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Characterization of chitosan iodate complex and its role on iodine uptake in tomato (*Lycopersicon esculentum*)

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Abstract
The bioavailability of iodine is influenced by the various types of iodine found in sediment as well as the time of iodine administration. The prevalence of iodine deficit is extensive; nevertheless, the bioavailability of iodine may be constrained due to the volatilization of iodine that is lost from the soil, leading to the occurrence of iodine shortage. The use of agronomic practises for iodine biofortification is a viable approach to mitigate iodine deficiency at a worldwide level. A multitude of research has been conducted to investigate the process by which plants uptake iodine from the soil. However, soil iodine stability is poorly understood. This study examined the relationship between tomato fruit iodine absorption and chitosan iodate complex formation. This evaluation encompassed several origins of chitosan and potassium iodate, both individually and in diverse combinations. The investigation was carried out through main and residual crop trials. The study found that added potassium iodate and chitosan complex increased iodine levels in the primary crop's leaves (24 ppm) and fruits (1 ppm). This combination also conserved residual crop iodine. Further the characterization results also revealed that there is a strong interaction between chitosan and iodate which prevent its leaching and volatilization losses. This work adds to the knowledge of soil iodine mobility and behaviour, especially when applied alone or with chitosan.

Keywords: Biofortification, Chitosan, Iodine, Mobility, Stability

Introduction

Earth's iodine distribution is broad but uneven. This unequal distribution is due to the scarcity of iodine, which is usually present as iodide salts. Antonyak et al. (2018) stated that iodine is essential for cognitive and physical development. Several vital foods have high iodine levels. Sea vegetables are rich in natural iodine, making them a good source. Iodide deposition in marine settings increases due to leaching, floods, and erosion of surface soil. Seawater iodide ions are converted to elemental iodine, which is discharged into the atmosphere as a volatile molecule. Precipitation returns this iodine to land. The iodine cycle is slow and incomplete in some areas, causing soil and drinking water iodine loss (MacKeown et al., 2022). The production of crops in these soils will deplete iodine, resulting in insufficient iodine intake for humans and animals. Thyroid hormone function stops if its iodine demands are not met. Due to low thyroid hormone levels in the circulation, iodine deficiency disorder (IDD) causes a variety of functional and developmental difficulties (Snart et al., 2019). Until salt iodization, crop biofortification, or food production in iodine-rich locations are done, iodine shortages remain (Olumet al., 2018). Global iodine fortification in salt is a well-accepted technique to combat iodine deficiency disorder. In recent

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decades, several nations have embraced this strategy, reducing the worldwide iodine deficit (Doggui et al., 2016). However, this strategy will not treat iodine deficiency. The instability of iodine in table salt causes it to volatilize faster (Dávila-Rangel et al., 2019). The iodization level during manufacture and losses from cooking or incorrect storage affect salt's iodine content (Desta et al., 2019). Up to 20% losses are normal throughout manufacturing, packing, transportation, and processing. Cooking may add 20% more loss. Agronomic biofortification methods, together with salt iodization, are promising and economically feasible ways to alleviate the iodine shortage. Fortification has reduced global iodine insufficiency in recent decades. Adding iodine-containing salts or organic resources like seaweed to soils helps crops absorb and store this element. Biofortification may provide iodine to plants. To avoid environmental losses, this procedure requires stabilized iodine in plants and soil.

Chitin, a polysaccharide-rich fibrous material, is found in crustacean exoskeletons, like prawns, lobsters, and crabs, and fungus cell walls (Ali et al., 2022). Chitosan improves cellular, tissue, physiological, and biochemical plant processes. It also alters gene expression at the molecular level (Zhang et al., 2020; Riseh et al., 2022). Iodine absorption is improved by chitosan-iodate complexes. Implementing effective cultivation strategies for individual species is crucial for attaining wide iodine biofortification (Smoleñ et al., 2016). This includes the chemical composition and manner of iodine delivery to plants. To address environmental pollution, especially in soil and surface rivers, it is necessary to avoid and mitigate its harmful effects on living organisms. The tomato, (*Solanum lycopersicum L.*,) is a worldwide vegetable crop valued in fresh markets because of its health advantages and economic importance (Abdelgawad et al., 2019). Due to their nutritious value, tomatoes are called "protective foods". Due to its extensive cultivation and home use, the tomato was chosen as the test crop in this study. This crop is sought after for fortification research due to its vast geographical range and attractiveness as a fresh food source (Duborska et al., 2020).

