Chitosan Biopolymer on Plant Growth

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The chitosan (CHT) biopolymer is a de-acetylated chitin derivative derived from the outer shell of shrimp, shellfish, lobster, or crabs, as well as the cell wall of fungi. Because of its biodegradability, environmental non-toxicity, and biocompatibility, it is an ideal resource for sustainable agriculture. The CHT emerged as a promising agent used as a plant growth promoter. It induces plant growth by influencing plant physiological processes like nutrient uptake, cell division, cell elongation, enzymatic activation, and synthesis of protein that can eventually lead to increased yield. It also alters plant defense responses by triggering multiple useful metabolic pathways. Depending on the structures, chitosan is useful for industrial and agricultural applications.

Keywords: Chitosan ; Biopolymer ; Plant ; Growth Promoter ; Development

1. Introduction

Chitosan (CHT) is a poly (1,4)-2-amino-2-deoxy-β-D glucose, a de-acetylation derivative of chitin, found in arthropod exoskeletons, which includes crustaceans like lobsters, shrimps and crabs, insects, mollusk radulae, beaks of cephalopod and fish, and lissamphibian scales [1]. The discovery of chitosan (pronounced as Kite-O-San) dates back to 1811 when a French Professor Henri Braconnot of Natural History first found “chitin” from which it is derived. He found a mushroom extract that would not dissolve in sulphuric acid, and he called it ‘fungine’ [2][3]. In 1823, it was named ‘chitin’ after another scientist Auguste Odier extracted it from cuticles of beetle and called it ‘chiton’. Chitin was the first man-identified polysaccharide, about 30 years prior to cellulose. The concept was further recognized when the existence of nitrogen in the chitin was demonstrated by Lassaigne in 1843. Professor C. Rouget undertook the alkaline treatment of chitin in 1859, resulting in an acid dissoluble substance, unlike chitin itself. Hoppe-Seiler gave the name “chitosan” to de-acetylated chitin [4]. Although chitin has long been an unused natural component, interest in this biopolymer and its derivatives like CHT has grown significantly in recent years due to its diversified biological properties.

The biopolymer CHT is safe, cheap and its chemical structure can easily be converted to develop relevant polymers for specified applications. These features make CHT a molecule of great significance in a wide range of potential users, from health care and biotechnological industries to farmers [5][6]. It is biodegradable, environment friendly for agriculture, and not toxic to humans or other organisms [7]. It has shown efficacy in increasing crop growth, yield, and quality. The CHT has been documented as an elicitor of plants’ natural defense response and has been utilized as a natural product to combat pathogenic diseases before and after harvest [8]. It functions as an antifungal [9], antibacterial [10], antiviral [11], and bionematicidal agent [12]. Chitosan has been widely utilized as a coating agent of different nuts, cereals, fruits, and vegetables to protect from post-harvest losses, and increase the duration of storage and preservation [13][14]. A wide range of studies showed that foliar application of CHT improves plant growth, yield and induces synthesis of secondary metabolites like polyphenolics, flavonoids, lignin, and phytoalexins in plants [15][16]. It influences seed plasma membrane permeability, enhances sugar and proline concentration, boosts peroxidase (POD), phenylalanine ammonia-lyase (PAL), tyrosine ammonialyase (TAL), and catalase (CAT) activities [17].

2. Effect of Chitosan Biopolymer on Plant Growth

Chitosan functions as a plant growth promoter in various crops such as beans, potato, radish, gerbera, soybean, cabbage, and other crops. As a result of plant growth promotion, it also enhances yield. Chitosan has a major influence on the growth rates of shoots, roots, flowering, and the number of flowers. As chitosan molecules are extremely hydrophilic, they reduce stress damage in plant cells by decreasing water content and accelerating several biological macromolecules’ activities. Three trials were conducted on orchids to determine the effect of CHT on organogenesis; the results showed that CHT could produce positive results at a very low concentration [18][19][20]. The results also suggested that CHT was working as a consequence of other metabolic processes rather than merely enhancing nitrogen nutritional quality or as a source of energy for the production of carbohydrates. Both Pornpeanpakdee et al. [19] and Nahar et al. [20] found that orchid growth (Dendrobium and Cymbidium) was stimulated by the supply of CHT to micro-propagated plants that grow under sterile conditions. This is corroborated by other findings showing increased growth in aseptic conditions like tissue cultured grapes [21] and the growth of Phyla dulcis in liquid bioreactors [22].

