CHITOSAN PRODUCTION FROM YEAST AND FUNGUS AND APPLICATION IN GREEN GRAM BEAN PRODUCTION

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Abstract

Low molecular weight chitosan can be used in agriculture to stimulate the growth and production of plants. The low molecular weight chitosan was produced from fungus, *A.niger* and yeast *S. cerevisiae* by enzymatic method. Free chitosan production from yeast cell wall after enzymatic method was 4.2g/100g of dried yeast, whereas the yield of chitosan-glucan complex was 17.3g/100g of dried yeast. The total yield of fungal chitosan after enzymatic extraction was 5.9 g/100g of dried mycelia. The effect of fungus chitosan on green gram plant *(Vigna radidta*) has been investigated as seed coating agent, growth promoter and to improve the yields of bean. The concentrations of fungus chitosan 200ppm, 150ppm, 100ppm, 50ppm, 0ppm were used to spray the fields of green gram plants. Among these concentrations, 100ppm showed the best growth and yield of green gram bean. Therefore the production of green gram bean can be increased by spraying of 100ppm fungus chitosan to the field of green gram plants.

Keywords: agriculture, fungal chitosan, green gram bean, seed coating, yeast chitosan

Introduction

Nowadays, Pesticides, insecticides, growth stimulators and fertilizers are being used all over the world at a large scale. The application of these chemicals in agriculture has caused many problems including environmental pollution, soil degradation, resistances to insects and disease pathogens, impaired food safety and quality and unstable ecosystems [1]. Applying bio-fertilizer, biostimulator and bio-insecticide may contribute to reduce these problems. Currently, chitosan is widely applied in agriculture as growth promoting agent, antifungal function and prevention of plant from insect attacks. It is, also used to stimulate germination of many vegetable seeds [2].

Chitin is a natural polysaccharide, which consists of a copolymer of N-acetyl-D-glucosamine and D-glucosamine residues, linked by β-1,4 glucosidic bonds. The deacetylated form of chitin is chitosan. chitosan has numerous applications in pharmaceutical, cosmetic, food, agriculture, textile, paper industry and in waste water treatment [3]. Chitosan with a molecular weight of 5-50 kDa was effective in cholesterol absorption [4], semipermeable membrane [5], antifungal and plant growth promotion [6] and enhance protocorm like body formation in orchid tissue culture [7]. The isolation of chitosan from fungal source could be permitted to obtain a low molecular weight chitosan (10-50 kDa with high polydispersity, 3-5)[8]. Therefore fungal source may be an alternative chitosan source for agriculture application.

In the present research, production of chitosan from fungus (*Aspergillus niger*) and yeast (*Saccharomyeces cerevisiae*) were carried out and used to stimulate the germination of green gram (*Vigna radiata*) seeds in seedbed preparation and to increase the yields of bean production.

Materials and methods

Extraction of chitosan from fungus and yeast

Aspergillus niger was obtained from the Department of Microbiology, Myanmar Pharmaceutical Factory. This strain was maintained on 3.9 % potato dextrose agar (PDA) slants at 4^{o}C. Spores from a 10-day culture of *A. niger* grown on PDA plates at 30^{o}C were harvested with sterile distilled water. The spores were gently scrapped off and the spore suspension was collected in a sterilized Erlenmeyer flask. Sweet potatoes were bought in the local market of Yangon, Myanmar. The sweet potato were cut into 1-1.5 x 1.5-2 cm pieces and washed with water. About 100 g crop pieces were arranged in a 600 ml beaker together with small pieces of plastic pipe (3 x 0.3 cm) for aeration and to keep distance between the solid substrates (SS) pieces. Each beaker was covered with aluminium foil, which has a hole filled with a cotton plug for aeration. The sweet potato pieces were sterilized together with mineral solution supplemented with urea 7.2g/kg of sweet potato pieces at pH 4.5 at 121^{o}C for 20 min, after that inoculated with spore solutions and incubated at 30^{o}C for 7 days. The mycelia were harvested at the end of the fermentation. The fermented contents were transferred to 5 liter beaker and stirred in excess water to separate the mycelia mat from the sweet potato pieces. The floating mycelia mass was collected from the water surface, while the remnant sweet potato debris settled down to the bottom of the beaker. The mycelia biomass was washed with water until all sweet potato pieces were removed. Finally mycelia were dried at 45^{o}C. Dried mycelia, 1g were treated with 40 ml of 11M NaOH for 13 h at 40°C. The alkaline insoluble material (AIM) was treated with 0.35M acetic acid at 95°C for 5 h. AIM suspension was adjusted to pH 4.5 and treated with α-amylase 4% (v/v) enzyme at 70°C and shaken at 200 rpm fro 3 h [9].

