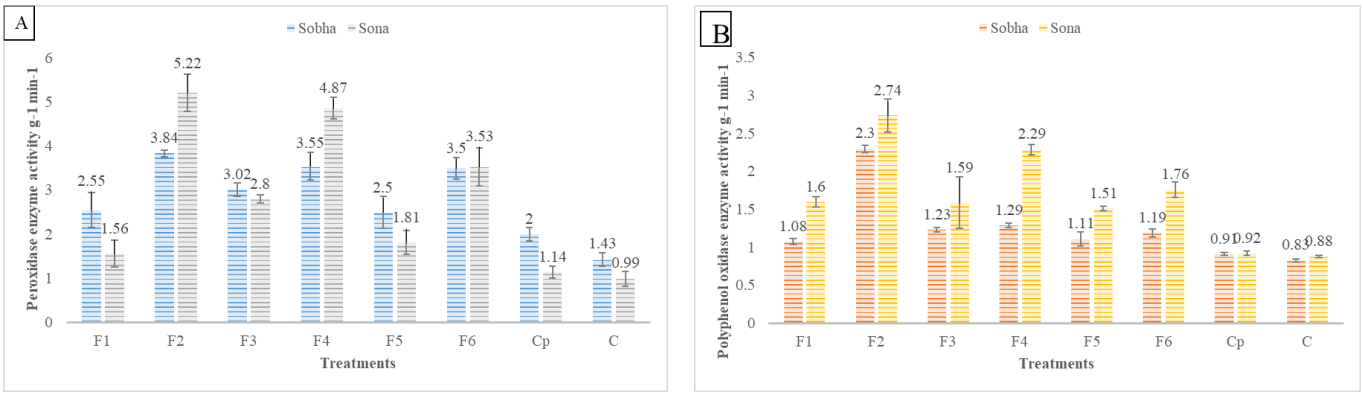

Fig. 3. Activities of defense enzymes in response to chitosan foliar application in turmeric varieties Sobha and Sona (A) Peroxidase and (B) Polyphenol

F_1: CTS 1 g L^{-1} monthly, F_2: CTS 2 g L^{-1} monthly, F_3: CTS 3 g L^{-1} bimonthly, F_4: CTS 4 g L^{-1} bimonthly, F_5: CTS 4 g L^{-1} trimonthly, F_6: CTS 5 g L^{-1} trimonthly, C_P: Primed control and C: Unprimed control.

Table 3. Rhizome spread and rhizome thickness in response to chitosan foliar application in turmeric varieties Sobha and Sona at harvest

Treatments	Rhizome spread (cm)		Rhizome thickness (cm)	
	Sobha	Sona	Sobha	Sona
F_1: CTS 1 g/L monthly	22.73 ± 0.16^{b}	22.34 ± 2.50^{abc}	2.11 ± 0.12^{bc}	1.79 ± 0.19^{cd}
F_2: CTS 2 g/L monthly	26.11 ± 1.70^{a}	25.57 ± 1.51^{a}	2.36 ± 0.06^{ab}	2.28 ± 0.03^{ab}
F_3: CTS 3 g/L bimonthly	22.76 ± 2.34^{b}	21.56 ± 0.16^{bc}	2.18 ± 0.13^{abc}	1.88 ± 0.63^{bcd}
F_4: CTS 4 g/L bimonthly	25.34 ± 0.75^{ab}	24.88 ± 0.59^{abc}	2.30 ± 0.21^{abc}	2.63 ± 0.13^{a}
F_5: CTS 4 g/L trimonthly	23.69 ± 0.85^{ab}	23.83 ± 4.14^{abc}	2.08 ± 0.10^{cd}	2.19 ± 0.06^{abc}
F_6: CTS 5 g/L trimonthly	24.54 ± 1.95^{ab}	25.31 ± 0.47^{ab}	2.42 ± 0.23^{a}	2.38 ± 0.25^{a}
C_p: Primed control	19.76 ± 0.56^{c}	21.38 ± 3.94^{cd}	1.84 ± 0.12^{de}	1.64 ± 0.39^{d}
C : Unprimed control	18.54 ± 1.74^{c}	17.53 ± 1.31^{d}	1.61 ± 0.12^{e}	1.56 ± 0.06^{d}
SEm (±)	0.887	1.274	0.087	0.153
CD (0.05)	2.69	3.865	0.263	0.464
CV	6.698	9.679	7.129	12.968

The data represents mean of 3 replications. Values that are followed by the same letter in a column do not differ significantly between them CTS : Chitosan

Fresh and dry weight of mother rhizome was significantly higher in F_4 (35.84 g and 4.76 g respectively) in variety Sobha. Whereas, in Sona, F_2 recorded significantly higher fresh weight (35.32 g) and F_4 recorded a significantly higher dry weight (4.33 g) of mother rhizome. With respect to the fresh and dry weight of primary fingers, F_2 recorded significantly higher value (98.42 g and 14.19 g respectively) in variety Sobha. In Sona also F_2 recorded significantly higher weight of primary fingers (97.48 g fresh and 15.96 g dry). In both the varieties, significantly higher fresh and dry weight of secondary fingers was recorded in F_4 with a value of 190.70 g and 23.28 g respectively in Sobha and 159.94 g and 23.93 g respectively in Sona. Significantly lower value with respect to the number of mother, primary and secondary finger was noted in unprimed control (Table 4).

