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Role of bioactive polymer coating on potato microtuber storage and field performance

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ABSTRACT

Potato microtubers produced from tissue culture were subjected to coating with three bio-polymers (chitosan, gelatin and chitin) in four doses. Gelatin at 0.75 and 1.00% is effective in reducing the storage loss considerably up to 125 days after harvest without affecting the germination. The storage losses were drastically reduced in gelatin and chitosan treated microtubers due to improvement in sprout thickness to the tune of 47 and 25 per cent when compared with control. The stand establishment of gelatin treated microtubers in the field was higher by 23 per cent over control which significantly resulted in higher yield by nearly 47 per cent than the untreated tubers.

Key words: Chitosan, chitin, gelatin, bio-polymers, potato microtubers.

INTRODUCTION

Potato (*Solanum tuberosum* L) microtubers are produced after several months under *in vitro* condition and thus produced tubers have multiple factors which affect their establishment under field condition such as size, low dry-matter concentration, very dormant, storage losses and low vigour. Microtubers which are produced in the controlled condition tend to give different size tubers. It was reported that larger the size (>0.04 g) of the micro-tubers, higher the survival rate.

Size increment in microtuber is a very slow process. Poly coating, a sophisticated application process, besides allowing a precise distribution of active ingredients on the seed surface without changing its shape also causes a weight gain of almost 2% (Kunwur *et al*., 8) because of additional products added to seed surface. The microtubers are produced inside the media almost in the wet condition throughout the development, do not store well and tend to be very soft with open lenticels (Ashwani *et al*., 1). These lenticels are responsible for the loss of moisture, in turn leads to poor storability. Chitosan is well demonstrated as seed coating material in several studies (Goosan Matteus, 6), which gave multiple effects over seeds like antifungal in few crops (Bhaskara Reddy *et al*., 2) through the segregation of phenolic and phytolaxin substances to reduce the microbial growth. Chitin is another variant of chitosan obtained from the same source but deviate in the manufacturing process, which is a macromolecule used for making artificial skins and sutures and assumed that it may cover effectively over the tuber because of its macro-molecule nature. Gelatin, a highly water soluble protein obtained from animal bones, on refinement widely used in food and pharmaceuticals and as bio-herbicide formulation (Zhang *et al*., 14). Gelatin is one of the bio-polymers, which has 19 amino acids joined together to make a peptide linkage and because of these properties it has been used for coating and micro-encapsulation of various drugs.

The present work was undertaken to minimize the storage loss, to prolong the shelf-life by coating the microtubers with bio-polymers, which are safe and more ecofriendly. The bio-polymers attempted are chitosan, gelatin and chitin because of their thin film making property and biodegradable nature.

MATERIALS AND METHODS

The study was carried out at ICAR-Central Potato Research Station, Muthorai, Tamil Nadu. Shoot cultures of potato were produced using 25 ml of Murashige and Skoog liquid medium. Cultures were kept under 16 h photoperiod of 3000-4000 lux with a temperature of 22 ± 2°C, for 25-28 days until shoots were formed. Then shoots were kept in tuber induction medium (MS basal salts and vitamins supplemented with 10 mg^{-1} benzyl adenine (BAP) and 8% sucrose at 5.8 pH. Micro-tubers were induced by incubating in darkness at 16 ± 1°C for 70 days. Thus, produced microtubers were allowed to become green at 16 h photoperiod at 22 ± 1°C for one week. Microtubers of 8 mm size after size grading were used for this experiment.

Biopolymers, namely, chitosan, gelatin and chitin were procured from Biogen (USA). Acetic acid with

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99.7% purity and hydroxyl sodium solution (1N) were procured from Merck (India) and it was used for dissolving the chitosan. Four concentrations were prepared in all the polymers. Chitosan powder was dissolved as per the methodology described by Khalid Ziani *et al*. (7) with some modifications as follows.

Table 1. Preparation of chitosan solution.