Ekott and Etukudo (2017) found that potassium iodate salt is more stable than potassium iodide owing to its sensitivity to oxidation and evaporation. Thus, potassium iodate with chitosan is preferred over potassium iodide in this study.

Materials and Methods

Characterization of chitosan and chitosan-iodate complex

The approach outlined by Limchoowong et al. (2018) was employed to create an edible film using chitosan and a chitosan-iodate complex.For chitosan and potassium iodate complex it was complexed in the ratio of 1% of chitosan and 0.01% potassium iodate. One gm of chitosan was dissolved in 100 mL acetic acid and 0.14 gm KIO3 (for chitosan iodate complex- 5 Kg ha^{-1}) and 0.28 gm KIO3 (for Chitosan iodate complex- 10 Kg ha^{-1}) were dissolved in 100 mL distilled water. The detailed examination of chitosan and chitosan iodate complex was conducted using sophisticated instruments, including the Scanning Electron Microscope (SEM) to analyse the fundamental structure of chitosan and chitosan iodate complex. Additionally, the interaction between chitosan and potassium iodate was explored using Fourier Transform Infrared Spectroscopy (FTIR).

Field experiments

Field experiments were conducted in *summer* and *Kharif* season of 2022 to determine the main and residual effects of biofortified iodine on enzymatic activity and its buildup in plants and fruit. The experimental crop was tomato, grown at Viraliyur hamlet in Coimbatore's Thondamuthur block. Iodine was biofortified through soil and foliar application using potassium iodate (KIO_3) and chitosan iodate complex mechanism. Chitosan product was purchased from Kerala Marine Hydrocolloids with a molecular weight of 501.486

g/mol and more than 75% deacetylation. Sigma Aldrich company supplied potassium iodate containing 59% iodine and 18% potassium.The plants were cultivated on black clay loam soil belonging to palaviduthi soil series characterised by a deficiency in nitrogen content but an abundance of organic carbon, phosphorus, and potassium. The tests were conducted using a randomised block design, with sixteen treatments and three replications, in the "Palaviduthi" soil series. Each plot had a gross size of '7m x 4m'. The hybrid tomato variety "Shivam" was utilised for the study.

The treatments were
T_1- KIO_3-Soil Application(SA)- 5 kg ha^{-1} ,
T_2- KIO_3- Soil Application(SA)- 10 kg ha^{-1},
T_3- Chitosan- KIO_3 Complex-5 kg ha^{-1},
T_4- Chitosan-KIO_3 Complex-10 kg ha^{-1},
T_5- Foliar Application (FA)-KIO_3-0.2% @ 60 and 90 DAT ,
T_6- Foliar Application (FA)-KIO_3-0.3% @ 60 and 90 DAT ,
T_7- KIO_3- Soil Application(SA)- 5 kg ha^{-1} + Foliar Application (FA)-KIO_3- 0.2% @ 60 and 90 DAT,
T_8- KIO_3- Soil Application(SA)- 10 kg ha^{-1} + Foliar Application (FA)-KIO^-_3- 0.2% @ 60 and 90 DAT,
T_9- Chitosan-KIO_3 Complex-5 kg ha^{-1} + Foliar Application (FA)-KIO_3-0.2% @ 60 and 90 DAT,
T_{10}-Chitosan-KIO_3 Complex-10 kg ha^{-1} + Foliar Application (FA)-KIO_3-0.2% @ 60 and 90 DAT,
T_{11}- KIO_3- Soil Application(SA)- 5 kg ha^{-1} + Foliar Application (FA)-KIO_3- 0.3% @ 60 and 90 DAT,
T_{12}- KIO_3- Soil Application(SA)- 10 kg ha^{-1} + Foliar Application (FA)-KIO_3- 0.3% @ 60 and 90 DAT.
T_{13}- Chitosan-KIO_3 Complex-5 kg ha^{-1} + Foliar Application (FA)-KIO_3-0.3% @ 60 and 90 DAT,
T_{14}- Chitosan-KIO_3 Complex-10 kg ha^{-1} + Foliar Application (FA)-KIO_3-0.3% @ 60 and 90 DAT,
T_{15}- Chitosan Spraying (control) and
T_{16}- Water Spraying (Absolute Control). The soil has a neutral pH and is non-saline.

