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Extraction of Bioactive Macromolecules from Locally Available Natural Resources and Application as Natural Tea Growth Promoter

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ABSTRACT
This study was designed to evaluate the potential uses of natural biomaterials as tea growth promoter and anti-fungal agent. Bio-macromolecules were extracted from locally available natural resources i.e. prawn shell, sea weed etc. Extracted macromolecules were treated with Co-60 gamma radiation at different doses and were used to evaluate the growth promoting activity on tea plants. The aim of this study was to measure the effects of various concentrations and ratios of irradiated bio-macromolecule's solution in order to get the best response on tea plants in terms of various growth attributes. Samples were applied through foliar spraying at 7 days interval. The growth attributes like- total number of buds, fresh and dry weight of buds, average leaf area, and weight per bud and anti-fungal activities were determined after the foliar application for 15 weeks. The results showed increase in productivity (about 50%, based on fresh weight of tea leaves) and it reduced the total fungal count dramatically (more than 100 times in contrast with the control).

Keywords: Natural growth promoter, Gamma radiation, Tea plant, Bio-macromolecules.

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INTRODUCTION

Today's over populated world demands more production of crops to fulfill its demand, within its limited hand. For instance, the use of chemical fertilizer for the production of more crops is popular in the recent times. However, the foods are produced using commercial chemical plant growth promoters, might have some harmful effects (Mollah et al., 2009). Chemical fertilizers which are used in agricultural land create environmental pollution (air and water) and facilitate the growth of weeds. For that, scientists are now interested to use natural polymer or bio-fertilizer as plant growth promoter instead of chemical fertilizer.

Biomaterials are new promising materials that possess important properties like biodegradability or lack of toxicity. The advantages of using these biomaterials are that, they are naturally available, cheap and have no destructive effect on overall environment including plants and animals which may be occurred in case of application of chemical fertilizers and pesticides.

Chitosan is a linear polysaccharide derived from chitin, a major component of the shell of crustaceans and the second most abundant biopolymer in nature next to cellulose and is commercially available [Kast et al., 2003]. It has the potential in agriculture with regard to controlling plant diseases and promoting the plant growth (Ghaouth et al., 1992, Ohta et al., 1999). These molecules were shown to display toxicity and inhibit fungal growth and development (Hadwiger, 1984).

On the other hand, alginates are widespread in nature; occurring in various organisms. Sargassum (brown algae) is the main source of sodium alginate (Na-alginate), which is abundantly found in the Coral Island, St. Martin's in Bangladesh (Aziz et al., 2001). Na-alginate, the natural growth promoter is friendly for environment. Alginate is the major structural polysaccharide extracted from brown seaweeds (Khan et al., 2010). Its empicical formula is $Na\text{-}C_6H_7O_6$. It is composed of three types of block polymers namely polyglucouronate (poly-G), polymannuronate (poly-M) and copolymer of poly-G and poly-M in random sequences (Hang et al., 1967).

Recently oligosaccharide derived from depolymerization of alginates and chitosan are promising candidate to the plant growth promotion applications. It was found that the influence of average molecular weight of chitosan on its fat-binding ability in vitro was very important and the reduction of molecular weight leads to a significant increase in the amount of fat bound by 1 g of chitosan (Czechowska-Biskup et al., 2005). Alginate of molecular weight less than 10 kD showed strong effect on the growth promotion of rice and peanut (Hien et al., 2010).

Several methods have been tried to produce low molecular weight chitosan, among them (oxidative degradation, acid hydrolysis, electrochemical process, ultrasonic treatment, thermal depolymerization,

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enzymatic methods, micro fluidization combined with an ultrafiltration treatment, ultraviolet degradation, synergetic degradation with ultraviolet light and hydrogen peroxide and gamma radiation) gamma radiation degradation is relatively simple one step and an eco-friendly process and can be employed for the large scale production of . Radiation processing is the most promising method, since the process is simple, it is carried out at room temperature and no purification of the product is required after processing which allows extensive modification without waste and is a powerful tool that can be used to reduce molecular weight through chain scission mechanism without causing alteration to the main backbone structure of chitosan (Chmielewski and HajiSaeid, 2004).

This research work is concerning on the effect of radiation processed chitosan and Na-alginate on tea plants in the field trial basis to make a comparative study among treated and untreated plants.

MATERIALS AND METHODS

Preparation of 2% Chitosan (CH) Solution

Extraction of chitosan from shrimp shells and preparation of 2% solution in 2% acetic acid were done at the laboratory of Institute of Radiation and Polymer Technology of Bangladesh Atomic Energy Research Establishment according to standard procedure [Freier, 2008].

Preparation of 2% Na-Alginate (AL) Solution

Extraction of Na-Alginate from brown seaweed Sargassum [Khan et al., 2010] (Collected from Saint Martins Island) and preparation of 2% solution in water were done at the laboratory of Institute of Radiation and Polymer Technology of Bangladesh Atomic Energy Research Establishment according to standard procedure.

Radiation Degradation

Chitosan and Na-alginate solutions were irradiated by gamma radiation emitted from a Co-60 source at 40 kGy and 20 kGy doses respectively for degradation with a view to preparing oligosaccharides from them.