Conc. (%)	Chitosan powder (g)	1% (w/w) acetic acid used (ml)	Time needed to dissolve (h)
0.25	0.25	10	4
0.50	0.50	15	4
0.75	0.75	20	6
1.00	1.00	25	6

*The final volume was made up to 100 ml with distilled water.

Gelatin powder weighing 0.25, 0.50, 0.75 and 1.00 g was dissolved in 100 ml of distilled water to get respective concentrations for T1, T2, T3 and T4. Chitin was dissolved in concentrated HCl and then the pH was adjusted to 7.0 as follows.

Table 2. Preparation of chitin solution.

Conc. (%)	Chitin powder (g)	HCl used (ml)
0.25	0.25	5.0
0.50	0.50	5.0
0.75	0.75	7.5
1.00	1.00	7.5

*The final volume was made up to 100 ml with double distilled water.

Distilled water was used as control. Total tuber immersing volume was calculated and is taken as T1, double the water volume is T2, triple the volume is T3 and four times the volume is T4.

For all the treatments a total of 15 tubers were selected and taken in to petridish and 5 ml of respective solution was added. Rotated gently by hand to get uniform coating. To eliminate the excess solution, drying process was conducted using conventional hand dryer. For multilayer treatment, the action was repeated two or three times. All the treatments were made in three replications and three coatings, *viz*., S1 = Single coating, S2 = Two coatings, S3 = Three coatings. The thickness of coating by polymers (thickness of the film) was measured by image analyzer. Images were treated using the software Prog Res® Capture pro, Version 2.7 × WIN. For each treatment, 15 seed tubers were placed in the petridish and stored in normal room conditions**.** The sprout percentages were assessed at every 15 days interval. Tuber weight loss was measured at regular interval of 15 days from the date of treatment and it is expressed in percentage. Sprout length was measured in 5 tubers in two replications (total 10 tubers) at random with the help of image analyzer as stated above. Measurements were taken from the point of sprout formation up to the length of the sprout. Method followed for the measurement is illustrated in Fig. 1. Sprout thickness was measured in three places leaving 0.25 ± 0.006 mm in both the sides and average was calculated and expressed in mm.

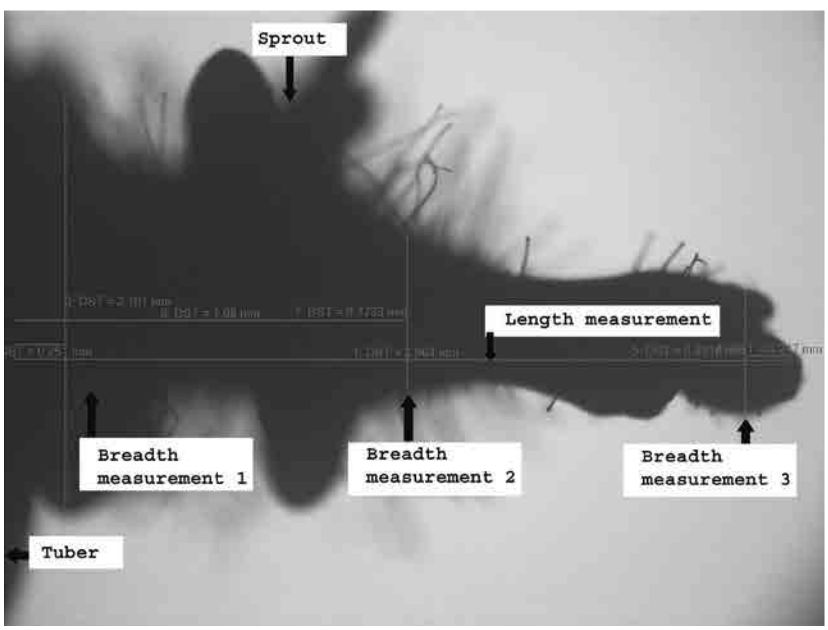

Fig. 1. Illustrating the procedure followed in the sprout length and thickness measurement using image analyser (Vertical line indicates the length and horizontal lines indicate the thickness).