Significant growth improvements have been found by several studies in daikon radishes [23], cabbage [24], soybean sprouts [25], sweet basil [26], and also in ornamental crops, including Gerbera [27] and Dendrobium orchids [18] by various modes of application such as in vitro, in vivo, soil application, pot application, and biofertilization. To increase maize yield, a mixture of CHT and plant-growth-promoting rhizobacteria can be utilized as biofertilizers [28]. It is utilized in potted freesia cultivation as a biostimulator [29]. Vasudevan et al. [30] reported that the use of CHT formulation could accelerate the length of root and shoot and yield of rice grain. It also promotes the growth of plants such as pepper, cucumber, and tomato raised in the nursery. Therefore, we have enlisted some important agricultural crops that showed improved plant growth and development due to the application of CHT (Table 1).

Table 1. Effects of chitosan (CHT) on plant growth and development.

Plant Species	CHT Effects	Mode of Application	References
Rice (*Oryza sativa* L.)	**Increased plant growth, higher photosynthesis rate**	**In vivo**	[31]
Soybean (*Glycine max*)	**Increased plant growth**	**Soil application**	[32]
Rape (*Brassica rapa* L.)	**Increased plant growth and content of leaf chlorophyll**	**Hydroponic pot application**	[33]
Maize (*Zea mays* L.)	**Increased plant growth and grain weight**	**Biofertilization**	[28]
	Improved seed germination	**In vivo**	[34]
	Improved seed germination and vigor index	**In vivo**	[35]
Potato (*Solanum tuberosum* L.)	**Increased tuber size**	**In vivo**	[36]
	Increased plant growth and yield	**In vitro and in vivo**	[37]
Tomato (*Solanum lycopersicum*)	**Improved fruit quality and productivity**	**In vivo**	[9][10]
	Increased seed germination and vigor index	**In vivo**	[38]
Daikon radishes (*Raphanus sativus*)	**Increased plant growth**	**In vivo**	[23]
Cabbage (*Brassica oleracea*)	**Increased plant growth**	**In vivo**	[24]
Soybean sprouts (*Glycine max*)	**Increased plant growth**	**In vivo**	[25]
Okra (*Hibiscus esculentus* L.)	**Increased plant growth, and yield**	**In vivo**	[39]
Eggplant (*Solanum melongena*)	**Increased plant growth, and yield**	**In vivo**	[40]
Bean (*Phaseolus vulgaris*)	**Increased leaf area, and carotenoids and chlorophylls levels**	**In vitro**	[41]
Chili (*Capsicum frutescence* L.)	**Increased plant growth, yield, and thousand seed weight**	**In vivo**	[42]
	Increased leaf area, canopy diameter, and plant height	**In vivo**	[43]
Bell pepper (*Capsicum annuum*)	**Increased fruit weight, diameter, and yield**	**In vivo**	[44]
Turmeric (*Curcuma longa*)	**Increased plant growth, and yield**	**In vivo**	[45]
Ajowan (*Carum copticum*)	**Increased seed germination, vigor index, dry weight, and radical length**	**In vivo**	[46]
Artichoke (*Cynara scolymus*)	**Improved seed germination and plant growth**	**In vivo**	[47]
Cucumber (*Cucumis sativus*)	**Increased plant growth and improved quality**	**In vivo**	[48]