Yield in terms of rhizome yield per plant differed significantly among the treatments tried in both the varieties (Fig. 4, 5). In variety Sobha, fresh rhizome yield per plant was found significantly higher (322.85 g) in plants sprayed with chitosan 4 g/L bimonthly (F_4). This was on par with F_2, chitosan 2 g/L monthly (312.89 g). Significantly lower yield per plant (113.09 g) was recorded in unprimed control (C) and was on par with primed control (C_P). Significantly higher dry rhizome yield per plant (41.11 g) was recorded in F_4 and was on par with all other treatments except F_1, C and C_P. Significantly lower dry weight per plant was observed in

Table 4. Below ground morphological parameters in response to chitosan foliar application in turmeric varieties Sobha and Sona at 6 MAT

Treatments	Weight of mother rhizome (g)				Weight of primary rhizome (g)				Weight of secondary rhizome (g)			
	Sobha		Sona		Sobha		Sona		Sobha		Sona	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
F_1: CTS 1 g/L monthly	26.14 ± 1.09^{c}	3.88 ± 1.10^{abc}	18.14 ± 2.01^{c}	3.73 ± 1.02^{abc}	69.56 ± 26.32^{bc}	11.33± 2.61^{c}	68.22 ± 23.75^{bcd}	10.88± 0.18^{b}	125.12 ± 13.62^{c}	18.93± 3.01^{a}	99.10 ± 2.04^{d}	16.53± 5.16^{bc}
F_2: CTS 2 g/L monthly	33.07 ± 4.06^{ab}	4.60 ± 0.86^{ab}	35.32 ± 1.33^{a}	4.17 ± 0.17^{a}	98.42 ± 18.40^{a}	14.19± 2.01^{a}	97.48 ± 24.07^{a}	15.96± 3.86^{a}	181.41 ± 9.94^{a}	22.29± 2.13^{a}	153.58 ± 2.66^{ab}	22.43± 0.51^{ab}
F_3: CTS 3 g/L bimonthly	27.02 ± 0.50^{bc}	4.05 ± 0.01^{ab}	18.31 ± 1.18^{c}	4.04 ± 0.26^{a}	81.85 ± 3.83^{abc}	12.29± 2.13^{bc}	84.79 ± 14.39^{ab}	13.27± 0.77^{ab}	136.40 ± 2.34^{bc}	20.61± 1.18^{a}	137.53 ± 9.55^{bc}	21.76± 3.87^{ab}
F_4: CTS 4 g/L bimonthly	35.84 ± 6.61^{a}	4.76 ± 0.24^{a}	32.39 ± 1.38^{a}	4.33 ± 0.37^{a}	96.32 ± 9.90^{a}	13.18± 1.96^{ab}	91.74 ± 13.36^{a}	15.24± 4.22^{a}	190.70 ± 14.70^{a}	23.18± 1.96^{a}	159.94 ± 15.57^{a}	23.93± 1.86^{a}
F_5: CTS 4 g/L trimonthly	25.58 ± 1.95^{c}	3.71 ± 0.43^{bc}	17.80 ± 2.16^{c}	3.87 ± 0.02^{ab}	73.02 ± 23.54^{abc}	11.89± 0.05^{bc}	78.19 ± 25.04^{abc}	12.59± 0.03^{ab}	128.42 ± 0.20^{c}	20.38± 4.50^{a}	125.12 ± 27.84^{c}	19.90± 1.30^{ab}
F_6: CTS 5 g/L trimonthly	31.51 ± 1.75^{abc}	4.32 ± 0.67^{ab}	27.58 ± 3.59^{b}	4.04 ± 0.30^{a}	86.11 ± 6.71^{ab}	13.16± 0.15^{ab}	87.26 ± 4.90^{ab}	14.30± 0.46^{a}	146.60 ± 1.35^{b}	20.84± 2.49^{a}	133.90 ± 15.87^{c}	21.05± 8.01^{ab}
C_P: Primed control	16.27 ± 3.59^{d}	2.99 ± 0.17^{c}	16.07 ± 1.84^{cd}	3.17 ± 0.57^{bc}	61.39 ± 11.38^{bc}	8.72± 0.85^{d}	59.17 ± 2.10^{cd}	10.12± 2.96^{b}	57.87 ± 6.31^{d}	11.16± 3.66^{b}	52.40 ± 3.73^{e}	12.14± 1.46^{cd}
C : Unprimed control	14.84 ± 2.41^{d}	2.98 ± 0.02^{c}	13.13 ± 1.75^{d}	3.15 ± 0.16^{c}	58.61 ± 3.12^{c}	8.04± 0.53^{d}	56.36 ± 0.73^{d}	10.03± 0.04^{b}	39.64 ± 6.64^{e}	8.50± 0.00^{b}	34.45 ± 4.59^{f}	7.15± 0.15^{d}
SEm (±)	2.007	0.342	1.202	0.234	8.558	0.589	6.924	1.122	5.024	1.668	5.669	2.074
CD (0.05)	6.087	1.037	3.647	0.709	25.959	1.788	21.003	3.405	15.237	5.061	17.196	6.29
CV	13.225	15.148	9.322	10.628	18.966	8.802	15.396	15.191	6.918	15.847	8.767	19.835

