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Effect of chitosan on growth, yield and curcumin content in turmeric under field condition

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ABSTRACT

Field experiments were conducted at the experimental field in Erode, TamilNadu to investigate the effect of foliar application of chitosan (a growth promoter) on growth, yield attributes and curcumin content of turmeric (Erode local). The chitosan (0.1%, w/v) was sprayed at a regular interval of 30 days up to 210 days. Results revealed that the growth parameters (shoot height, leaf number/plant, plant fresh weight) were increased with application of chitosan. Foliar application of chitosan induced the activity levels of defense enzymes such as protease inhibitors (PI), β-1,3 glucanases, peroxidases (PO) and polyphenol oxidases (PPO) in the leaves and rhizomes of turmeric plants. Chitosan treatment to turmeric plants results in high yield and curcumin content. The results suggest that chitosan can be used as an eco-friendly compound to induce defense responses as well as the growth and curcumin content of turmeric plants.

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1. Introduction

Turmeric (*Curcuma longa* L.) is an important common flavoring spice of daily use. A recent report indicates that, the export and demand of Indian turmeric have increased due to increased food as well as non-food uses (Ray et al., 2016). Turmeric contains the main active constituent curcumin has a wide range of biological activities including antioxidant, anti-inflammatory, antimutagenic, anti-carcinogenic and anti-angiogenic properties (Kunnumakkara et al., 2007; Li et al., 2009). The use of turmeric and its value added products is recognized globally and hence the production has to be increased to meet the requirements. However, its cultivation is affected by several fungal diseases. Among the fungal diseases, rhizome rot causes a severe yield reduction and reduce the quality (Rathaiah, 1980). Therefore, finding the effective method to solve this problem should be considerably focused.

Chitosan, a biopolymer, has been reported to stimulate the immune system involved in plant resistance to pathogen infection (Pichyangkura and Chadchawan, 2015). In addition, chitosan has been widely used to stimulate growth, germination and enhance yield in many crop species such as in orchid (Nge et al., 2006), faba bean (El-sawy et al., 2010), cucumber (Sheheta et al., 2012) and corn (Boonlertnirun et al., 2011; Lizárraga-Paulín et al., 2011). Faoro et al. (2008) showed that the chitosan applied as a foliar spray on barley reduced locally and systemically the infection by powdery mildew pathogen *Blumeria graminis* f. sp. *hordei*. Considering the above facts, the present research work was undertaken to evaluate the foliar application of chitosan on growth, curcumin content and in control of rot disease in turmeric plants under field condition.

2. Materials and methods

2.1. Biological material and experimental design

The field experiment was conducted in the Oonjalur village of Erode District, Tamil Nadu, where the cultivation of turmeric is highly practiced. The widely cultivated turmeric cultivar Erode local (*Curcuma longa*) was used as test crop. The experimental field was prepared properly with ploughing and laddering. The trials were laid out in a randomized block design (RBD) with net plot size of 4 × 2 m. Rhizomes (each rhizome with 3 nodes) of cultivar Erode local were planted on row ridges (4 m long; 25 plants/row) spaced 40–60 cm apart and 15 cm between plants for all the treatments at the same time. Each treatment consisted of 3 replications in the field experiment. Turmeric plants (30 day old) were sprayed with chitosan (0.1%, w/v) at regular interval of 30 days up to 210 days (6 ml/plant) and water sprayed plants served as control. Rhizomes treated with mancozeb (0.3%) for 30 min at the time of sowing and spraying of tilt 25 EC (propiconazole, 0.1%)