Planting was done inside the net house with a spacing of 50 cm × 20 cm. Tubers were placed manually at a depth of 5 ± 1 cm. Recommended cultural practices were followed. Establishment percentage, plant height, number of leaves and number of stems at 60th and 75th day after planting and yield data in four grades were recorded. Statistical analysis was done for field data using RBD and lab data using CRD. Data were analyzed using Analysis of Variance with mean separation by LSD Test. Numbers are rounded off to 1 decimal point.

RESULTS AND DISCUSSION

To confirm that the tubers had been coated, an observation was taken with the image analyser (Fig. 2). Observation confirmed that all solutions formed thin layer of film coating on the surface of the tubers. The chitosan and chitin formed a layer like structure, whereas gelatin formed granular structure all over the tubers (Fig. 2). This observation confirms the adherence of polymers to the tubers. Nevertheless, through image analyzer, differentiation on the number of layers could not be observed. A major component of managing potato quality in storage is slowing down of the sprout growth. Increased weight loss, reduced tuber quality and impeded air movement through the potato pile in storage due to sprouting is reported. In our study, though we have not kept the tubers in pile, the weight loss is warranted due to the movement of water from tubers. No significant differences were noticed among the different layers of coating.

In stored potatoes loss of weight was mainly due to two physiological processes namely transpiration and respiration. Potato tuber contains 80 per cent water and being a living organism, respires fast and more. During this process, starch is converted into water, CO_2 and heat energy. Being a high water containing body, potato looses more water including bounded water when it is covered with more heat, which in turn leads to reduction in the dry matter content and weight loss. Reports indicate a direct correlation between respiration, size of the tuber and physiological age of the seed tubers (Rastovski and van, 10). In case of microtubers, the maturity index or physiological