The data represents mean of 3 replications. Values that are followed by the same letter in a column do not differ significantly between them CTS : Chitosan

Fig. 4. Rhizome yield per plant in response to chitosan foliar application in turmeric varieties A) Sobha; B) Sona

F_1: CTS 1 g/L monthly, F_2: CTS 2 g/L monthly, F_3: CTS 3 g/L bimonthly, F_4: CTS 4 g/L bimonthly, F_5: CTS 4 g/L trimonthly, F_6: CTS 5 g/L trimonthly, C_P: Primed control and C: Unprimed control. CTS: Chitosan

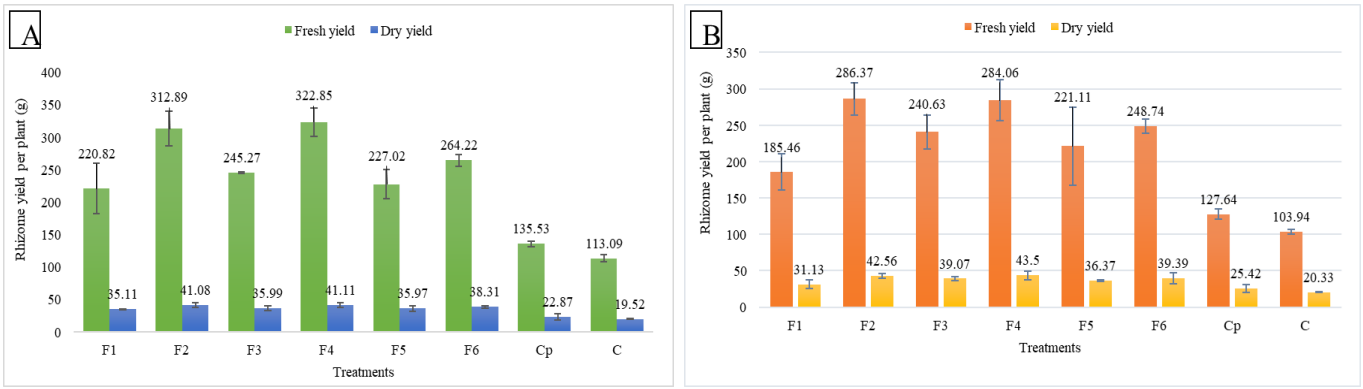

Fig. 5. Rhizome yield/plant in response to chitosan foliar application in turmeric varieties (A) Sobha and (B) Sona

F_1: CTS 1 g L^{-1} monthly, F_2: CTS 2 g L^{-1} monthly,F_3: CTS 3 g L^{-1} bimonthly, F_4: CTS 4 g L^{-1} bimonthly, F_5: CTS 4 g L^{-1} trimonthly, F_6: CTS 5 g L^{-1} trimonthly, C_P: Primed control and C: Unprimed control.

unprimed control with a value of 19.52 g. In case of Sona, F_2 recorded significantly higher fresh rhizome yield per plant (286.37 g) and was on par with F_4 (284.06 g). Significantly lower fresh rhizome yield was recorded in unprimed control (103.94 g), which was on par with C_P (127.64 g). The dry rhizome yield per plant showed significantly higher value (43.50 g) in F_4, which was on par with F_2, F_3 and F_6. Significantly lower value (20.33 g) was recorded in unprimed control treatment (C). The fresh rhizome weight enhanced by 85 % in Sobha and 75 % in Sona over the control, while dry rhizome weight enhanced by 11 % in Sobha and 14 % in Sona over the control.

Uptake of major nutrients

Various treatments showed significant difference in the uptake of plant nutrients in both the varieties and the data is illustrated in Table 5. In Sobha, treatment F_2 recorded the highest values (5.06, 0.50 and 4.82 g per plant, respectively) of nitrogen, phosphorous and potassium uptake and significantly lower reading (2.07, 0.13 and 1.05 g per plant, respectively) was recorded in unprimed control.

In case of Sona, significantly higher uptake of nitrogen (4.81 g per plant) was observed in F_2 and was on par with F_4 (4.25 g per plant). Significantly lower value (3.00 g per plant) of nitrogen uptake was obtained in C_P, which was on par with F_1, F_3, F_5 and unprimed control. The uptake of phosphorus was significantly higher in F_2 (0.44 g per plant). The highest potassium uptake (5.03 g per plant) was recorded in F_2. The lowest value of P (0.17 g per plant) and K (0.97 g per plant) uptake were recorded in unprimed control.