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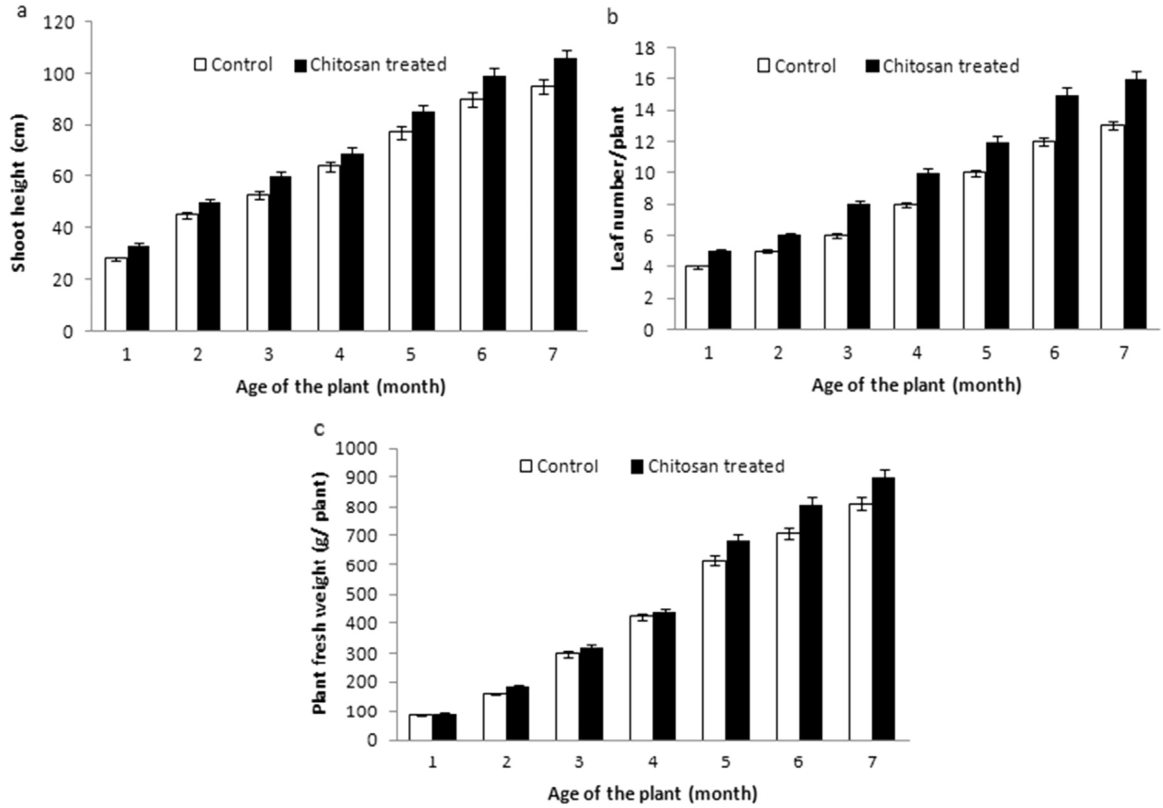

Fig. 1. Shoot height (a), leaf number/plant (b) and plant fresh weight (c) of turmeric plants treated with chitosan under field condition. Data represent Mean ± Standard Error.

along with Dithane M-45 75 WP (0.25%) thrice at 20 days interval served as fungicide treated. Plants were irrigated at regular intervals. The leaves were removed after 210 days and the rhizomes were left for another 30 days to harvest.

2.2. Effect of chitosan on plant growth

Growth parameters such as leaf number, shoot height, plant fresh weight in control and treated plants were monitored at different age levels up to 215 days.

2.3. Protein extraction, estimation and enzyme activities

Leaves and rhizomes were collected from control and GNP treated turmeric plants at regular intervals. They (1 g /2 ml) were homogenized with potassium phosphate buffer (0.02 M, pH 7.6) and centrifuged at 10,000*G* for 10 min at 4° C. The clear supernatant was used as a source of protein, enzymes. The protein concentration of the supernatant was estimated by Bradford's method (1976) using BSA fraction V (Sigma Chem. Co., USA) as a standard.

β-1,3 glucanase, Peroxidase (PO), Polyphenol Oxidase (PPO) and Protease inhibitor (PI) activity were assayed as described previously (Anusuya and Sathiyabama, 2015).

2.4. Effect of chitosan on rhizome yield and curcumin content

Rhizome yield was determined at the time of harvest. The average fresh and dry weight of rhizome per plant was expressed in terms of gram. The yield increase percentage was calculated using the following formula; Yield increase (%)=[treatment yield − control yield]/ control yield × 100 (Tariq et al., 2010).

For curcumin analysis, rhizomes (1 g/10 ml) were extracted with methanol at 60° C for seven hours in a Soxhlet apparatus and dried using a rotary evaporator (Yamato RE 601). 1 mg of extracted sample was mixed with methanol and OD was taken at 425 nm using curcumin (Sigma Chem Co., USA) as a standard (Chauhan et al., 1999). Curcumin content of control and treated plants were expressed as milligram per plant.

2.5. Disease incidence

Turmeric plants (control, chitosan treated, fungicide treated) showing typical symptoms of rotting under field conditions were assessed at the time of harvest. Disease incidence was determined on the basis of disease score, an estimate of the area decayed using a five-class scale (Campbell and Madden, 1990) as follows: 0=No disease (none affected); 1=Slight rot or discoloration (less than 30% affected tissue); 2=Moderate rot or discoloration (30–70% affected tissue); 3=Severe rot or discoloration (more than 70% affected tissue); 4=Complete rot. The percentage of disease incidence was calculated as described by Guo et al. (2004). Rot incidence=([Scale × Number of plants infected]/[Highest scale × Total number of plants]) × 100.