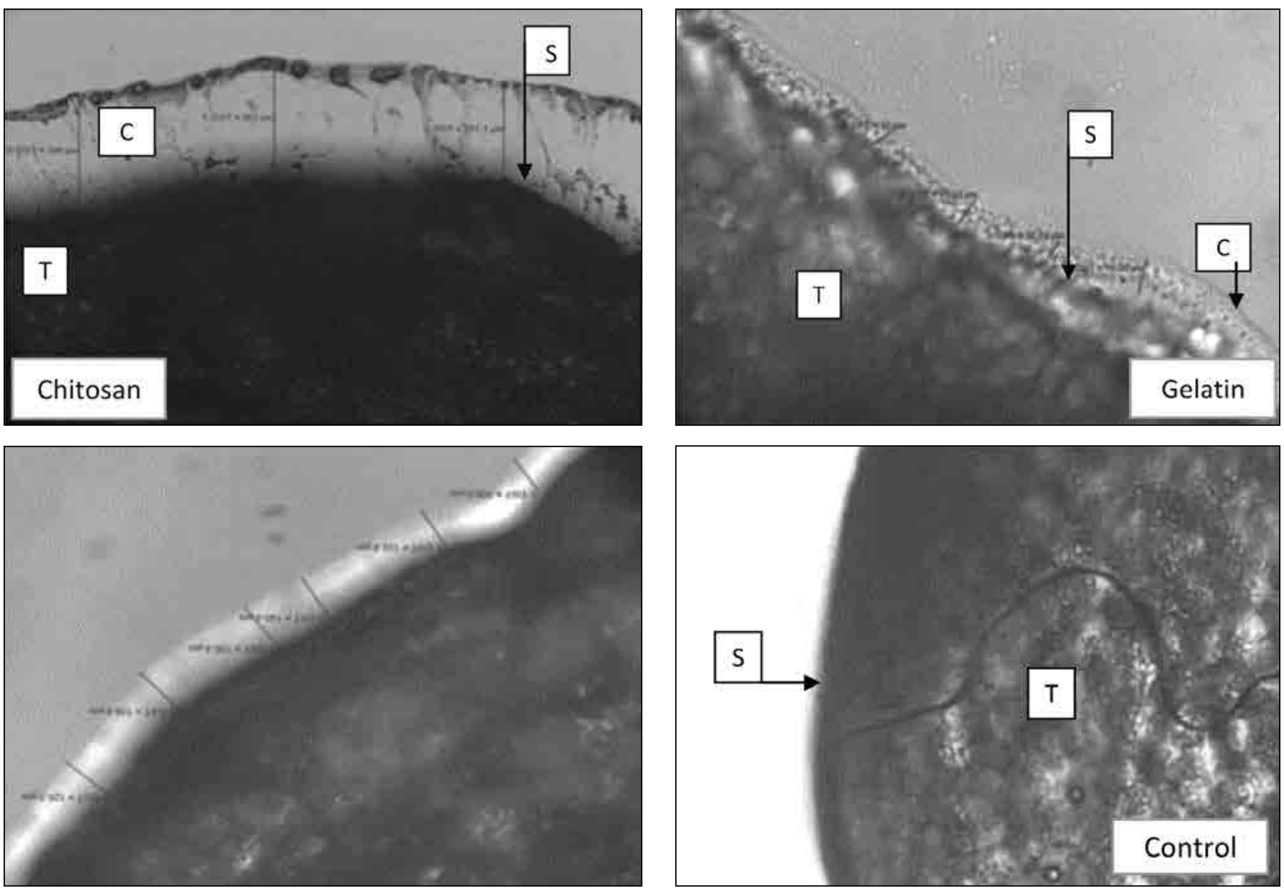

Fig. 2. Polymer coated skin thickness as visualized in image analyzer at 10 X magnification.

Where, T = Microtuber; S = Tuber skin ; C = Polymer coating.

maturity is not mentioned anywhere. The harvesting is based on the days inside the media. Hence, the respiration rate corresponding to maturity *in vivo* may vary during storage. In our study, the weight loss is more drastic after 45 days of storage in control as well as polymer treated microtubers. The tuber weight loss was significantly reduced over control in all the polymer coated treatments from 30 days of storage onwards. Among the different treatments the lowest weight loss was recorded in chitosan 1.00% treatment which was almost 50% less than that of control. Among the three polymers tried, chitosan and gelatin were found effective in reducing weight loss of tubers in storage in comparison with chitin. This might be due to reduced water evaporation from microtubers as chitosan is having film making character to retain water and keep moisturizing (Meyers *et al*., 9), whereas, gelatin form thermo reversible gels. Further, periderm thickness also plays a major role in determining weight loss since 98% of the moisture loss could be attributed to water transport through periderm (Van Es and Hartmans, 13). Chitosan and chitin are a form of shell from the crab and contains calcium, which might have played a role in stopping the loss of cell integrity, ion transport regulation and water loss.

With reference to coating procedure, weight loss was more in single time coated tubers than the two and three time coated irrespective of bio-polymers tried. More weight loss was noticed in 1.00% concentration in chitosan and gelatin irrespective of number of layers coated (Fig. 3). Sprout growth in potato is an indication of release of dormancy and start of plant growth. Short or rudimentary sprout is not fit for giving generation as plant due to lack of necessary energy, vitamins, minerals, protein and all needed desirable nutritional changes (Chavan *et al*., 4). As microtubers are miniature structure of potato tubers, the nutritional capacity to give a better sprout is remote and it needs exogenous application. In potato, the sprout performance is judged through the thickness and length of the sprouts. Sprout thickness is important for potato seed tubers for further better performance in the field. The nutrients available to the bud along with environment may have a role in the sprout thickness. In this, the expectation was gelatin may play a role in sprout thickness as it is having many amino acids such as glycine, proline, hydroxyproline, glutamic acid etc. (Stevens, 12). However, in our experiment, sprout thickness was improved with chitosan treatment with maximum value being in 1.00% concentration (8.95 mm) and in case of gelatin the maximum thickness of sprouts was recorded in 0.75% concentration (8.02 mm). Chitin had shown negative effect on improving the thickness of sprouts in comparison with control (Fig. 4).

