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Research Application Summary

Effect of pre-harvest application of Chitosan and Silicon on growth, Lycopene content and shelf-life of tomato

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Abstract

Post-harvest losses account for 25-30% of horticultural produce in Kenya. The actual post-harvest loss for tomato is about 20-50%. This can even be higher depending on the season and level of production. This study investigated the role of chitosan and silicon on growth, quality and shelf-life of tomato fruits grown under greenhouse conditions. The experiments were laid out using a completely randomized design (CRD) involving four treatments of chitosan, silicon, chitosan-silicon complex and a control with three replications. The experiments were carried out in a greenhouse and laboratories at Jomo Kenyatta University of Agriculture and Technology (JKUAT) for two seasons. An elite hybrid variety, i.e., Bravo was grown with proper routine practices maintained. Data on germination rate, days to flowering, days to ripening, yield, lycopene content and days to deterioration of the harvested fruits were taken. The collected data were subjected to ANOVA for parameterization and means separated using protected LSD0.05. The study showed that tomato plants grown using chitosan, silicon and chitosan-silicon complex had significantly ($P<0.05$) better results in terms of germination rate, days to flowering, yield and post-harvest shelf of the fruits. Also, the treated plants had significantly higher chlorophyll and lycopene contents than control. Treatments with chitosan-silicon complex had the best performance in most aspects. The study recommends consideration of chitosan-silicon complex as an important input during tomato production for increased yield, quality and post-harvest shelf life.

Keywords: Chitosan, chlorophyll, lycopene, silicon, tomato plants

Résumé

Les pertes après récolte représentent 25 à 30% des produits horticoles au Kenya. Les pertes réelles après récolte pour les tomates sont d'environ 20 à 50%. Cela peut même être plus élevé selon la saison et le niveau de production. L'étude a examiné le rôle du chitosane et du silicium sur la croissance, la qualité et la durée de conservation des fruits de tomate cultivés en serre. Les expériences ont été réalisées selon un plan en blocs complètement randomisés (CRD) comprenant quatre traitements de chitosane, de silicium, de complexe chitosane-silicium et un témoin, avec trois répétitions. Les expériences ont été menées en serre et dans des laboratoires à l'université Jomo Kenyatta de l'agriculture et de la technologie (JKUAT) pendant deux saisons. Une variété hybride d'élite, Bravo, a été cultivée en suivant les pratiques routine appropriées. Les données sur le taux de germination, les jours jusqu'à la floraison, les jours jusqu'à la maturation, le rendement, la teneur en lycopène et les jours jusqu'à la détérioration des fruits récoltés ont été collectées. Les données collectées ont été soumises à une analyse de variance (ANOVA) pour la paramétrisation, et les moyennes ont été séparées à l'aide d'un test LSD0.05 protégé. L'étude a montré que les plants de tomates cultivés avec du chitosane, du silicium et du complexe chitosane-silicium présentaient des résultats significativement meilleurs ($P<0,05$) en termes de taux de germination, de jours jusqu'à la floraison, de rendement et de durée de conservation après récolte des fruits. De plus, les plants traités présentaient des teneurs en chlorophylle et en lycopène significativement plus élevées que le témoin. Les traitements avec le complexe chitosane-silicium présentaient les meilleures performances dans la plupart des aspects étudiés. L'étude recommande de prendre en compte le complexe chitosane-silicium comme un intrant important lors de la production de tomates pour augmenter le rendement, la qualité et la durée de conservation après récolte.

Mots-clés: Chitosane, chlorophylle, lycopène, silicium, plants de tomate

Introduction

Kenya is a leading producer of tomato (*Lycopersicon esculentum* M. 1768) and is ranked 6th in Africa. Tomato accounts for 14% of the total vegetable produce and 6.72% of the total horticultural crops (Food and Agriculture Organization, 2012; Government of Kenya, 2012). Despite the importance of this vegetable crop as food and cash crop among most farmers, huge losses are incurred during the production and distribution phase. The losses are attributed to poor agronomic practices (10-20 %), pest and diseases (20-30%), poor transportation methods (10 %) and post-harvest losses during marketing (25-30%) (Masinde *et al.,* 2001; Food and Agriculture Organization, 2012; Mujuka *et al.,* 2019). The post-harvest losses are attributed to biotic and abiotic factors. These factors can be associated with the field and storage conditions such as pest and diseases, genetic quality, plant nutrition, routine practices, method of harvesting and post-harvest handling. The role of each factor in post-harvest deterioration has not been well documented but there are records indicating that pathological and physiological losses associated with growing conditions accounts for a high percentage of the losses incurred (Masinde e*t al.,* 2001; HCDA, 2014).