2.6. Statistical analysis

All the data were subjected to one-way analysis of variance to determine the significance of individual differences in $p < 0.01$ and 0.05 levels. All statistical analysis was conducted using SPSS 16 software support.

3. Results and discussion

Foliar application of chitosan to turmeric plants increased the number of leaves/plant, shoot height and plant fresh weight when

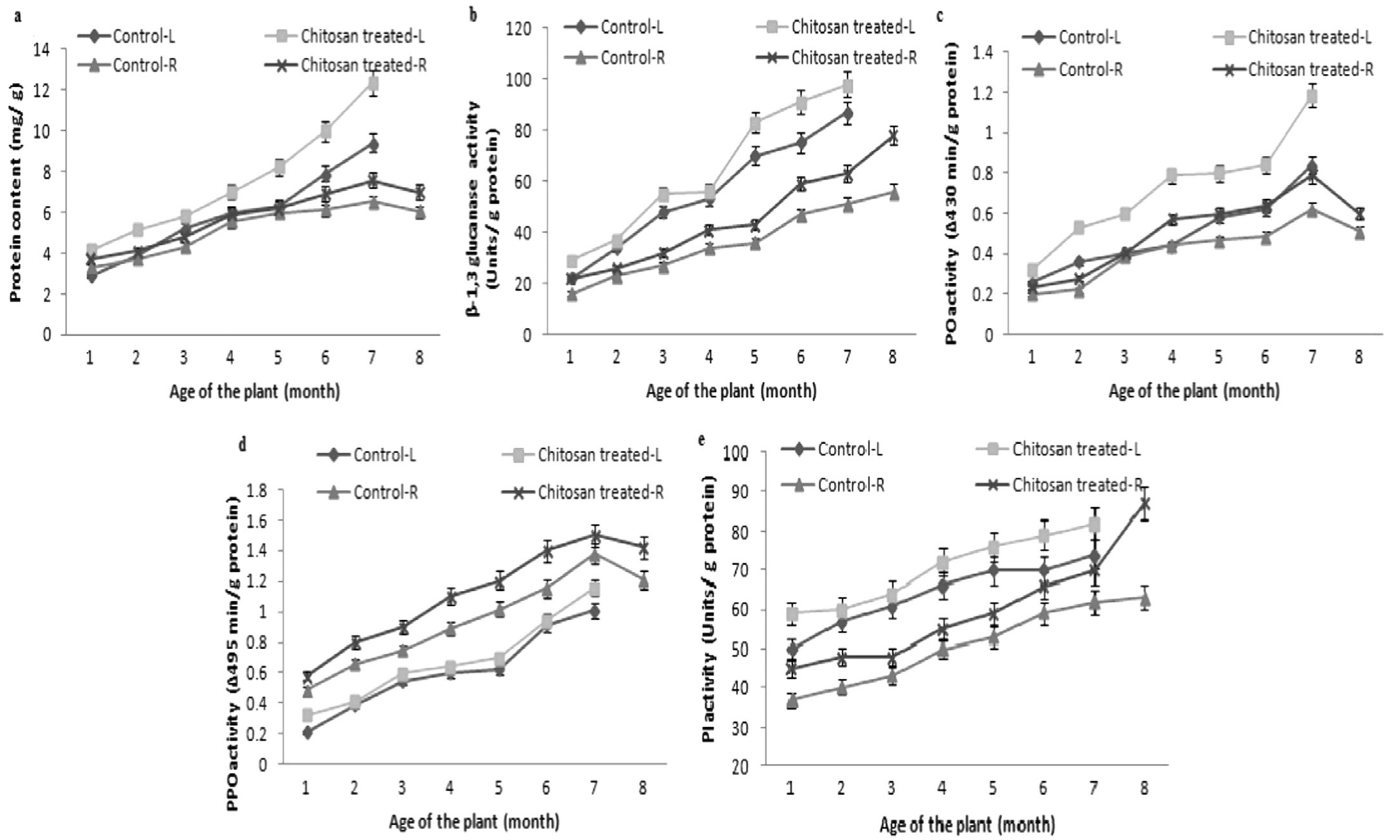

Fig. 2. Protein content and enzyme activities of turmeric plants at different age levels under field condition: L – Leaf; R – Rhizome; PO – Peroxidase; PPO – Polyphenol oxidase; PI – Protease inhibitor; Data represent Mean ± Standard Error; Bars without standard error showed negligible values.

compared to control under field condition (Fig. 1). Plants sprayed with chitosan showed an increase in protein content in leaves as well as in rhizomes at all age levels when compared to control. In general, the protein content was higher in leaves of treated plants than rhizomes of turmeric plants (Fig. 2a).