Curcumin content

There was significant difference in curcumin content, among the treatments tried in both the varieties (Fig. 6). In variety Sobha, out of all the treatments, F_2 (chitosan 2 g/L monthly foliar spray) recorded significantly higher curcumin content of 6.63 %, which was on par with F_4 (6.42 %). The unprimed control treatment (C) recorded significantly lower curcumin content of 3.50 %. In variety Sona, F_2 (chitosan 2 g/L monthly foliar spray) recorded the highest curcumin content of 7.35 %. Significantly lower curcumin content (4.76 %) was recorded in unprimed control (C). Thus, monthly application of chitosan 2 g/L enhanced the curcumin content by 89 % in Sobha and 54 % in Sona over the unprimed control.

Table 5. Uptake of major plant nutrients in response to chitosan foliar application in turmeric varieties Sobha and Sona

Treatments	Nitrogen (g plant^{-1})		Phosphorus (g plant^{-1})		Potassium (g plant^{-1})	
	Sobha	Sona	Sobha	Sona	Sobha	Sona
F_1: CTS 1 g/L monthly	2.81 ± 0.45^{d}	3.12 ± 0.14^{cd}	0.28 ± 0.02^{e}	0.30 ± 0.02^{cd}	1.51 ± 0.19^{e}	1.59 ± 0.10^{e}
F_2: CTS 2 g/L monthly	5.06 ± 0.18^{a}	4.81 ± 0.43^{a}	0.50 ± 0.03^{a}	0.44 ± 0.08^{a}	4.82 ± 0.17^{a}	5.03 ± 0.04^{a}
F_3: CTS 3 g/L bimonthly	3.17 ± 0.49^{d}	3.61 ± 0.04^{cd}	0.34 ± 0.00^{d}	0.38 ± 0.02^{ab}	1.88 ± 0.01^{d}	2.01 ± 0.01^{d}
F_4: CTS 4 g/L bimonthly	4.43 ± 0.45^{b}	4.25 ± 0.04^{ab}	0.47 ± 0.01^{b}	0.40 ± 0.07^{ab}	3.40 ± 0.14^{b}	3.39 ± 0.07^{b}
F_5: CTS 4 g/L trimonthly	2.86 ± 0.35^{d}	3.11 ± 0.13^{bcd}	0.33 ± 0.01^{d}	0.38 ± 0.05^{ab}	2.01 ± 0.39^{d}	1.72 ± 0.20^{e}
F_6: CTS 5 g/L trimonthly	3.78 ± 0.23^{c}	3.72 ± 0.18^{bc}	0.43 ± 0.01^{c}	0.33 ± 0.01^{bc}	2.76 ± 0.24^{c}	2.72 ± 0.06^{c}
C_P: Primed control	2.28 ± 0.07^{e}	3.00 ± 0.54^{d}	0.24 ± 0.01^{f}	0.25 ± 0.01^{d}	1.33 ± 0.10^{ef}	1.34 ± 0.12^{f}
C : Unprimed control	2.07 ± 0.06^{e}	3.10 ± 0.73^{cd}	0.13 ± 0.01^{g}	0.17 ± 0.05^{e}	1.05 ± 0.08^{f}	0.97 ± 0.06^{g}
SEm (±)	0.153	0.215	0.009	0.023	0.114	0.05
CD (0.05)	0.465	0.653	0.026	0.07	0.346	0.152
CV	8.023	10.385	4.407	12.024	8.42	3.701

The data represents mean of 3 replications. Values that are followed by the same letter in a column do not differ significantly between them CTS : Chitosan

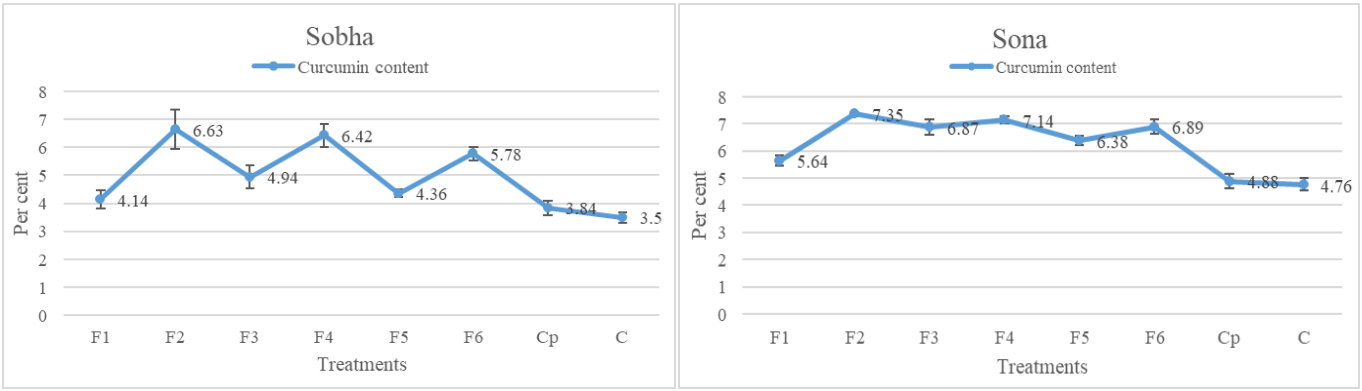

Fig. 6. Curcumin content in response to chitosan foliar application in turmeric varieties (A) Sobha and (B) Sona