There are some compounds that are known to improve the growth and development of tomato. These agents affect the crop production during the pre-harvest and post-harvest phases once accumulated in the plant system. Such materials include silicon which is an ubiquitous element in the earth's crust. However, silicon is not considered a major element in plant nutrition but it availability affect absorption of other nutrients such as Ca, Mg, N, P, K and micro-nutrients which enhances crop performance. Availability of the element to the plant is affected by the form it exists as it can only be utilized in ionic form. This form is easily leached thus requires supplementation for optimal crop development (Bloodnick, 2018).

Another important agent is chitosan which is a deacetylated and soluble form of chitin whose role in crop production cannot be overemphasized. The compound acts as a plant nutrient because it is rich in carbon and nitrogen. It also has antimicrobial effect on pathogens and increases host-plant resistance (Aider, 2010; Gatahi *et al.,* 2016). The agent is assimilated within the plant system triggering complex biochemical reactions such as increased growth, higher nutrition value, increased phytochemicals content and prolonged shelf-life (Li and Yu, 2000; Elsabee and Abdou, 2013). The two materials can be combined to form a complex with enhanced effects on crops. This is because chitosan acts as a strong cationic copolymer with adsorptive effect on most elements. These two compounds are rich in bonds such as hydroxyl, silanol and hydrogen-carbon which easily interlink to form the product. The product is safe for the produce, environment and consumers hence, can be used for sustainable agriculture (Zaman *et al.,* 2007; Mohammed *et al.,* 2017; United State Department of Agriculture, release 28). This study aimed at enhancing tomato growth, yield, quality and produce shelf-life of hybrid tomato by use of the innovate dubbed chitosan-silicon complex.

Methodology

Materials. Tomato seed (Anna) was obtained from Seminis Ltd while peat moss, polyfeed, planting trays and pesticides were obtained from Amiran (K) Ltd. All materials were at least analytical grade and included; acetic acid and sodium hydroxide pellets obtained from Sigma Aldrich. Chitin was obtained from fish scales collected from lake Naivasha.

The study adopted the following protocols: a) deacetylation of chitin and synthesis of chitosan silica nanoparticles; b) preparation of biological control suspensions (*Bacillus subtilis*, effective micro-organisms, *Trichoderma viridae, Glomus mosseae* and *R. solanacearum-phage*) and adsorption on Chitosan immobilized silica nanocomposites; and c) efficacy trials, tomato plants were treated with the bio-nanocomposites inoculated with *R. solanacearum* pathogen to evaluate performance in a greenhouse. Subsquently, characterization of the product and produce were done using FTIR and UV-Vis. Data on tomato performance in terms of germination, growth rate, induced resistance, yield, shelf-life were collected over three growing seasons at Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Results and Discussion

Fourier Transform Infra-Red Spectrometer (FTIR) Characterization. The synthesized chitosan-silicon complex was characterized using FTIR. Formation of chitosan-silicon complex (c) from chitosan (a) and silicon (b) molecules was confirmed by the marked changes in the functional bonds (Fig. I). This was expressed by shifting to right and left of the wave numbers due to stretching or vibrational forces on the functional bonds (-OH, C-H, N-H and Si-OH) (Silverstein *et al*., 1981). In addition, new bonds were also formed in complex due to integration of different substances in a complex matrix. This is because the free amino groups in chitosan macromolecules easily bond with the silanol groups contained in the silicon solution resulting in a hybrid inorganic/organic hydrogel known as chitosan-silicon complex (Shandina *et al.,* 2015; Shipovskaya *et al.,* 2016). The product is usually stabilized due to the formation of a network of intermolecular hydrogen bonds among the participating functional groups. Shipovskaya *et al*. (2016) hypothesized a scheme of the intermolecular interactions in the chitosan-silicon-containing hydrogel (Fig. 2). The compounds were also observed under Nixon Microscope which showed physical transformation (Plate I).