Tomato hybrid seedlings were transplanted at a spacing of '45 cm x 30 cm' when they were around 25 days old. Potassium iodate was administered both as soil and as foliar application. The chitosan and potassium iodate combination was formed by combining chitosan and potassium iodate in a ratio of 1% chitosan to 0.01% potassium iodate. Samples of fruit and plants were gathered at various phases of tomato ripening, namely at the green, pink, and red harvest stages. These samples were subsequently subjected to additional analysis. The iodine content was determined by inductively coupled plasma–optical emission spectrometry, following the methodology outlined by Knapp et al. (1998). A plant/fruit sample weighing 0.5g was subjected to a mixture containing 1mL of Tetramethyl Ammonium Hydroxide with a concentration of 25% and 10mL of double distilled water. The resulting mixture was then heated to a temperature of 900°C and kept at this temperature for a duration of 3 hours. The data collected underwent a one-way analysis of variance (ANOVA). The statistical software programme IBM SPSS® Statistics, specifically version 25, was utilised to conduct all of the statistical analyses.

Results and Discussion

I. Characterization of chitosan iodate complex
Scanning electron microscope (SEM)
The characterization of the complex formation between chitosan and potassium iodate was conducted using a scanning electron microscope (SEM). Figure 1 illustrates the surface appearance of both chitosan alone and the chitosan iodate complex, seen at a magnification of 5000x.The thickness of the chitosan film exhibited a range of 1.282-1.807ìm. The thickness of the films

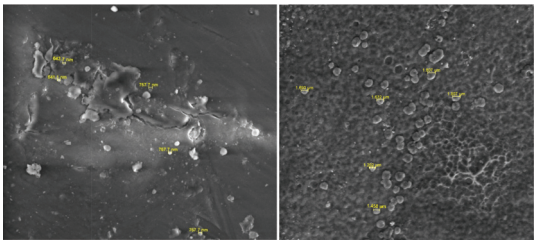

Figure 1. A. SEM image of Chitosan alone at 5000x magnification and B. SEM image of Chitosan Iodate Complex at 5000x magnification

comprising chitosan-iodate complexes exhibited a range of 0.759-0.990ìm. The thickness of the chitosan iodate complex is seen to be lower in comparison to that of chitosan alone, suggesting that the chitosan molecule undergoes degradation in order to form a complex with iodate. The chitosan films displayed transparency and a uniform surface devoid of any particulate contaminants. The chitosan iodate exhibits a consistent reticular nanoporous structure. The iodide was evenly distributed throughout the chitosan. A decrease in pore size was seen subsequent to the treatment of chitosan with iodate (Limchoowong et al., 2018). This reduction suggests that some iodate molecules were effectively incorporated into the porous framework of chitosan.

Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was utilized to determine the presence of certain functional groups inside a molecule and to examine the interaction between chitosan and iodate. The peaks in the absorption bands observed in the infrared spectrum are as follows : at 3283 cm^{-1}, indicating the vibratory stretching of hydroxyl (-OH) and symmetric elongation of NH bonds of the amino group; at 1644 cm^{-1}, corresponding to the C=O stretching of the amide I band; at 1554 cm^{-1}, representing the NH bending at 1372 cm^{-1}, associated with the symmetric deformation of CH; at 1024 cm^{-1}, indicating the C=O stretching in ether groups resulting from the deacetylation of the chitosan; and at 893 cm^{-1}, relating to the CH wagging motion in the saccharide structure of chitosan (Figure 2 and 3).The peaks seen in the chitosan iodate complex exhibited similarities to those observed in the chitosan alone, but with minor changes. The identification of a peak at 2879 cm^{-1} indicates the existence of CH stretching, whilst the peak at 1312 cm^{-1} signifies a combination of CH wagging, in-plane deformation, and amide (III) stretching in the spectra of the chitosan iodate complex. The wave number in the chitosan iodate spectra exhibited a modest reduction when compared to the spectra of chitosan alone. The observed modifications in the chitosan iodate complex indicate that the primary site of interaction for iodate was the amine group, which carries a positive charge. This is consistent with the well-known tendency of iodate to form charge transfer complexes with electron acceptors, as shown by Limchoowong et al. (2021).