F_1: CTS 1 g L^{-1} monthly, F_2: CTS 2 g L^{-1} monthly, F_3: CTS 3 g L^{-1} bimonthly, F_4: CTS 4 g L^{-1} bimonthly, F_5: CTS 4 g L^{-1} trimonthly, F_6: CTS 5 g L^{-1} trimonthly, C_p: Primed control and C: Unprimed control.

Expression profile of curcumin synthase gene

Rhizomes from the treatment that yielded the highest curcumin content among the different treatments *i.e.* chitosan 2 g/L monthly foliar application and the rhizomes from unprimed control treatment of both the varieties were used to study the expression profile of curcumin synthase gene. This treatment showed the highest curcumin content in both the varieties, Sobha and Sona. The purity of the isolated RNA ranged from 1.97 to 2.02, with concentrations varying between 0.094 and 0.228 µg/µl. The synthesized cDNA when subjected to real-time PCR with reference gene *actin*, qRT-PCR product was observed as distinct bands in agarose gel (1.5 %) (Fig. 7). The results of qPCR indicated that the monthly foliar application of chitosan at 2 g/L led to a 1.48 fold increase in the expression of the curcumin synthase 1 (*CURS1*) gene in Sobha and a 1.77 fold increase in Sona compared to the untreated control (Fig. 8).

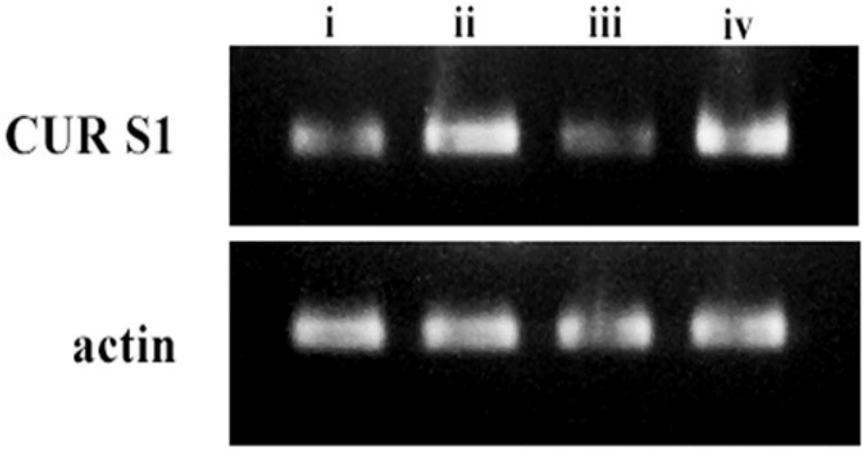

Fig. 7. Gel profile of *CURS1* and *Actin*

Lane i - Tc of Sona, ii -Tt of Sona, iii - Tc of Sobha, iv -Tt of Sobha, Tc : Untreated control, Tt : Chitosan treated sample