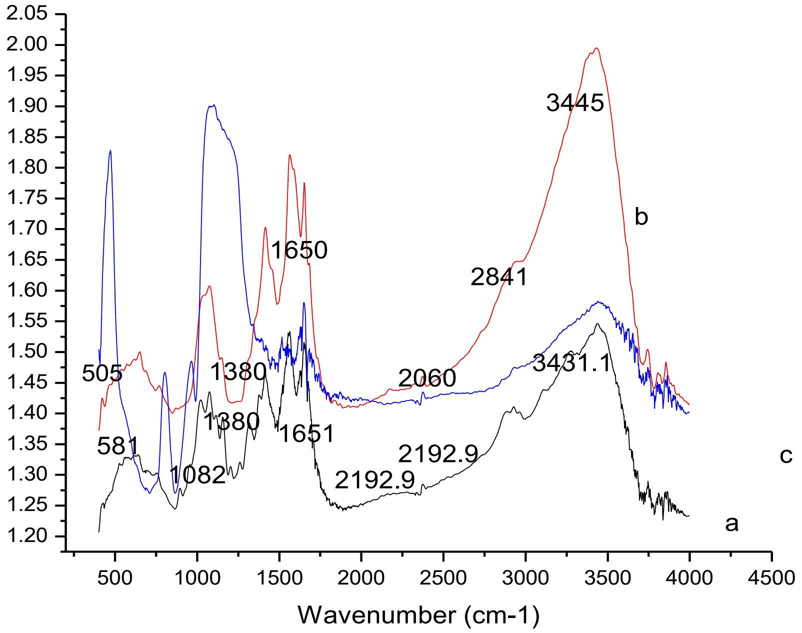

Figure 1. Spectrum of chitosan, silicon and chitosan-silicon complex
a = chitosan molecules, b = silicon molecules and c = chitosan-silicon complex

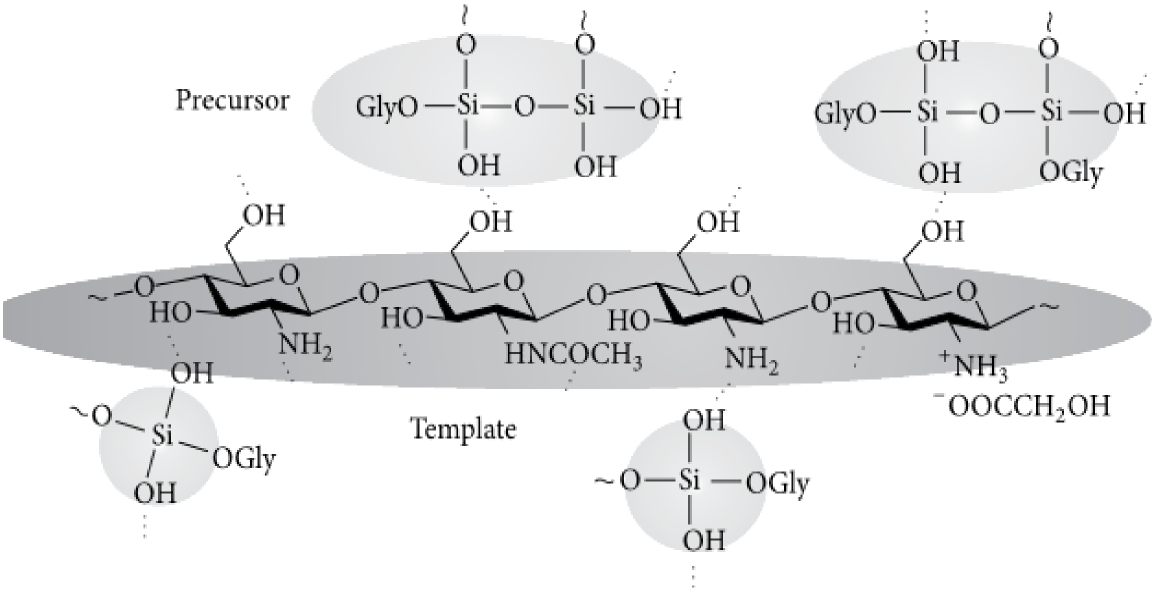

Figure 2. A hypothesized scheme of the intermolecular interactions in the chitosan-silicon-containing hydrogel (Shipovskaya ***et al.*****, 2016)**