Application of chitosan also demonstrated the enhanced level of defense related enzymes such as protease inhibitors, β-1,3 glucanases, peroxidases, polyphenol oxidases in leaves and also in rhizomes under field condition (Fig. 2b–e). High level expression of defense-related proteins such as peroxidase (PO) and polyphenol oxidase (PPO) in chitosan treated plants might involve in the formation of lignin to restrict the entry and movement of fungal pathogens in the plant system. Increased PO and PPO activity has been shown in a number of resistant interactions involving plant pathogenic fungi, bacteria and viruses (Chen et al., 2000; Nandakumar et al., 2001). The systemic induction of PIs has been demonstrated in rice (Xu et al., 1993) and maize (Eckelkamp et al., 1993).

In control plants, disease symptoms were observed in 75 day old seedlings. Rot incidence increased with increase in age level and 95% rot incidence was observed at the 8th month (at the time of harvest) in control plants, whereas the disease symptom was observed after 120 days and the rot incidence was only 30% at the 8th month in chitosan treated plants (Table 1). Foliar application of chitosan significantly reduced the rot disease incidence (up to 68%) compared to control plants in field condition. There is a correlation between enhanced activities of defense enzymes and suppressed disease symptom in chitosan treated plants at field level. It may be possible that enhanced defense enzyme activities in leaf and rhizome might have played a role in curtailing the disease development in chitosan treated turmeric plants. Application of chitosan has induced systemic resistance in host plants and provides sustainable disease control as suggested by van Loon et al. (1998). It is suggested that chitosan can be used commercially for controlling tomato root rot diseases under field conditions (El-Mougy et al., 2006).

There was a significant variation in yield under field conditions due to foliar application of chitosan when compared to control. Foliar application of chitosan increased the rhizome yield up to 60% by fresh weight and 50% increase by dry weight over the

Table 1
Disease incidence (%) in turmeric plants under field condition.

	Treatment	Age of the plant (month)							
		1	2	3	4	5	6	7	8
Disease score (0–4 scale)	Control	0	0	1	1	2	2	3	3
	Chitosan treated	0	0	0	0	0	1	1	1
	Fungicide treated	0	0	0	0	0	1	1	1
Disease incidence (%)	Control	0	0	5.83 ± 0.02	9.16 ± 0.043	20 ± 0.007	27 ± 0.054	50 ± 0.003	62 ± 0.102
	Chitosan treated	0	0	1.23 ± 0.10	1.8 ± 0.045	9.27 ± 0.9	14.2 ± 0.2	21 ± 0.078	30 ± 0.09
	Fungicide treated	0	0	1.09 ± 0.03	1.1 ± 0.023	9.41 ± 0.7	12.2 ± 0.9	20 ± 0.099	29 ± 0.006