Plant Species	CHT Effects	Mode of Application	References
Chickpea (*Cicer arietinum*)	Increased plant growth	In vivo	[49]
	Increased seed germination and vigor index	In vivo	[50]
Coffee (*Coffea arabica*)	Increased plant height and leaf area	In vivo	[51]
Strawberry *(Fragaria × annanasa*)	Increased fruit yield and total antioxidant activities	In vivo	[14]
	Increased fruit yield	In vivo	[52]
Watermelon (*Citrullus lanatus*)	Increased plant growth	In vivo	[53]
Mango (*Mangifera indica*)	Increased plant growth, fruit size and weight	In vivo	[54]
Grapevine (*Vitis vinifera* L.)	Increased plant growth	In vivo	[21]
Basil (*Ocimum ciliatum* and *Ocimum basilicum*)	Increased plant growth and phenol content	In vivo	[26]
Phyla dulcis	Increased plant growth	In vitro	[55]
Freesia (*Freesia corymbosa*)	Increased plant growth	In vivo	[29]
Gerbera jamesonii	Increased plant growth	In vivo	[27]
Dendrobium aggregatum	Increased plant growth	In vitro	[18]
Cymbidium insigne	Increased plant growth	In vitro	[20]
Kemiri sunan (*Reutealis trisperma*)	Increased plant growth	In vivo	[56]
Scots pine (*Pinus sylvestris* L.)	Increased plant growth	In vivo	[57]

3. Concluding Remarks and Future Perspectives

Chitosan, a chitin derivative, is the second most widely distributed abundant natural polymer. Over the last decades, the number of uses of CHT and its derivatives has significantly increased. The availability of information on biocompatible and biological characteristics of CHT makes it a potential bioactive substance for agriculture. CHT is a versatile nontoxic compound with multiple modes of action to positively impact plant health. Its application can mitigate the broad use of chemical pesticides, at least in part. To date, there is ample evidence to suggest that plants may achieve improved growth and development after the application of CHT, suggesting that the utilization of natural elicitors like CHT may be an essential component of sustainable agriculture.

While a lot of work has been done, several issues still remain unclear pertaining to the mechanisms of inducing plant immunity, accelerating plant growth, and development. In that regard, research and development should pay attention to discovering new derivatives of CHT, as their effective chemical alteration might significantly improve its chemical and physical characteristics, and enhance its field applicability by ensuring low mammalian toxicity. CHT and its derivatives apparently rely on their molecular weight for the majority of physiological activity and functionality. In addition, further study is needed to confirm whether biopolymers like CHT have the ability to influence physiological processes.

Therefore, future studies should also concentrate on understanding the details at the molecular levels, which can offer an insight into the unknown biochemical mechanisms of CHT. Combined proteome and transcriptome study of known proteins and genes would enhance our knowledge of the complex CHT-mediated signal pathway and allow for improving biotechnological approaches in plant growth promotion. A better understanding of CHT's mode of action in plants and pathogens would improve the possibility of its effective application. Furthermore, the collaboration and participation of research organizations, government regulatory authorities, and industries will be the primary key to the success of CHT use by unraveling its innate immunity-induced activities, growth enhancement in plants, and biotechnological prospects for sustainable agriculture.