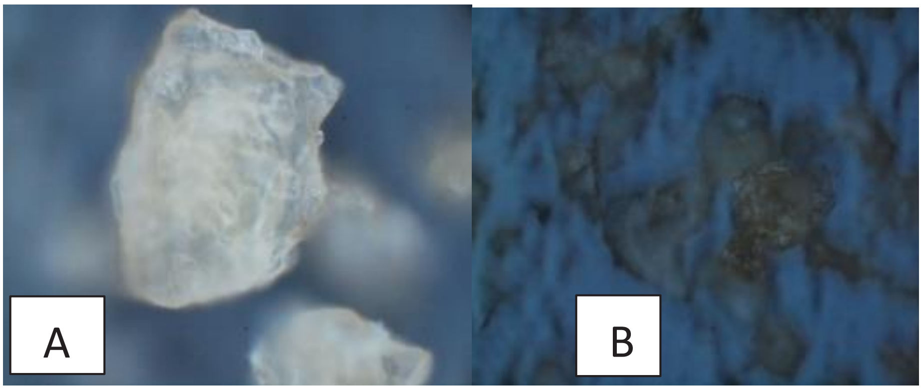

Plate I. Chitosan (A) and Chitosan-Silicon gel (B)

Effect of Chitosan-Silicon complex on seed germination. All treatments had significantly ($P<0.05$) higher germination rate when compared with the control. However, chitosan-silicon complex treatment showed relatively higher germination rate than other treatments (Figure 2 and Plate II).

The enhanced germination was attributed to the role of chitosan gel and silicon mineral in seed physiology (Guan *et al*., 2009; Algam *et al*., 2010). Chitosan has an amino group ($-NH_2$) that makes it hygroscopic and when it gets in contact with water, becomes protonated changing into ammonia ($-NH_3$). The chemical change makes chitosan more hygroscopic causing seeds or plants to trap more moisture. In addition, chitosan increases the water utilization efficiency of plants, increasing mineral uptake and stimulates growth. Water is a critical agent of seed germination and basic for plant growth and development (Yang *et al*., 2001).

Also, silicon though not a macro element in crop development, plays a major role in intracellular transport, seed-water permeability and air uptake. Air and water are important agents in seed germination (Kubata *et al*., 2005). In related studies, Gao *et al*. (2006) and Moussa (2006) observed that silicon increased seed germination in wheat and maize. In the current study, the chitosan-silicon complex displayed the highest germination rate which can be attributed to synergy between the two molecules. This can be inferred to qualify the classification of the complex as a germination stimulant.