Sprout length did not differ significantly with the application of polymers. However, the increased concentration of chitosan showed a reduction in sprout length and in case of gelatin, longest sprouts were observed in 0.50% concentration (1.33 mm). Growth regulators like GA_3, which is used to break the tuber dormancy, has shown significant increase in the length of sprouts (Salimia *et al*., 11). In our study, correlations were established between sprout length, sprout thickness and yield (Fig. 5). In all the bio polymer treatments there existed a negative correlation between sprout length and yield. But, sprout thickness is positively correlated with the yield. It indicates that for better performance of the micro tubers, the sprouts should be sturdy and not too long.

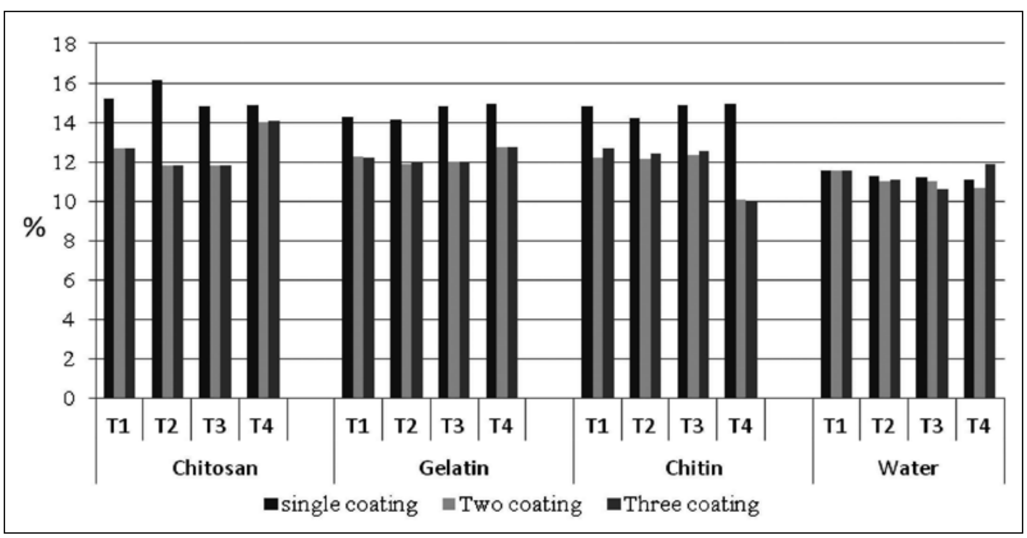

Fig. 3. Effect of bio-polymer coating on micro-tuber weight loss (%).

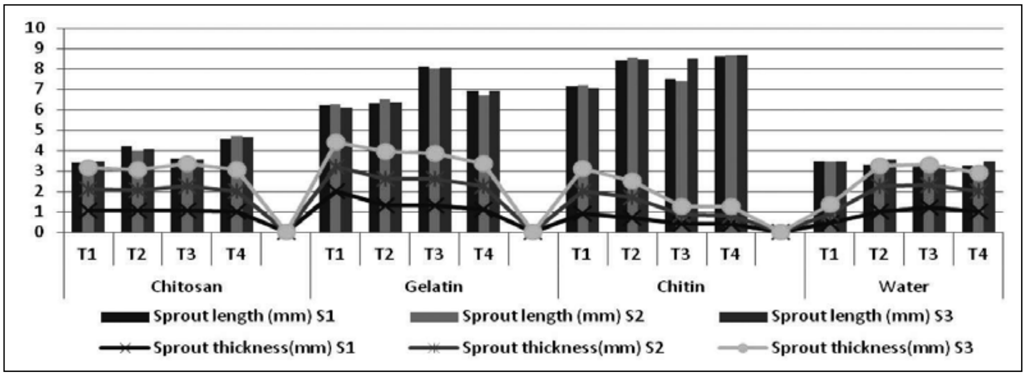

Fig. 4. Effect of bio-polymer coating on sprout length (mm) and thickness (mm) in potato.