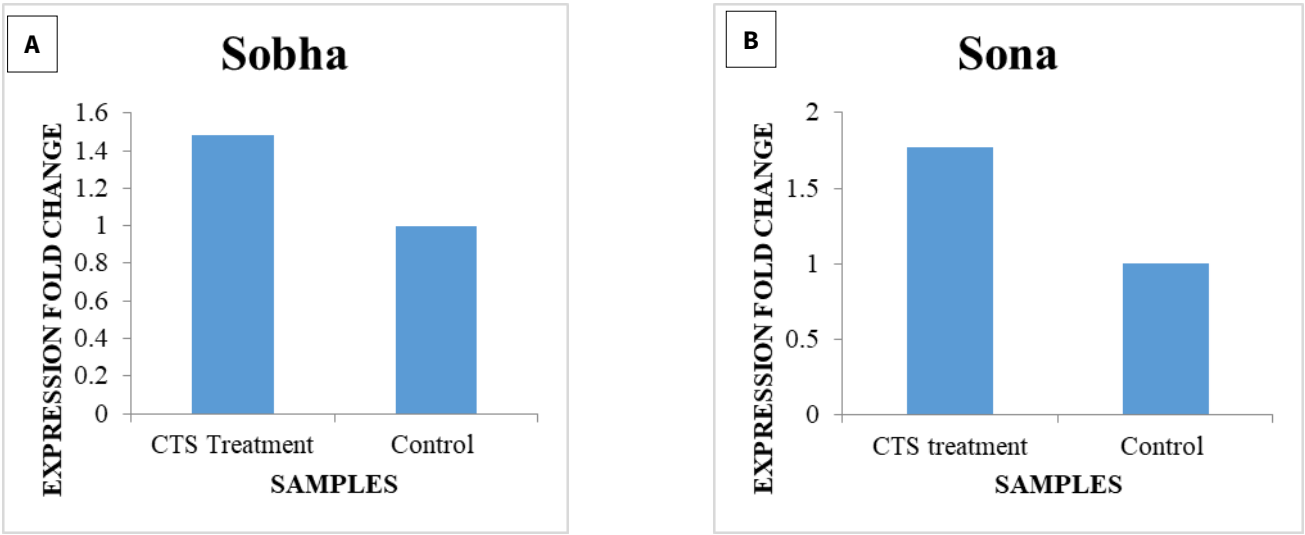

Fig. 8. Expression pattern of *CURS1* in response to chitosan foliar application (2 g/L bimonthly) in turmeric varieties (A) Sobha and (B) Sona (CTS : Chitosan)

Discussion

The foliar spray application of chitosan increased the plant above ground parameters compared to control. In both the varieties, the tallest plants were observed in F_2 (chitosan 2 g/L monthly application) and unprimed control plants (C) recorded significantly lower plant height. Ahmed (20) noted that applying chitosan as foliar spray (at concentrations of 4 and 6 ml/L) at 30, 45, 60 and 75 days after planting boosted garlic plant height. According to him, growth enhancement following chitosan foliar spray may be due to certain amino acid components provided by chitosan, which is essential for plant development. Sofy et al. (21) proposed that the amino groups within chitosan aid chlorophyll synthesis, thereby improving photosynthesis and ultimately enhancing plant height. Contrary to our results, Janmohammadi et al. (22) found no significant difference in plant height of lentil on foliar application of 0.1% w/v chitosan at 30 days after sowing over the untreated control. This variation in the response to chitosan in different plant species could be related to the concentration and frequencies of application of chitosan. The number of tillers did not show any significant variation among the various treatments tried.

Chitosan application significantly enhanced the leaf number and leaf area might have facilitated increased nutrient absorption by plants. This, in turn, might have promoted cell enlargement, chloroplast development and chlorophyll production (23). Similarly, Mondal et al. (24) observed that fortnightly foliar application of chitosan 100-125 mg/L improved plant height and leaf number in okra. Guan et al. (25) also opined that chitosan induced the augmentation of leaf dimensions, which would lead to increased water and nutrient uptake by plants. This consequently, enhanced the activity of enzymes crucial for nitrogen metabolism and facilitated improved nitrogen transportation within the plant. These mechanisms, in turn, could accelerate photosynthesis, overall growth and plant development.

Malekpoor et al. (26) investigated the impact of chitosan on morphological parameters in sweet basil (*Ocimum basilicum*). Among various concentrations (0.0, 0.2 and 0.4 g/l) of chitosan tested, significantly higher shoot weight was observed when treated with chitosan at 0.4 g/l. Conversely, Lei et al. (27) found no significant effect on the growth of *Artemisia annua* in terms of weight of plant following foliar application of chitosan. Manjusha et al. (28) documented notable improvements in various growth parameters of turmeric when subjected to foliar spray with chitosan at 100 ppm. This treatment, applied at 60, 90 and 120 days after transplanting (DAT), resulted in significantly superior plant height, number of leaves per plant, leaf length, leaf width, number of tillers per plant and fresh weight per plant. Similarly, Sheikha and Al-Malki (29) reported that chitosan application enhanced the leaf area, shoot length as well as fresh and dry weights of shoots in beans (*Phaseolus vulgaris*). The monthly foliar application of chitosan 2 g/L increased the plant below ground parameters viz. rhizome spread, rhizome thickness, number of fingers and rhizome weight compared to control in both the varieties. In the present investigation, monthly foliar application of chitosan at a concentration of 2 g/L (F_2) was demonstrated superior outcomes in terms of morphological parameters such as plant height, leaf area, shoot weight and rhizome spread in both the varieties.

Chitosan regulates various physiological and biochemical processes in plants including enhancing stress resistance and nutrient uptake. Chitosan aids in the removal of reactive oxygen species (ROS), possibly by activating antioxidant enzymes and safeguards the functions of the bio-membrane. This protective action enhances various physiological fucntions in the crop (30, 31). Leaf chlorophyll content serves as a vital indicator of a plants physiological status, closely linked to its photosynthetic function (32). The treatment F_2, chitosan 2 g/L monthly application which recorded a significantly higher chlorophyll content was also observed to give better results in terms of plant growth parameters. An increased capacity for photosynthesis might be the key factor behind the enhanced growth of chitosan treated plants (33). Limpanavech et al. (34) and Ahmed et al. (35) reported that chloroplast are primary targets for chitosan, which enhances chloroplast function and improves chlorophyll synthesis. Increased number of chloroplast per cell (36) and higher availability of amino compounds due to chitosan treatment (37) enhances chlorophyll synthesis. Additionally, chitosan boosts photosynthetic pigments by elevating endogenous levels of cytokinins, which in turn stimulate chlorophyll synthesis, as highlighted by El-Khair (38).