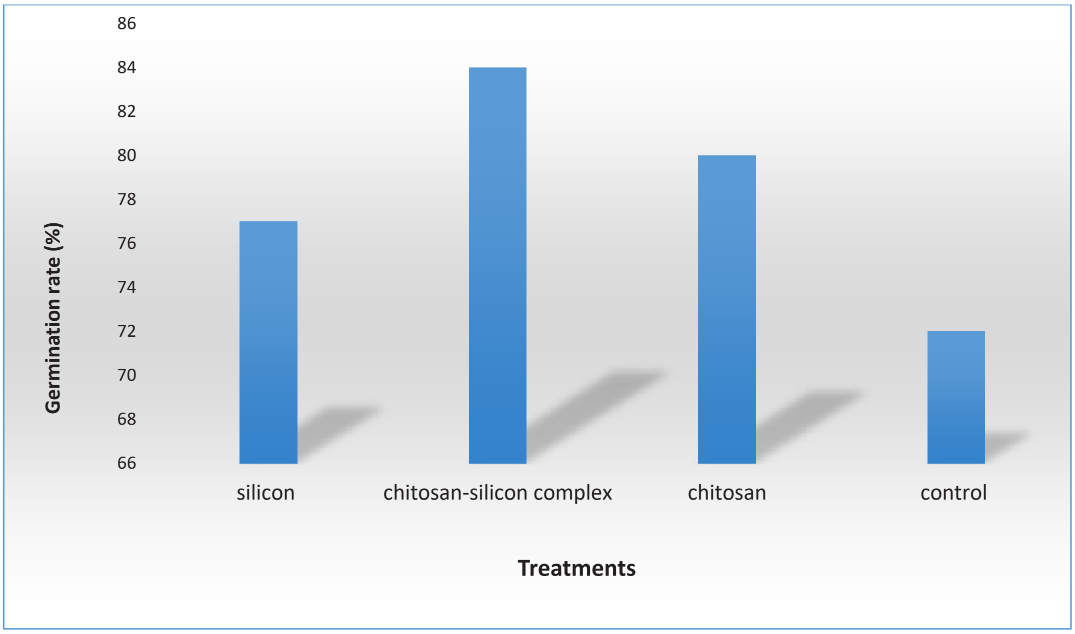

Figure 2. Germination rates of tomato seeds
Means significant at L.S.D 0.05 (F-test) ı-LSD bar

Plate II. Treated seedlings in the propagation unit
Means significant at L.S.D 0.05 (F-test) ¹-LSD bar

Effect of Chitosan-silicon complex on Chlorophyll content. Treatment of tomato seedlings with chitosan, silicon, chitosan-silicon complex increased the chlorophyll content significantly (P≤0.05) when compared to the control (Table I).

Table I. Chlorophyll content in tomato seedlings

Treatment	Chlorophyll Content level
Control	29.9 a
Chitosan	35.3 b
Silicon	36.7 b
Chitosan–silicon complex	38.6 bc

Means followed by the same letter are not significantly different atLSD$_{0.05}$

The increased chlorophyll is caused by accelerated biochemical activities in the tomato plants triggered by the glucosamine units in chitosan. Chitosan is rich in N and C which affect have a direct influence on chlorophyll content. Further, hydration of chitosan results in formation of urea which plays a role in plant nutrition. Also, silicon has a positive role in increasing chlorophyll accumulation in plants. For instance, according to Zeng *et al*. (2007), Silicon was observed to enhance chlorophyll efficiency in rice, barley, wheat and sugarcane. Silicon also improves photosynthetic capacity by enlarging the size of chloroplasts and increasing number of grana in leaves (Jian, 2004; Soratto *et al.,* 2012). Application of silicon results in increased chlorophyll content which is associated with a change in the pigment composition of the photosynthetic apparatus towards a solar-type chloroplast hence higher photosynthesis (Yang, 2010). Hybridization of the two, i.e., chitosan and silicon into a complex, enhanced the efficacy of the product as observed from the treatment. The complex ensured slow release of silicon over a prolonged period of time due to encapsulation within the chitosan matrix, thus higher chlorophyll content in the treatments where the chitosan-silicon complex was applied (Ma *et al.,* 2012; Wang *et al.,* 2015).

Effect of chitosan-silicon complex on flowering and ripening and shelf life of tomato fruits. Tomato plants treated with chitosan-silicon complex had significantly ($P \leq 0.05$) shorter times to days to 50% flowering and ripening. In addition, there was also a significant ($P \leq 0.05$) increase in the shelf life of tomato fruits (Tables 2, 3 and 4).

Table 2. Days to flowering and ripening of fruits in tomato hybrids

Treatment	Days to flowering	Ripening
Chitosan	24 a	63 a
Silicon	25 a	63 a
Chitosan-silicon complex	26 b	65 ab
Control (Distilled water)	29 c	74 c

Means followed by the same letter along a column are not significantly different $LSD_{0.05}$

Table 3. Shelf life (days) of tomato fruits

Treatment	Days
Control	18. 0 a
Chitosan	27.0 b
Silicon	26.0 b
Chitosan-silicon complex	27.0 b

Means followed by the same letter along a column are not significantly different $LSD_{0.05}$

The accelerated growth, flowering and increased shelf life of the tomato fruits observed in the study was attributed to nutritional and physiological effect of the treatments on the plants. Silicon for instance, influences uptake of the macro-nutrients such as N, P, K and Ca in plants. The macro nutrients are associated with growth and development therefore any activity that enhances their absorption increases development. Particularly, potassium plays a critical role in flower development, pollination and ripening. In addition, calcium treatment has been shown to decrease respiration, reduce ethylenen production and slow down the onset of ripening in many fruits including tomato (Sellars, 2010).