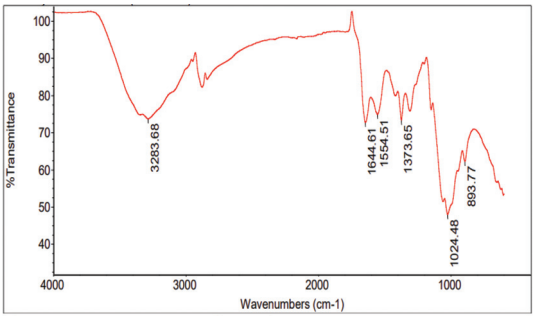

Figure 2. FT-IR spectrum of Chitosan alone

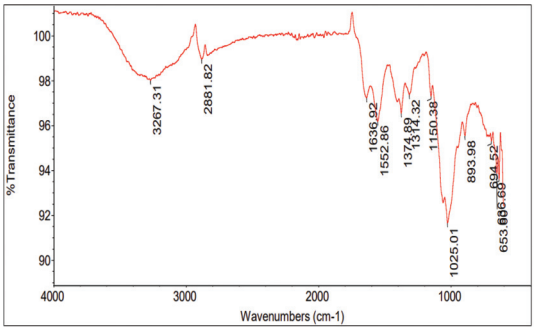

Figure 3. FT-IR spectrum of Chitosan-Iodate complex

II. Partitioning of iodine

Iodine partitioning in leaves and fruits of tomato

The iodine content of the leaves and fruit grew from the green stage to the pink stage (14.03 mg kg^{-1} to 14.53 mg kg^{-1} and 0.50 mg kg^{-1} to 0.52 mg kg^{-1}) of the main crop, but it declined (14.53 mg kg^{-1} to 13.75 mg kg^{-1} and 0.52 mg kg^{-1} to 0.44 mg kg^{-1}) when the fruit reached the red ripen stage (Fig. 4-7). On the other hand, the iodine content in leaves and fruits of residual crop decreased as the harvest stages progressed (12.32 mg kg^{-1} to 7.48 mg kg^{-1} and 0.37 mg kg^{-1} to 0.23 mg kg^{-1}).Treatments that involve potassium iodate alone, chitosan potassium iodate complex alone, and chitosan alone do not follow the trend described above. Because there was a shortage of iodine in the leftover crop, the iodine

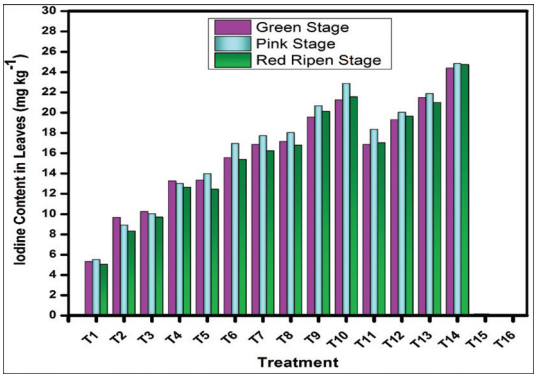

Figure 4. Effect of potassium iodate and iodine chitosan complex on leaves iodine content (mg kg^{-1}) at different stages of harvesting of main crop

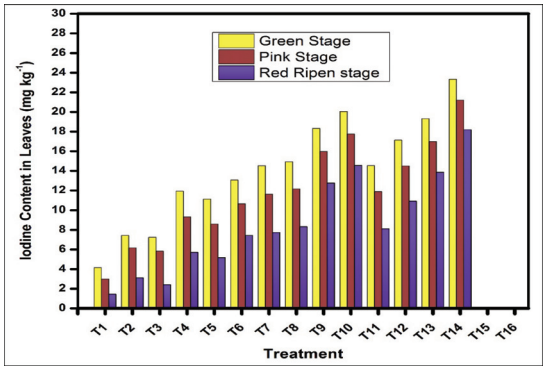

Figure 5. Effect of potassium iodate and iodine chitosan complex on leaves iodine content (mg kg^{-1}) at different stages of harvesting of residual crop