Chitosan 2 g/L monthly application also recorded a significantly higher activity of defense enzymes among the various treatments tried. Peroxidase and polyphenol oxidase are vital antioxidant enzymes that play a crucial role in mitigating oxidative damage and enhancing plant growth. The application of chitosan has been shown to boost the activity of these enzymes. Anusuya and Sathiyabama (14) reported that foliar spray of 0.1% w/v chitosan induced the expression of defense enzymes such as peroxidase and polyphenol oxidase in both leaves and rhizomes of turmeric. Zong et al. (39) found that foliar application of chitosan enhanced the performance of antioxidant enzymes like superoxide dismutase, catalase and peroxidase in *Brassica rapa*. By elevating the levels of these defense enzymes, chitosan effectively reduces reactive oxygen species, thereby minimizing cell damage (40) and stimulates defense mechanisms within plants (41). This enhanced activity of defense enzymes due to chitosan treatment helps the plants to withstand environmental stresses, both biotic and abiotic, leading to improved plant growth and yield.

In the present study, significantly higher fresh rhizome yield per plant was obtained from 2 g/L monthly and 4 g/L bimonthly chitosan foliar applications. A study by Anusuya and Sathiyabama (14) demonstrated that monthly application of chitosan 0.1 % w/v to turmeric plants resulted in substantial increase in the yield, approximately 60 % higher compared to those treated with water spray alone. Similarly, in garlic, foliar spraying with chitosan 4 and 6 ml/L, at different intervals after planting led to improved yield and related parameters such as bulb fresh weight, number of cloves per bulb and bulbing ratio (20). In potato, the foliar

application of hydrolyzed chitosan derivative at a rate of 200 mg/ha significantly boosted tuber yield by 15 % to 30 % over untreated control by enhancing both the number and total mass of tubers (42).

In the present study, frequent application of chitosan (at monthly and bimonthly intervals) at lower rate (2 and 4 g/L) improved plant growth parameters and rhizome yield. Kra et al. (43) found that chitosan at a concentration of 100 mg/L resulted in increased shoot and root growth, whereas growth was hindered at a concentration of 125 mg/L in cassava (*Manihot esculenta*). This aligns with the present study's findings, suggesting that optimal concentrations of chitosan enhance growth, while higher concentrations may inhibit it. Guan et al. (25) indicated that the effects of chitosan on plant growth depend on the timing of its application, which supports the approach in the present study of applying lower rates more frequently. In a study by da Silva et al. (11) a 0.125% concentration of chitosan promoted shoot and root growth and increased total dry weights in *Mentha arvensis.* However, higher concentrations (0.25% and 0.75%) caused injuries to the plants. These findings indicate that the growth responses of plants are influenced by the concentrations of chitosan used.

The notable rise in crop yield resulting from the application of chitosan, specifically the treatments F_2 and F_4 could be attributed to enhancement in photosynthetic pigments, synthesis of photosynthates, and biochemical plant processes. These might have facilitated the redirection of a higher quantity of photosynthates towards the rhizomes (44). The activity of defense enzymes, both peroxidase and polyphenol oxidase were recorded significantly higher in treatments F_2 and F_4. This also might have helped in the better establishment of crop in the field. However, varietal variation on plant growth and yield parameters in response to chitosan application in turmeric was evident in the present study. Manjusha et al. (28) reported varietal differences in turmeric varieties Roma and Mydukur following foliar application of chitosan at a concentration of 0.01 g/L at 60, 90 and 120 days after planting.

The application of chitosan resulted in increased uptake of major nutrients, including nitrogen, phosphorus and potassium. The treatment, F_2, chitosan 2 g/L monthly application recorded significantly higher uptake of major nutrients. Chitosan serves as a carbon source for soil microbes, hastening the conversion of organic matter into inorganic forms and aiding roots in absorbing nutrients more efficiently from the soil (45). The enhanced uptake of nitrogen by the shoots could be attributed to the amino components present in chitosan and the improved ability of plants to absorb nitrogen from the soil as chitosan degraded. The increased uptake of potassium facilitates the translocation of photosynthates from leaves to storage organs, thereby improving their quality (38). Mahdavi and Rahimi (41) proposed that chitosan enhances plant growth by augmenting the availability and absorption of water and essential nutrients, potentially by regulating cell osmotic pressure. Chitosan molecules exhibit significant hydrophilicity, allowing them to regulate osmotic pressure within plant cells by enhancing the absorption of water and essential nutrients (25). Additionally, chitosan promotes the division of root cells by activating plant hormones such as auxin and cytokinin, thereby further enhancing nutrient uptake (46). The enhanced uptake of major nutrients helps in the better plant growth in turmeric. The treatments, F_2 and F_4 recorded significantly higher uptake of major nutrients and also recorded significantly higher plant growth and yield.