Additionally, chitosan confers longevity to harvested fruits when applied while in the field. The ability of chitosan to enhance shelf life of other fruits was described by Soesiladi *et al*. (2015) where field application of 2.5% chitosan solution enhanced the shelf-life and fruit quality of

Cavendish banana. The modified atmosphere created by the chitosan coating retarded ethylene production rate, which caused a delay in ripening, chlorophyll degradation and carotenoid synthesis, ultimately delaying color change of fruits. Banana and tomato are easily compared in their post-harvest physiology because they are climacteric fruits (Widodo *et al*., 2013; Widodo *et al*., 2015). Other studies on papaya and carambola fruits by Islam *et al*. (2018) confirmed extended shelf-life of the fruits when treated with low concentration of chitosan solution. Chutichudet and Prasit (2014) also demonstrated increased shelf life of other climacteric fruits treated with chitosan before harvesting.

Effect of chitosan-silicon complex on tomato yield. Tomato yield expressed in form of fruit size and weight was increased significantly ($P \leq 0.05$) when chitosan, silicon and chitosan-silicon complex were used (Table 4).

Table 4. Yield of tomato fruits in terms of circumference (cm) and weight (kg/ plant)

Treatments	Circumference (cm)	Weight (kg/plant)
Control	15.0 a	8.7 a
Chitosan	17.2 b	11.9 b
Chitosan-silica complex	17.3 b	14.2 bc
silicon	17.7 b	13.2 b

Means followed by the same letter along a column are not significantly different. LSD $_{0.05}$

The high yield could be attributed to the increased chlorophyll content, increased growth and flowering which resulted after application of chitosan and silicon compounds. For instance, Cao *et al*. (2013) found out that chitosan contains oligosaccharides that act as phytohormones which increase plant growth and development. Chitosan also increases water utilization efficiency by promoting availability and uptake of water. Water is very essential in plant growth by ensuring nutrients uptake adjusting osmotic pressure during photosynthesis (Li *et al*., 2010). Silicon on the other hand, plays a role in availability of essential nutrients N, P, K, Ca and other micro-nutrients. Despite, the mineral being a minor element and tomato a silicon non-accumulator, inadequate silicon level results in floral abortion, abnormal flower development, low pollen fertility, reduced fruit set and malformed fruits (Liang, 1999; Locke, 2007). Thus, applying adequate quantity of Silicon enhanced yield in tomato plants. Combination of the chitosan and silicon into one compound conferred benefits of the two molecules simultaneously thus higher yield (Miyake and Takahashi, 2012).

Effect of Chitosan-silicon complex on Lycopene content. There was significant ($P<0.05$) difference in lycopene content between chitosan, silicon and chitosan-silicon complex treated tomato plants and the control (Table 5). However, the lycopene content was not significantly different between the three main treatments. This shows that the three, i.e., chitosan, silicon and chitosan-silicon complex have a stimulatory effect in production of lycopene compound.

Lycopene is an important natural antioxidant naturally occurring in ripe tomato. Formation of this carotene biochemical requires optimal conditions such as pH. The mild alkaline pH is easily achieved when chitosan is applied due to the ubiquitous amino groups and silicon solution associated with the hydroxyl groups (Shipovskaya *et al*., 2016). Further, application of chitosan encourages accumulation of phytochemical substances (Mondal *et al.,* 2016). These biochemicals trigger a series of secondary metabolites synthesized through the pathway of shikimic acid or malonic acid. Lycopene is one of the biochemicals formed through this pathway. Lycopene is also affected by plant stress, both chitosan and silicon reduce plant stress, hence may increase the lycopene concentration in fruits (Anthon *et al.,* 2011; Hernández-Hernández *et al.,* 2018). Chitosan and silicon are known to increase phenolic compounds in plants. In particular, high concentration of silicon in tomato increases the beta-carotene and lycopene contents, which are important quality aspect of tomato fruit quality (De Pascale *et al.,* 2001; Beckles, 2012).