The dried yeast was obtained from a local market of Yangon, Myanmar that was sold for barker yeast. Dry yeast, 1g were treated with 40 ml of 11M NaOH for 3 h at 130°C. The alkaline insoluble material (AIM) was treated with 0.35M acetic acid at 95°C for 5 h. AIM suspension was adjusted to pH 4.5 and treated with α-amylase 4% (v/v) enzyme at 70°C and shaken at 200 rpm for 3 h [9].

The degree of deacetylation of chitosan was determined by IR method [10, 11].

Effect of fungal chitosan on green gram bean production

The chitosan, 1g, was dissolved in 100ml of 1% acetic acid and used as stock solution for this research. For seed coatings, a 500 times diluted chitosan solution was prepared from the stock chitosan solution and the seeds were soaked in diluted chitosan for 6 h for green gram bean. The CTS-O coated seed was taken out from the solution and planted in the field after puddling. After that the chitosan solutions (0 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm) were used to spray the plants at 2 weeks intervals and each experiment was carried out 6 replicates. The field trial was completed in 10 weeks. Non-chitosan treated field served as control. Determination of effectiveness of chitosan on green gram was carried out at Government Technological College, Kyaukse, Myanmar. Data analysis was performed by using Microsoft Excel 7.0 software.

Results and discussions

Production of chitosan from A. niger and S. cerevisae

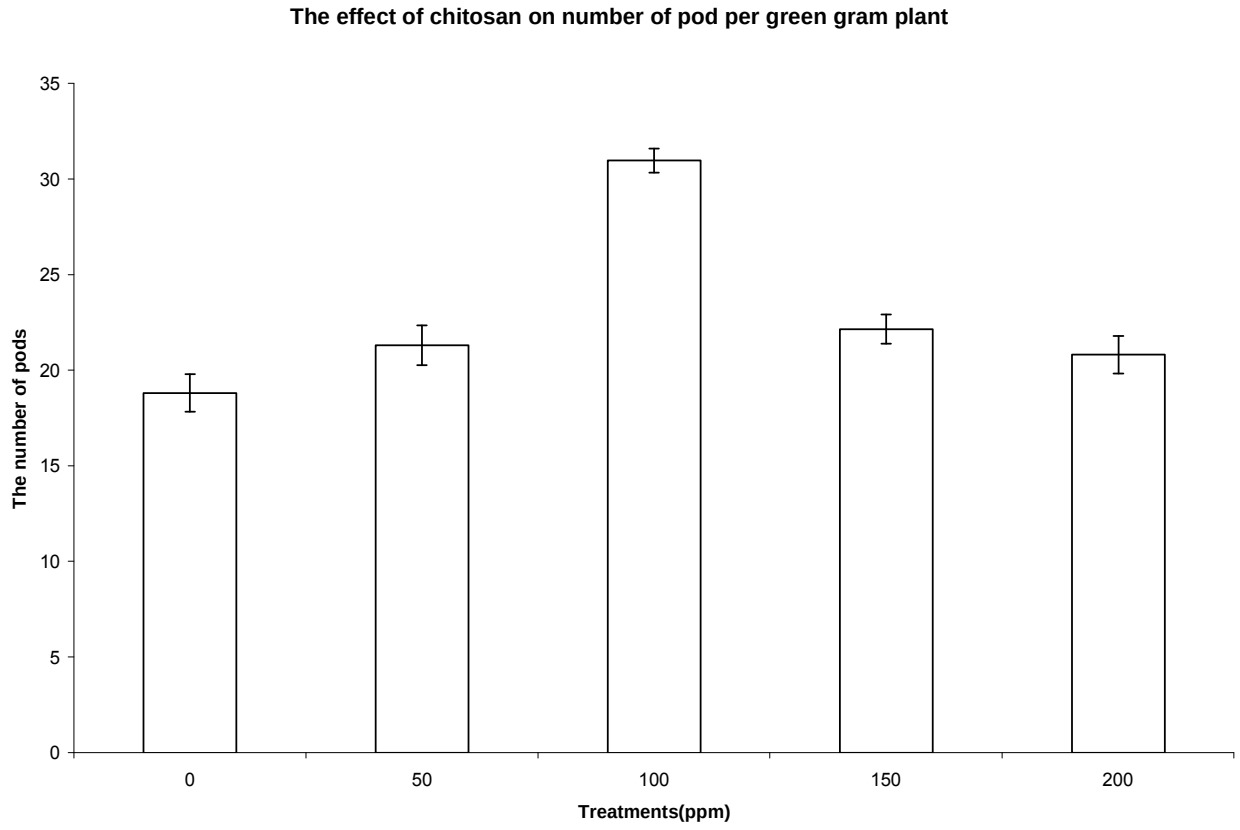
The acid/alkali extraction of *S. cerevisiae* cell wall results in three fractions (1) alkali soluble fraction (2) alkali and acid insoluble fraction and (3) alkali insoluble and acid soluble fraction. The