The treatment, F_2 (chitosan 2 g/L monthly application) recorded the highest curcumin content in both the varieties, Sobha and Sona. However, all the chitosan foliar spray treatments were observed to give significantly higher curcumin content over the control plants. Chitosan, acting as a bio-elicitor, has the potential to boost the production of secondary metabolites and promote plant growth across various spices and medicinal plants, as noted by Yin et al. (47) and Lopez-Moya et al. (48). Aswini (15) demonstrated that foliar application of chitosan 1 g/L effectively increased curcumin content and yield in turmeric, with an increase of 52.96 % in curcumin yield over the control. Similarly, Sathiyabama et al. (49) found that application of chitosan 0.1 % stimulated curcumin accumulation and doubled its production in turmeric. Gorelick et al. (50) observed that treating aswagandha plants with chitosan at a concentration of 100 mg/ml led to a significant increase in withaferin A content by approximately 69 % compared to the control. Furthermore, chitosan enhances the activity of the phenylalanine lyase (PAL) enzyme, a crucial regulator in the phenylpropanoid pathway responsible for inducing secondary metabolite production (51).

Key enzyme genes essential for curcuminoid biosynthesis include diketide-CoA synthase (DCS) and curcumin synthases CURS1, CURS2 and CURS3 (52). Sheeja et al. (53) proposed that along with this, two novel polyketide synthase genes (*clpks1* and *clpks2*) are also involved in the curcuminoid biosynthesis pathway of *C. longa*. This study focuses on elucidating the impact of chitosan application on the expression of the curcumin synthase 1 (*CURS1*) gene in the rhizomes. The rhizome from the plants sprayed monthly with chitosan 2 g/L, that yielded the highest curcumin content also showed 1.48 fold increase in the expression of the curcumin synthase 1 (*CURS1*) gene in Sobha and 1.77 fold increase in Sona over the untreated control. Ayer et al. (54) observed lower expression of the *CURS1* gene in the low curcumin yielding cultivar NDH-98, while expressions were higher in the high curcumin yielding cultivar GNT-2. Abhijit (55) reported a 1.87 fold enhancement in the expression of the piperine synthase gene in *Piper longum* following a chitosan foliar spray at 1 g/L. Sandeep et al. (56) highlighted that soil environmental factors play a significant role in influencing the expression of the curcumin synthase gene, which is closely associated with curcumin yield in turmeric cultivars. This suggests that environmental conditions can impact the production of curcumin. Kim et al. (51) suggested that chitosan can efficiently stimulate the production of phytochemicals in plants. This indicates that chitosan may offer a viable and efficient alternative to genetic modification for enhancing phytochemical production in plants. Lei et al. (27) found that chitosan induced the expression of *ADS* and *DBR2* genes involved in the artemisinin biosynthesis pathway,

consequently leading to increased artemisinin content in *Artemisia annua* plants. Similarly, according to Fooladi Vanda et al. (57), treatment with chitosan resulted in elevated expression levels of *PAL1, TAT* and *RAS* genes in *Melissa officinalis*, which are associated with rosmarinic acid biosynthesis. Furthermore, Al Ghamdi (58) observed increased expression of genes related to essential oil composition, (*CYP71D179/182* and *CYP71D178 PII)*, in marjoram plants after an 8-week foliar application of chitosan at 50, 200 and 500 ppm.

Chitosan serves as a regulatory molecule in signal transduction across various signaling pathways. Upon activation by chitosan, specific receptors located either on the cell membrane or intracellularly initiate signaling cascades wherein one or more second messengers relay the signal to the cell. This process triggers a spectrum of physiological responses and the signal can be amplified, leading to the development of a complex signaling network (59). Even at lower concentrations, chitosan acts as a signaling molecule capable of stimulating plant growth, eliciting physiological responses and ultimately enhancing the yield. This underscores its potency as a regulator of plant development and productivity. The repeated application of inorganic fertilizers, with restricted degradability, has led to increased soil toxicity, creating negative impact on beneficial soil microorganisms and soil properties. The use of chitosan at low concentrations as a bio-fertilizer emerges as a safe and effective alternative, mitigating the risks associated with the use of inorganic fertilizers and could be effectively incorporated in organic production system of crops.

Conclusion

Chitosan, the most commonly used derivative of chitin in agriculture, is non-toxic, bio-compatible and biodegradable biostimulant recommended to enhance plant growth, yield, and quality attributes. In the current study, foliar application of chitosan improved growth, yield, nutrient uptake, photosynthesis, defense enzymes activity and secondary metabolite production in turmeric. Among various concentrations and application frequencies, a monthly foliar spray of chitosan at 2 g/L yielded the best results for plant growth, yield and curcumin content. Chitosan application also regulates the expression of curcumin synthase 1 (*CURS1*) gene involved in the curcuminoid biosynthesis pathway of turmeric. Thus, chitosan proves to be an effective biostimulant for enhancing growth, yield and curcumin content, supporting overall productivity in turmeric.

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Authors' contributions

Conceptualization, Methodology and Supervision by DSN. Formal analysis and investigation by SSN. Manuscript preparation, Editing and Review by SSN, DSN, SGS, SPP, SA, AKN and AJ. Resources supplied by DSN, SA and AKN. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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