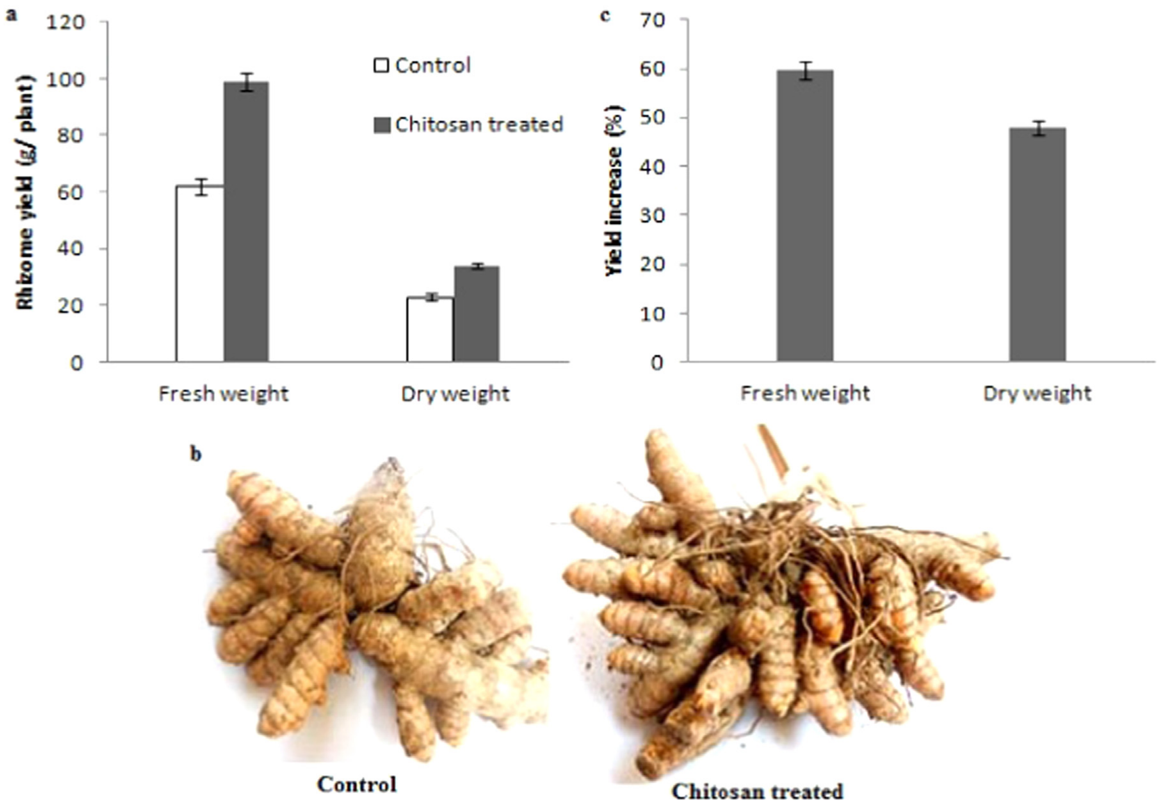

Fig. 3. Rhizome yield of turmeric plants (a, b) and yield increase (%) over control (c) under field condition.

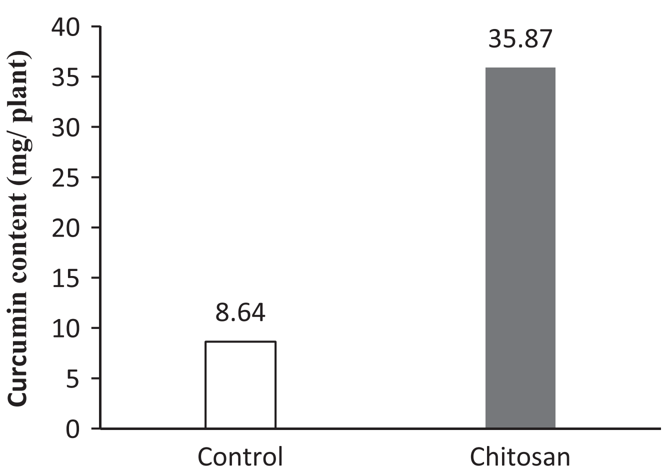

Fig. 4. Curcumin content of turmeric plants under field condition.

control (Fig. 3a–c). Increased numbers of nodes in rhizomes were also observed in chitosan sprayed plants (Fig. 3c). Chitosan treatment elicited 4-fold increase in curcumin content compared to control (Fig. 4). Mondal et al. (2012) reported the effect of foliar application of chitosan on growth and yield in okra. Trials conducted in tomatoes (Walker et al., 2004) showed that foliar applications of chitosan resulted in yield increase of nearly 20% and a significant improvement in powdery mildew disease control. Thus chitosan, a nontoxic, biodegradable material can be used under field condition, as an ecofriendly compound to enhance growth and yield of turmeric.

4. Conclusion

It is concluded that foliar application of chitosan at vegetative stage enhanced the plant growth and development, which results in increased rhizome yield in turmeric. Chitosan treated plants also showed a significant improvement in control of rhizome rot disease. Hence, chitosan can be used as an ecofriendly compound to protect turmeric plants as well as to enhance yield and curcumin content under field condition.

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References

Anusuya, S., Sathiyabama, M., 2015. Foliar application of β-d-glucan nanoparticles to control rhizome rotdisease of turmeric. Int. J. Biol. Macromol. 72, 1205–1212.

Boonlertnirun, S., Suvannasara, R., Promsomboon, P., 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 the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Campbell, C.L., Madden, L.V., 1990. Plant Disease Epidemiology. John Wiley & Sons, New York, p. 532.