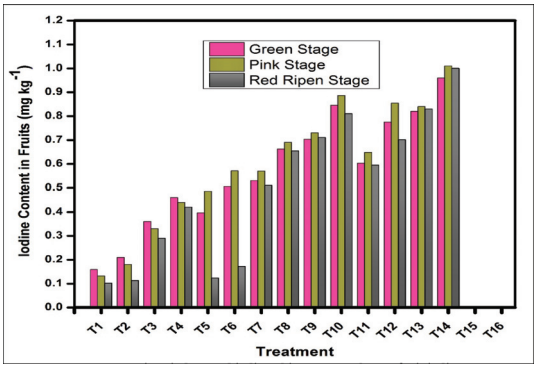

Figure 6. Effect of potassium iodate and iodine chitosan complex on fruit iodine content (mg kg^{-1}) at different stages of harvesting of main crop

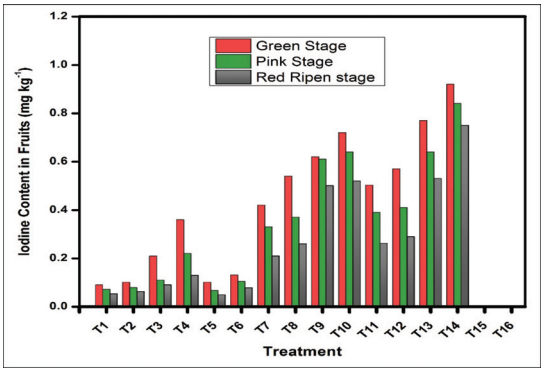

Figure 7. Effect of potassium iodate and iodine chitosan complex on fruit iodine content (mg kg^{-1}) at different stages of harvesting of residual crop

content of the leaves and fruit reduced as the plant progressed from the green to the red ripen stage. The absorption of iodine in leaves and fruits was increased in treatments that were given with chitosan iodate complex. This was owing to the fact that there was no leaching or volatilization of iodate ion, which was caused by the strong contact between the cationic amino group of chitosan and the iodate ion (Limchoowong et al., 2021). It was IO_3^- that was the most important iodine species that chitosan complexed. The transfer of iodine from roots is mostly accomplished through xylem vessels by mass flow that is driven by the transpiration of leaves. For this reason, the buildup of iodine in the leaves is greater than that in the roots and stems of the plant when chitosan is provided to the plant (Gonzali et al., 2017). When compared to treatments that consisted of KIO_3 soil application alone and chitosan iodate complex alone, the foliar application of KIO_3 resulted in an increase in the amount of iodine that was present in the leaves. Iodine that was administered to the leaves using the foliar method entered the plant through the cuticular waxes.Because xylem transport is preferred by plants, it is the most effective means of transporting iodine over long distances. Phloem is not as effective. Because of this, leaves often have higher iodine content than low transpiring fruits, which are favored by the phloem, because leaves transpire more than low transpiring fruits (Gupta et al., 2022). The leaves of the plant have a tendency to become increasingly concentrated with iodine as the plant matures.The addition of potassium iodate and chitosan complex led to a rise in the levels of iodine

in the leaves of the primary crop (24 ppm) and in the fruits (1 ppm). According to Li *et al*. (2022), the complexity of chitosan is directly proportional to the amount of amino groups it contains, whereas the degree of deacetylation is indirectly connected to the complexing capacity of chitosan.

Conclusion

The significant buildup of iodine in the main crop compared to the residual crop might be attributable to the elevated bioavailability of iodine provided in various ways. In the case of residual crop, the chitosan iodate treatment exhibits a higher retention of applied iodine. This can be attributed to the electrostatic contact that occurs between chitosan and iodate, which enhances the retention process. The potassium iodate provided iodine is susceptible to significant volatilization losses, resulting in just a minimal quantity of iodine present in the fruits of the primary and residual crops. The chitosan molecule possesses inherent stability and preservative properties, rendering it a favourable candidate for the biofortification of iodine in agricultural products. The biofortification of iodine in the form of iodate chitosan complex has been shown to enhance the stability of iodine and the iodine content in the resulting fruit.

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