References

1. Kurita, K. Chitin and chitosan: Functional biopolymers from marine crustaceans. Mar. Biotechnol. 2006, 8, 203–226.
2. Braconnot, H. Sur la nature des champignons. Annu. Chem. 1811, 79, 265–304.
3. Labrude, P.; Becq, C. Pharmacist and chemist Henri BraconnotLe pharmacien et chimiste Henri Braconnot. Rev. Hist. Pharm. 2003, 51, 61–78.
4. Hoppe-Seiler, F. Ueber chitosan und zellulose. Ber. Dtsch. Chem. Ges. 1894, 27, 3329–3331.
5. Anitha, A.; Sowmya, S.; Kumar, P.T.S.; Deepthi, S.; Chennazhi, K.P.; Ehrlich, H.; Tsurkan, M.; Jayakumar, R. Chitin and chitosan in selected biomedical applications. Prog. Polym. Sci. 2014, 39, 1644–1667.
6. Malerba, M.; Cerana, R. Chitosan effects on plant systems-A review. Int. J. Mol. Sci. 2016, 17, 996.
7. Park, Y.; Kim, M.H.; Park, S.C.; Cheong, H.; Jang, M.K.; Nah, J.W.; Hahm, K.S. Investigation of the antifungal activity a nd mechanism of action of LMWS-chitosan. J. Microbiol. Biotechnol. 2008, 18, 1729–1734.
8. Wang, B.; Zhang, S.; Wang, X.; Yang, S.; Jiang, Q.; Xu, Y.; Xia, W. Transcriptome analysis of the effects of chitosan on the hyperlipidemia and oxidative stress in high-fat diet fed mice. Int. J. Biol. Macromol. 2017, 102, 104–110.
9. Sathiyabama, M.; Charles, R.E. Fungal cell wall polymer based nanoparticles in protection of tomato plants from wilt di sease caused by Fusarium oxysporum f. sp. lycopersici. Carbohydr. Polym. 2015, 133, 400–407.
10. Sathiyabama, M.G.; Akila, R.; Einstein, C. Chitosan-induced defence responses in tomato plants against early blight dis ease caused by Alternaria solani (Ellis and Martin) Sorauer. Arch. Phytopathol. Plant Prot. 2014, 47, 1777–1787.
11. Kulikov, S.N.; Chirkov, S.N.; Ilina, A.V.; Lopatin, S.A.; Varlamov, V.P. Effect of the molecular weight of chitosan on its ant iviral activity in plants. Appl. Biochem. Microbiol. 2006, 42, 200–203.
12. Silva, M.; Nunes, D.; Cardoso1, A.R.; Ferreiral, D.; Britol, M.; Pintadol, M.E.; Vasconcelos, M.W. Chitosan as a biocontr ol agent against the pinewood nematode (Bursaphelenchus xylophilus). For. Pathol. 2014, 44, 420–423.
13. Sun, D.; Liang, G.; Xie, J.; Lei, X.; Mo, Y. Improved preservation effects of litchi fruit by combining chitosan coating with ascorbic acid treatment during postharvest storage. Afr. J. Biotechnol. 2010, 9, 3272–3279.
14. Rahman, M.; Mukta, J.A.; Sabir, A.A.; Gupta, D.R.; Mohi-ud-din, M.; Hasanuzzaman, M.; Miah, M.G.; Rahman, M.; Isla m, M.T. Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. PLoS ONE 2018, 13, e0203769.
15. Emami Bistgani, Z.; Siadat, S.A.; Bakhshandeh, A.; Ghasemi Pirbalouti, A.; Hashemi, M. Interactive effects of drought s tress and chitosan application on physiological characteristics and essential oil yield of Thymus daenensis Celak. Crop J. 2017, 5, 407–415.
16. Xoca-Orozco, L.-Á.; Cuellar-Torres, E.A.; González-Morales, S.; Gutiérrez-Martínez, P.; López-García, U.; Herrera-Estr ella, L.; Vega-Arreguín, J.; Chacón-López, A. Transcriptomic analysis of avocado hass (Persea americana Mill) in the in teraction system fruit-chitosan-Colletotrichum. Front. Plant Sci. 2017, 8, 956.
17. Guan, Y.; Hu, J.; Wang, X.; Shao, C. Seed priming with chitosan improves maize germination and seedling growth in rel ation to physiological changes under low temperature stress. J. Zhejiang Univ. Sci. B 2009, 10, 427–433.
18. Chandrkrachang, S. The applications of chitin in agriculture in Thailand. Adv. Chitin Sci. 2002, 5, 458–462.
19. Pornpeanpakdee, P.; Pichyangkura, R.; Chadchawan, S.; Limpanavech, P. Chitosan effects on Dendrobium ‘Eiskul’ Pro tocorm-like body production. In Proceedings of the 31st Congress on Science and Technology of Thailand, Nakornrach aseema, Thailand, 18–20 October 2005; pp. 1–3.
20. Nahar, S.J.; Kazuhiko, S.; Haque, S.M. Effect of Polysaccharides Including Elicitors on Organogenesis in Protocorm-lik e Body (PLB) of Cymbidium insigne in vitro. J. Agric. Sci. Technol. 2012, 2, 1029–1033.
21. Barka, E.A.; Eullaffroy, P.; Clément, C.; Vernet, G. Chitosan improves development, and protects Vitis vinifera L. agains t Botrytis cinerea. Plant Cell Rep. 2004, 22, 608–614.
22. Sauerwein, M.; Flores, H.M.; Yamazaki, T.; Shimomura, K. Lippia dulcis shoot cultures as a source of the sweet sesquit erpene hernandulcin. Plant Cell Rep. 1991, 9, 663–666.
23. Tsugita, T.; Takahashi, K.; Muraoka, T.; Fukui, H. The application of chitin/chitosan for agriculture (in Japanese). In Proc eedings of the Special Session of the 7th Symposium on Chitin and Chitosan, Fukui, Japan, 6 March 1993; Japanese Society for Chitin and Chitosan: Fukui, Japan, 1993; pp. 21–22.
24. Spiegel, Y.; Kafkafi, U.; Pressman, E. Evaluation of a protein-chitin derivative of crustacean shells as a slow-release nitr ogen fertilizer on Chinese cabbage. J. Hortic. Sci. 1988, 63, 621–628.