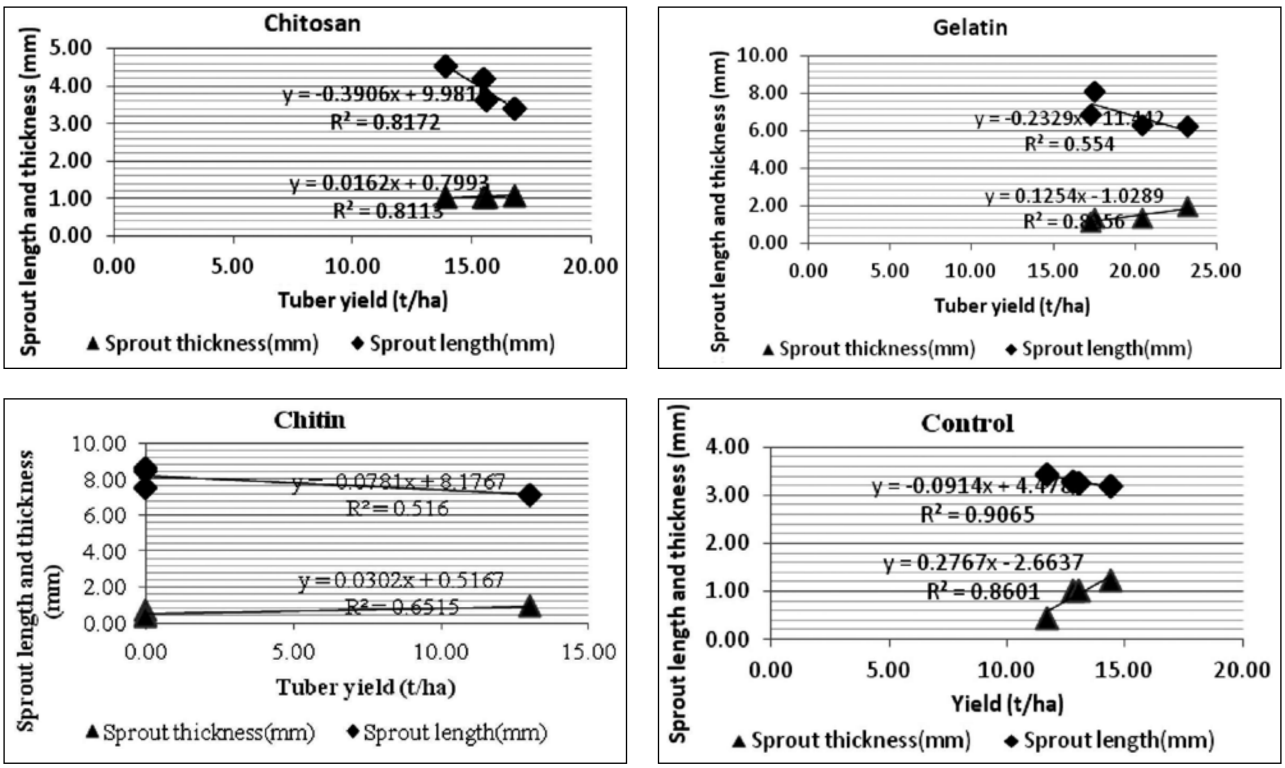

Fig. 5. Correlations between sprout length and thickness with tuber yield in potato.