Chauhan, S.K., Singh, B.P., Agrawala, S., 1999. Estimation of curcuminoids in *Curcuma longa* by HPLC and spectrophotometric method. Ind. J. Pharm. Sci. 61, 58–60.

Chen, C., Belanger, R.R., Benhamou, N., Paulitz, T.C., 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting Rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol. Mol. Plant Pathol. 56, 13–23.

Eckelkamp, C., Ehmann, B., Schopfer, A., 1993. Wound induced systemic accumulation of a transcript coding for a Bowman-Birk trypsin inhibitor related protein in maize (*Zea mays* L.) seedlings. FEBS Lett. 323, 73–76.

El-Mougy, N.S., El-Gamal, N.G., Fotouh, Y.O., Abd-El-Kareem, F., 2006. Evaluation of different application methods of chitin and chitosan for controlling tomato root rot disease under greenhouse and field conditions. Res. J. Agric. Biol. Sci. *2*, 190–195.
El-Sawy, N.M., El-Rehim, H.A.A., Elbarbary, A.M., Hegazy, E.A., 2010. Radiation-induced degradation of chitosan for possible use as a growth promoter in agricultural purposes. Carbohydr. Polym. 79, 555–562.
Faoro, F., Maffi, D., Cantu, D., Iriti, M., 2008. Chemical-induced resistance against powdery mildew in barley: the effects of chitosan and benzothiadiazole. BioControl 53, 387–401.
Guo, J.H., Qi, H.Y., Guo, Y.H., Ge, H.L., Gong, L.Y., Zhang, L.X., Sun, P.H., 2004. Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. Biol. Control 29, 66–72.
Kunnumakkara, A.B., Guha, S., Krishnan, S., Diagaradjane, P., Gelovani, J., Aggarwal, B.B., 2007. Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kB-regulated gene products. Cancer Res. 67, 3853–3861.
Li, J., Jiang, Y., We, J., Fan, G., Wu, Y., Zhang, C., 2009. A rapid and simple HPLC method for the determination of curcumin in rat plasma: assay development, validation and application to a pharmacokinetic study of curcumin liposome. Biomed. Chromatogr. 23, 1201–1207.
Lizarraga-Paulin, E., Torres-Pacheco, G., Moreno-Martinez, E., Miranda-Castro, S.P., 2011. Chitosan application in maize (Zea mays) to counteract the effects of abiotic stress at seedling level. Afr. J. Biotechnol. 10, 6439–6446.
Mondal, M.M.A., Malek, M.A., Puteh, A.B., Ismail, M.R., Ashrafuzzaman, M., Naher, L., 2012. Effect of foliar application of chitosan on growth and yield in okra. Aust. J. Crop Sci. 6, 918–921.
Nandakumar, R., Babu, S., Viswanathan, R., Sheela, J., Raguchander, T., Samiyappan, R., 2001. A new bio formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced gain yield in rice. Biol. Control 46, 493–510.
Nge, K.L., Nwe, N., Chandrkrachang, S., Stevens, W.F., 2006. Chitosan as a growth stimulator in orchid tissue culture. Plant Sci. 170, 1185–1190.
Pichyangkura, R., Chatchawan, S., 2015. Biostimulant activity of chitosan in horticulture. Sci. Hortic. 195, 49–65.
Rathaiah, Y., 1980. Leaf blast of turmeric. Plant Dis. 64, 104–105.
Ray, M., Dash, S., Shahbazi, S., Achary, K.G., Nayak, S., Singh, S., 2016. Development and validation of ELISA technique for early detection of rhizome rot in golden spice turmeric from different agroclimatic zones. Food sci. Technol. 66, 546–552.
Sheheta, S.A., Fawzy, Z.F., 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.
Tariq, M., Yasmin, S., Hafeez, F.Y., 2010. Biological control of potato black scurf by rhizosphere associated bacteria. Brazilian. J. Microbiol. 41, 439–451.
Van Loon, L.C., Bakker, P.A.H.M., Pieterse, C.M.J., 1998. Ann. Rev. Phytopathol. 36, 453–483.
Walker, R., Morris, S., Brown, P., Gracie, A., 2004. Evaluation of potential for chitosan to enhance plant defense. A Report for the Rural Industries Research and Development Corporation. RIR DC Publication, Australia. 04.
Xu, D., McElroy, D., Thornburg, R.W., Wu, R., 1993. Systemic induction of a potato pin2 promotor by wounding, methyl jasmonate and abscisic acid in transgenic rice plants. Plant Mol. Biol. 22, 573–588.