Table 5. Lycopene content in tomato fruits

Treatment	Lycopene
Chitosan	4.72 b
Silicon	4.47 b
Chitosan–silicon complex	4.95 b
Control	3.91 a

Means followed by the same letter are not significantly different LSD $_{0.05}$

Conclusion

Chitosan-silicon complex had synergistic effect on tomato production, shelf-life and quality aspects. The study therefore recommends use of the Chitosan-silicon complex under greenhouse conditions to enhance productivity of tomatoes.

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References

Algam, S., Xie, G., Li, B., Yu, S., Su, T. and Larsen, L. 2010. Effects of paenibacillus strains and chitosan on plant growth promotion and control of *R. solanacearum* wilt in tomato. *Journal of Plant Pathology* 92 (3): 593-600.

Anthon, E., LeStrange, M. and Barrett, M. 2011. Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes. *J. Sci. Food Agric.* 91:1175–1181.

Beckles, M. 2012. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biol. Technol.* 63 (1):129–140.

Bloodnick. E. 2018. Role of Silicon in Plant Culture. PROMIX

Chutichudet, B. and Chutichudet, P. 2014. Effects of chitosan or calcium chloride on external postharvest qualities and shelf-life of‘ Holland’ Papaya Fruit. *Journal of Agricultural Science* 6 (11): 160-177.

Dennis, G., Harrison, W., Agnes, K. and Erastus, G. 2016. Effect of biological control antagonists adsorbed on chitosan immobilized Silica Nanocomposite on *Ralstonia solanacearum* and growth of tomato seedlings. *J. Advances in Research* 6 (3): 1-23.

Fish, W., Perkins-Veazie, P. and Collins, K. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *J. Food Comp. Anal*. 15: 309/317.

Food and Agricultural Organization (FAO). 2012. Statistical Database. Retrieved from http://www.faostat.fao.org

Gao, X., Zou, C., Wang, L. and Zhang, F. 2006. Silicon decreases transpiration rate and conductance from stomata of maize plants. *Journal of Plant Nutrition* 29: 1637-1647

Government of Kenya. 2012. National Horticulture Policy. Ministry of Agriculture, Kilimo House, Nairobi: Government Printer.

Guan, J., Wang, J. and Shao, X. 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univascie B* 10: 427-433.

Horticultural Crop Development Authority (HCDA). 2014. Horticulture Validated Report. Nairobi, Kenya: Ministry of Agriculture.

Hinkelmann, K. and Kempthorne, O. 2008. Design and analysis of experiments. I and II (2nd ed.). Wiley, 470, 38551-7.

Hernández-Hernández, H., González-Morales, S., Benavides-Mendoza, A., Ortega-Ortiz, H., Cadenas-Pliego, G. and Juárez-Maldonado, A. 2018. Effects of Chitosan–PVA and Cu Nanoparticles on the growth and antioxidant capacity of tomato under saline stress molecules.

Jian, F. 2004. Role of silica in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition* 50 (1): 11-18.

Kubata, M., Matsui, M., Chiku, H., Kasashima, N., Shimojoh, M. and Sakaguchil, K. 2005. Cell adsorption and selective desorption for separation of microbial cells by using chitosan immobilized silica. *Appl. Environ Microbial* 71 (12): 8895-8902.

Liang, Y. 1999. Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. *Plant and Soil* 209: 217-224

Locke, J. 2007. Silicon boosts yields, disease suppression in media. GMPro Magazine (8):58.

Islam, T., Afrin, N., Parvin, S., Dana, H., Rahman, S., Zzaman, W. and Islam, N. 2018. The impact of chitosan and guava leaf extract as preservative to extend the shelf-life of fruits. *International Food Research Journal* 25 (5): 2056-2062

Ma, L., Li, Y., Yu, C., Wang, Y., Li, X., Li, N., Chen, Q. and Bu, N. 2012. Alleviation of exogenous oligochitosanon wheat seedlings growth under salt stress. *Protoplasma* 249: 393–399.

Masinde, A., Anastacia, O., Kwambai, K. T. and Wambani, N. H. 2001. Evaluation of tomato (*Lycopersicon esculentum* L.) variety tolerance to foliar diseases at Kenya Agricultural Research Institute Centre-Kitale in North west Kenya. *African Journal of Plant Science* 5 (11): 676-681.

Minolta Camera Co. Ltd.1989. Chlorophyll meter SPAD-502. Instruction Manual. Radiometric Instruments Divisions, Osaka, Minolta, 22pp.