Selection of the experimental plots

The plant growth promotion activity was experimented in the field trial basis in Uttarbag-Indranagar teas state, Moulavibazar, Sylhet. Nine Plots having healthy tea trees from two divisions were selected. Among these nine plots 3 plots were for control, 3 plots were for Chitosan treatment and 3 plots were for Na-alginate treatment. The number of bushes per plot were counted for both divisions.

Evaluation of Growth Promoting Activity

The irradiated solutions of chitosan and Na-alginate were diluted to 500 ppm and 300 ppm respectively using pond water for testing the viability and reassuring it at the peasant level. The solutions were sprayed by using normal hand sprayer in the morning when photosynthesis of trees start. Foliar applications of biomaterials were done at every 7 days interval.

Evaluation of plant growth promotion activity

The leaves produced for each plant per plucking, their average numbers per plot and their average weight per plucking for each plot were calculated. Data collection started from the beginning of the first plucking. Measurements were recorded approximately at every 2 weeks interval and continued until 17th plucking. The results were finally tabulated and summed up using Microsoft Excel 2013 software. Then the results were graphically represented to evaluate the promotion activity.

RESULTS & DISCUSSION

The results for the field trial growth promotion experiments for division 1 and 2 are shown in the graphs 1 and 2 respectively. The graphs contain data for yields in Kg per plucking per plot.

Two varieties of tea trees were treated for this experiment. In division 1, tea seedlings of age 30 years and in division 2 tea variety TV23 of age 15 years were taken for this present work. From the graphs it is apparent that, for two varieties of tea trees, effectiveness of the two plant growth promoters (PGPs) is time dependent.

For both divisions, best results were found with alginate treatment. From figure 1, maximum yield with alginate treatment is found to be 21.67 % higher than that for control. However from figure 2, i.e. division 2 more prominent result with alginate treatment was found, and which was 71.47 % higher than that for control.

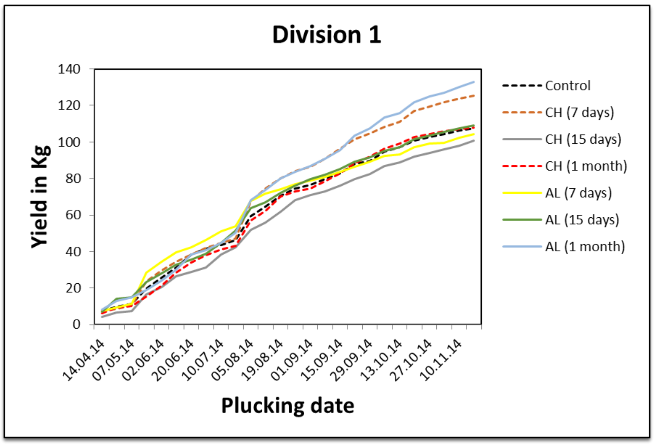

Figure 1. Yield of Tea leafs in Kg per plot of Division 1.

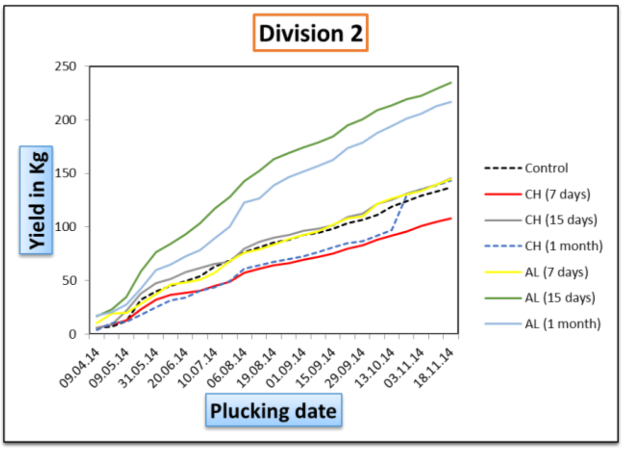

Figure 2. Yield of Tea leafs in Kg per plot of Division 2.

For chitosan treatment the results were not so much significant as that was for alginate. For division 1, yield of tea from seedlings of 30 yeas age, was found to be 14.757 % higher than that of control. On the other hand, for division 2 containing TV23 variety tea plants, the yield was only 5.632 % higher compared to the control.
From figure 1, it can be concluded that the optimized application intervals with alginate and chitosan treatment are 1 month and 7 days respectively. Whereas for division 2 15 days for alginate and 1 or half month for chitosan treatment are recommended.
There are some other conclusions that can be drawn from the figures, that, for TV23 tea plants both chitosan and alginates can be used as growth promoter; and for seedlings in division 1, alginate is the best solution.
Some negative trends in the tea yields for both division were also observed. For both chitosan and alginate treatment, from figures, low yields of tea are clear in contrast to the yield of the control, for some plots, may be due to the lack of due time spraying and red spider attack, as reported by the monitoring personnel in the tea state.

CONCLUSION

Field trial of tea growth promotion activities by the natural PGPs has been successful in spite of some inadvertent situations. Increase of budding, reduction of harvesting time were remarkable in this experiment. It can be summed up for the results found from this work as, these PGPs should be used for other promising fruits, that can bring more and more foreign revenues for 3rd world country like us. Moreover gamma radiation treatment, as a greener way to produce effective plant growth promoters should be taken under mass consideration by the research organizations as well the government to reduce the poisoning the environment by reducing the use of harmful chemicals.

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