25. Lee, Y.S.; Kim, Y.H.; Kim, S.B. Changes in the respiration, growth, and vitamin C content of soybean sprouts in respons e to chitosan of different molecular weights. Hortic. Sci. 2005, 40, 1333–1335.

26. Pirbalouti, A.G.; Malekpoor, F.; Salimi, A.; Golparvar, A. Exogenous application of chitosan on biochemical and physiolo gical characteristics, phenolic content and antioxidant activity of two species of basil (Ocimum ciliatum and Ocimum ba silicum) under reduced irrigation. Sci. Hortic. 2017, 217, 114–122.

27. Wanichpongpan, P.; Suriyachan, K.; Chandrkrachang, S. Effects of Chitosan on the growth of Gerbera flower plant (Ge rbera jamesonii). In Chitin and Chitosan in Life Science, Proceedings of the Eighth International Chitin and Chitosan Co nference and Fourth Asia Pacific Chitin and Chitosan Symposium, Yamaguchi, Japan, 21–23 September 2000; Uraga mi, T., Kurita, K., Fukamizo, T., Eds.; Kodansha Scientific: Tokyo, Japan, 2001; pp. 198–201.

28. Choudhary, R.C.; Kumaraswamy, R.V.; Kumari, S.; Sharma, S.S.; Pal, A.; Raliya, R.; Biswas, P.; Saharan, V. Cu-chitos an nanoparticle boost defense responses and plant growth in maize (Zea mays L.). Sci. Rep. 2017, 7, 9754–9765.

29. Salachna, P.; Zawadzinska, A. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. J. Ecol. E ng. 2014, 15, 93–102.

30. Vasudevan, P.; Reddy, M.S.; Kavitha, S.; Velusamy, P.; Paul Raj, R.S.D.; Priyadarisini, V.B.; Bharathkumar, S.; Kloeppe r, J.W.; Gnanamanickam, S.S. Role of biological preparations in enhancement of rice seedling growth and seed yield. C urr. Sci. 2002, 83, 1140–1143.

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

32. Chibu, H.; Shibayama, H.; Arima, S. Effects of chitosan application on the shoot growth of rice and soybean. Jpn. J. Cr op Sci. 2002, 71, 206–211.

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

34. Shao, C.X.; Hu, J.; Song, W.J.; Hu, W.M. Effects of seed priming with chitosan solutions of different acidity on seed ger mination and physiological characteristics of maize seedling. Agric. Life Sci. 2005, 31, 705–708.

35. Saharan, V.; Kumaraswamy, R.; Choudhary, R.C.; Kumari, S.; Pal, A.; Raliya, R.; Biswas, P.J. Cu-Chitosan nanoparticl e mediated sustainable approach to enhance seedling growth in maize by mobilizing reserved food. Agric. Food Chem. 2016, 64, 6148–6155.

36. Falcón-Rodríguez, A.B.; Costales, D.; Gónzalez-Peña, D.; Morales, D.; Mederos, Y.; Jerez, E.; Cabrera, J.C. Chitosans of different molecular weight enhance potato (Solanum tuberosum L.) yield in a field trial. Span. J. Agric. Res. 2017, 15, e0902.

37. Kowalski, B.; Jimenez Terry, F.; Herrera, L.; Agramonte Peñalver, D. Application of soluble chitosan in vitro and in the gr eenhouse to increase yield and seed quality of potato minitubers. Potato Res. 2006, 49, 167–176.