Miyake, Y. and Takahashi, E. 2012. Effect of silicon on the growth of solution-cultured cucumber plant. *Soil Science and Plant Nutrition* 29 (1):71-83

Mondal, A., Puteh, B. and Dafader, C. 2016. Foliar application of chitosan improved morphophysiological attributes and yield in summer tomato (*Solanum lycopersicum*). *Pak. J. Agric. Sci*. 53 (2): 339-344.

Mujuka, E., Mburu, J., Ogutu, A. and Ambuko, J. 2019. Returns to investment in postharvest loss reduction technologies among mango farmers in Embu County, Kenya. *Food Energy Secur.* 00:e195.

Olivas, I. and Barbosa-Cánovas, V. 2005. Edible coatings for fresh-cut fruits. *Critical Reviews in Food Science and Nutrition* 45 (7-8): 657-670.

Puvvada, S., Vankayalapati, S. and Sukhavasi, S. 2012. Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry. *International Current Pharmaceutical Journal* 1 (9): 258-263.

Sellars, B. 2010. Calcium chloride uses on tomatoes. Retrieved from http://www.ehow.com/list_6082729_calcium-chloride-uses-tomatoes. html

Ramirez, A., Rodriguez, T., Alfonso, L. and Peniche, C. 2010. Chitin and its derivatives as biopolymers with potential agricultural applications. *Biotecnología Aplicada* 27 (4): 270-276.

Shadrina, E.V., Malinkina, O.N., Khonina, T.G., Shipovskaya, A.B., Fomina, V.I., Larchenko, E.Y., Popova, N.A., Zyryanova, I.G. and Larionov, L.P. 2015. Formation and pharmacological activity of silicon—chitosan containing glycerohydrogels obtained by biomimetic mineralization. *Russian Chemical Bulletin* 64 (7): 1633–1639.

Shipovskaya, A., Malinkina, O., Zhuravleva, Y. and Rogacheva, S. 2016. Synthesis of silicon-containing chitosan hydrogels in a glycolic acid medium. A*dvances in Materials Science and Engineering* 1-8 pp.

Shchipunov, Y. and Karpenko, T. 2004. Hybrid polysaccharide silica nanocomposites prepared by the sol-gel technique. *Langmuir* 20 (10): 3882–3887.

Silverstein, R.M. and Bassler, G.C. 1962. Spectrometric identification of organic compounds. *Journal of Chemical Education* 39 (11): 546.

Soratto, R., Crusciol, C., Castro, G. and Claudi, C. 2012. Leaf application of silicic acid to white oat and wheat. *Agron. J*. 36 (5): 1538–1544.

USDA National Nutrient Database for Standard Reference. Release 28.

Wang, D., Romer, F. and Connell, L. 2015. Highly flexible silica/chitosan hybrid scaffolds with oriented pores for tissue regeneration. *Journal of Materials Chemistry B* 3 (38): 7560–7576.

Widodo, E., Zulferiyenni, G. Y., Fazri, F. and Saputra, D. 2015. Effects of 1-Methylcyclopropene and Chitosan on the fruit shelf-life and qualities of two different ripening stages of 'Cavendish' banana. *Journal of Food and Nutrition Sciences* 3 (1-2): 54-59.

Widodo, S.E. and Arista, R. 2013. Coating effects of chitosan and plastic wrapping on the shelf-life and qualities of Mutiara and Crystal guavas. *Journal of International Society for Southeast Asian Agricultural Sciences* 19 (1): 1-7.

Yang, Y., Zhou, Y., Qi, Y., Wang, X., Zhang, Z. and Shi, Y. 2001. Effect of chitosan on physiological activities in germinating seed and seedling leaves of maize. *Journal of Hebei Vocationtechnical Teachers College* 15 (4): 9-12.

Yang, X. 2010. Effect of N Si fertilizer on the growth and yield of Chinese cabbage, MSc. Thesis, Shan Dong Agricultural University, China.

Zeng, X., Liang, J. and Tan, Z. 2007. Effects of silicate on some photosynthetic characteristics of sugarcane leaves. *Journal of Huazhong Agricultural University* 26 (3): 330–334.