As there was no significant difference in the single and multiple layers of coating on sprout growth, only single coated microtubers were carried forward to field inside the net house. Gelatin and chitosan performed better in terms of stand establishment. Chitosan performed well due to anti fungal and moisture retaining capacity along with the vigour maintained during the storage with proper physiological and bio-chemical activities. Barring 0.25% concentration, no establishment was noticed in chitin treated tubers. This can be correlated with its performance during storage. More weight loss is the main cause as potato cannot with stand the desiccation. In seed tubers, water loss leads to modification of many physio-biochemical activities like reduction of cellular volume, unregulated metabolism and bio physical damages to macromolecular structure. Though the potato tuber is not designated as recalcitrant seed, few characters of recalcitrant seed suits to it as a propagating material. The desiccation sensitivity of potato seed could be highly correlated with recalcitrant seeds.

Significant differences were observed in all the biometric parameters due to treatments. Gelatin performed well in all the concentrations followed by chitosan. Among the concentrations, 0.25% gelatin produced more number of stems per plant (1.67), number of leaves per plant (19.75) and plant height (57.78 cm) (Fig. 6). Chitin performance was almost at par with control. The reason for poor performance of chitosan and chitin in the field might be due to the presence of calcium. In the field, after degradation of bio polymers, calcium remains stuck to the tubers and hampers the activity of stomata leading to imbalance between photosynthesis and respiration. Thus, catabolism is higher than anabolism, which implies slow metabolism and plant growth.

The higher dry matter content (11.29 g plant^{-1}) of 75-day-old crop from gelatin treated tubers indicates that the biomass conversion was more effective in gelatin treatment. The same trend was maintained throughout the productive phase of the microtuber in gelatin treatment. Gelatin performed well in spite of less sprout length and less sprout thickness due to the presence of amino acids. Amino acids are well known as a means to increase yield and overall quality of crops as they can directly or indirectly influence the physiological activities of the plant. Potato tuber is considered as balanced and nutritionally favorable but the essential amino acids methionine and cysteine are under represented (Burten, 3). Galili and Hofgen (5) reported that the seeds accumulate amino acids to be used later as sources of nitrogen and energy for further growth. Hence, the amino acids supplied through gelatin might have given a support for the better performance in the field by these microtubers. A better healthy crop can have a good source sink movement which results in the bulking of tubers. Chitosan at all the concentrations and gelatin at 0.75 and 1.00% produced significantly superior yield over control in terms of tuber number as well as yield (Fig. 7). Chitin was not better than control.

Biopolymers, a multi action bioagents are used in many fields. Attempts were made to utilise some of their properties for the improvement in the storage life and viability of potato microtubers. Chitisan @ 1.00% followed by gelatin @ 0.75% single coating on the potato microtubers enhances the shelf-life and viability of the microtubers. However, the performance of gelatin polymer coated microtubers in terms of all biometric parameters and yield is better than the chitosan and due to the easy availability and dissolving property in water, gelatin is recommended to be used as shelf-life protectant of potato microtubers up to a period of 125 days after harvesting. The study has opened a way for attempting other biopolymers for the storage of potato microtubers. Further, we suggest that study on the mechanism of biopolymers during storage at molecular level is needed.