38. Saharan, V.; Sharma, G.; Yadav, M.; Choudhary, M.K.; Sharma, S.; Pal, A.; Raliya, R.; Biswas, P. Synthesis and in vitro antifungal efficacy of Cu–chitosan nanoparticles against pathogenic fungi of tomato. Int. J. Biol. Macromol. 2015, 75, 34 6–353.

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

40. Sultana, S.; Islam, M.; Khatun, M.A.; Hassain, M.A.; Huque, R. Effect of Foliar Application of Oligo-chitosan on growth, yield and quality of tomato and eggplant. Asian J. Agric. Res. 2017, 11, 36–42.

41. Pereira, A.E.S.; Silva, P.M.; Oliveira, J.L.; Oliveira, H.C.; Fraceto, L.F. Chitosan nanoparticles as carrier systems for the plant growth hormone gibberellic acid. Colloids Surf. B Biointerfaces 2017, 150, 141–152.

42. Akter, J.; Jannat, R.; Hossain, M.M.; Ahmed, J.U.; Rubayet, M.T. Chitosan for plant growth promotion and disease supp ression against anthracnose in chilli. Int. J. Agric. Environ. Biotechnol. 2018, 3, 806–817.

43. Chookhongkha, N.; Miyagawa, S.; Jirakiattikul, Y.; Photchanachai, S. Chili growth and seed productivity as affected by chitosan. In Proceedings of the International Conference on Agriculture Technology and Food Sciences (ICATFS'2012), Manila, Philippines, 17–18 November 2012; pp. 17–18.

44. Mahmood, N.; Abbasi, N.A.; Hafiz, I.A.; Ali, I.; Zakia, S. Effect of biostimulants on growth, yield and quality of bell peppe r cv. Yolo wonder. Pak. J. Agric. Sci. 2017, 54, 311–317.

45. Anusuya, S.; Sathiyabama, M. Effect of chitosan on growth, yield and curcumin content in turmeric under field conditio n. Biocatal. Agric. Biotechnol. 2016, 6, 102–106.

46. Batool, M.; Asghar, R. Seed priming with chitosan improves the germination and growth performance of ajowan (Carum copticum) under salt stress. Eurasia J. Biosci. 2013, 7, 69–76.

47. Ziani, K.; Ursúa, B.; Maté, J.I. Application of bioactive coatings based on chitosan for artichoke seed protection. Crop P rot. 2010, 29, 853–859.

48. Shehata, S.; Fawzy, Z.; El-Ramady, H. Response of cucumber plants to foliar application of chitosan and yeast under g reenhouse conditions. Aust. J. Basic Appl. Sci. 2012, 6, 63–71.

49. Mahdavi, B.; Safari, H. Effect of chitosan on growth and some physiological characteristics of chickpea under salinity st ress condition. J. Plant Process Funct. 2015, 4, 117–127.

50. Anusuya, S.; Nibiya Banu, K. Silver-chitosan nanoparticles induced biochemical variations of chickpea (Cicer arietinum L.). Biocatal. Agric. Biotechnol. 2016, 8, 39–44.

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

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

53. González Gómez, H.; Ramírez Godina, F.; Ortega Ortiz, H.; Benavides Mendoza, A.; Robledo Torres, V.; Cabrera De la Fuente, M. Use of chitosan-PVA hydrogels with copper nanoparticles to improve the growth of grafted watermelon. Mol ecules 2017, 22, 1031.

54. Zagzog, O.A.; Gad, M.M.; Hafez, N.K. Effect of nano-chitosan on vegetative growth, fruiting and resistance of malforma tion of mango. Trends Hortic. Res. 2017, 6, 673–681.

55. Sauerwein, M.; Yamazaki, T.; Shimomura, K. Hernandulcin in hairy root cultures of Lippia dulcis. Plant Cell Rep. 1991, 9, 579–581.

56. Irawati, E.B.; Sasmita, E.R.; Suryawati, A. Application of chitosan for vegetative growth of kemiri sunan plant in margina l land. IOP Conf. Ser. Earth Environ. Sci. 2019, 250, 012089.

57. Trzcinska, A.; Bogusiewic, A.; Szkop, M.; Drozdowski, S. Effect of chitosan on disease control and growth of scots pine (Pinus sylvestris L.) in a forest nursery. Forest 2015, 6, 3165–3176.