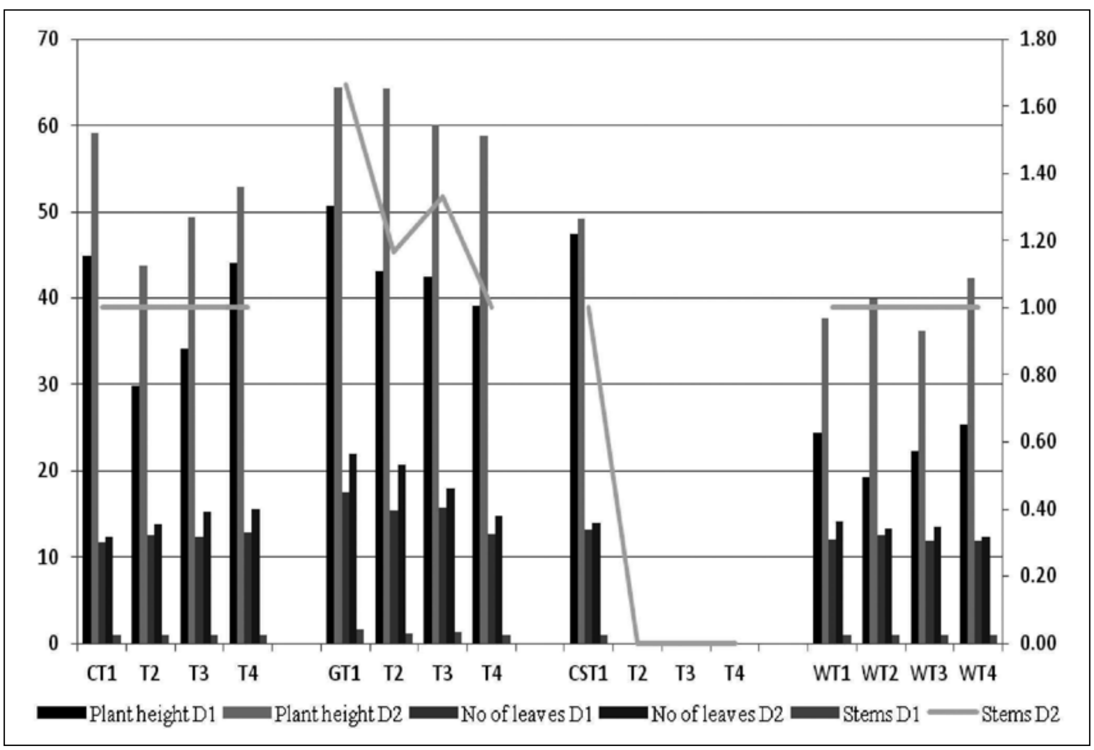

Fig. 6. Effect of bio-polymer coated microtubers in the field on plant height (cm), number of leaves and number of stems during 60th (D1) and 75th (D2) day after planting.

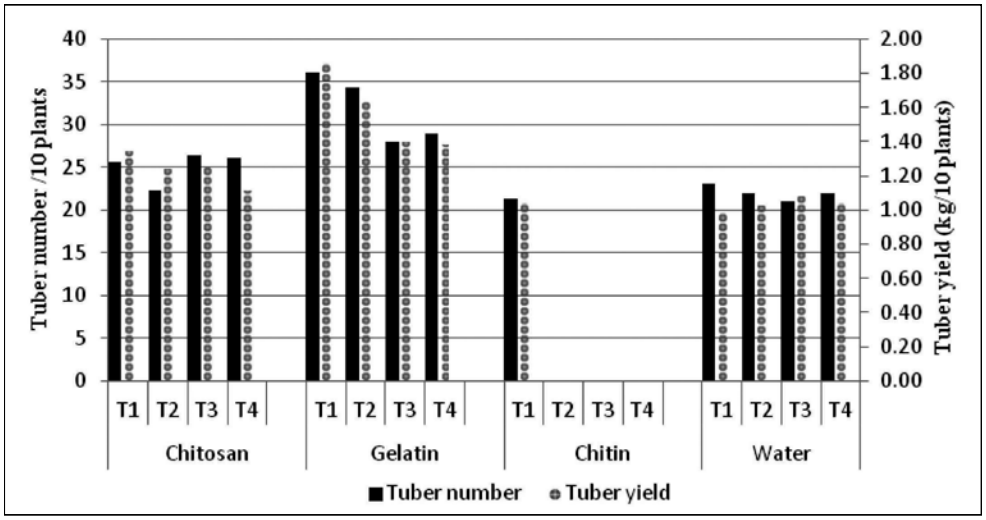

Fig. 7. Effect of polymer coating on field performance of potato microtubers.

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