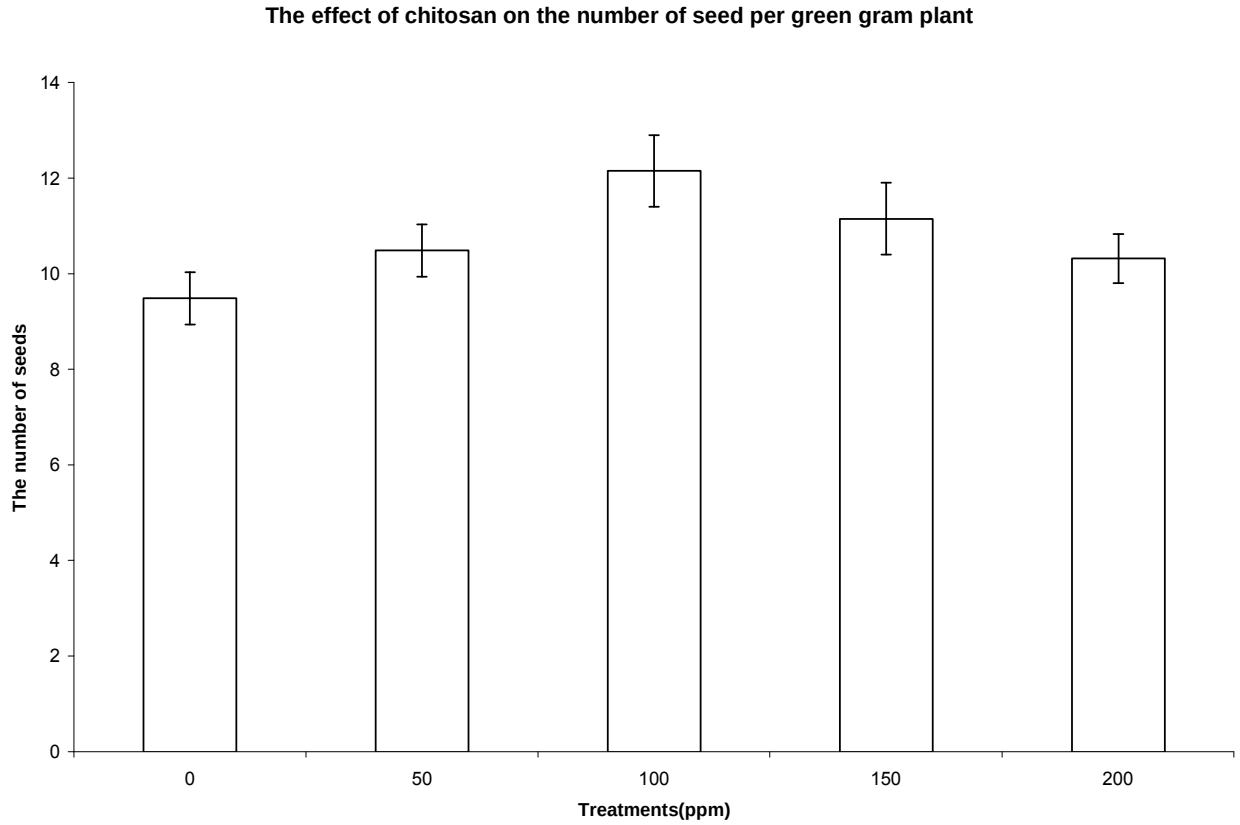
total yield of chitosan from yeast cell wall was 4.2 g/100g of the dried yeast, whereas the chitosan-glucan complex was 17.3 g/100g of dried yeast.

The chitosan from fungus after enzymatic extraction were 5.9 g/g of dried mycelia. The chitosan-glucan complex after repeated acid extraction was 17-21g/100g dried mycelia. Muzzarelli, *et al.,* (1980) reported that the yield of chitosan 11-14 g/100 of *A. niger* biomass under comparable condition of alkali extraction [12].

Application of fungal chitosan in green gram bean production

Green gram bean seeds were soaked in the dilute chitosan solution for 6 h. After that the seeds were sowed and bio-stimulation activity of chitosan on the germination of green gram bean was studied. Chitosan soaked seeds were germinate after two days but control seeds were germinate after four days. The chitosan soaked seedlings were found 3-5 cm taller than that of untreated seedling. The effect of chitosan on the number of pods, the number of seeds and the production yield are shown in Figure 1.





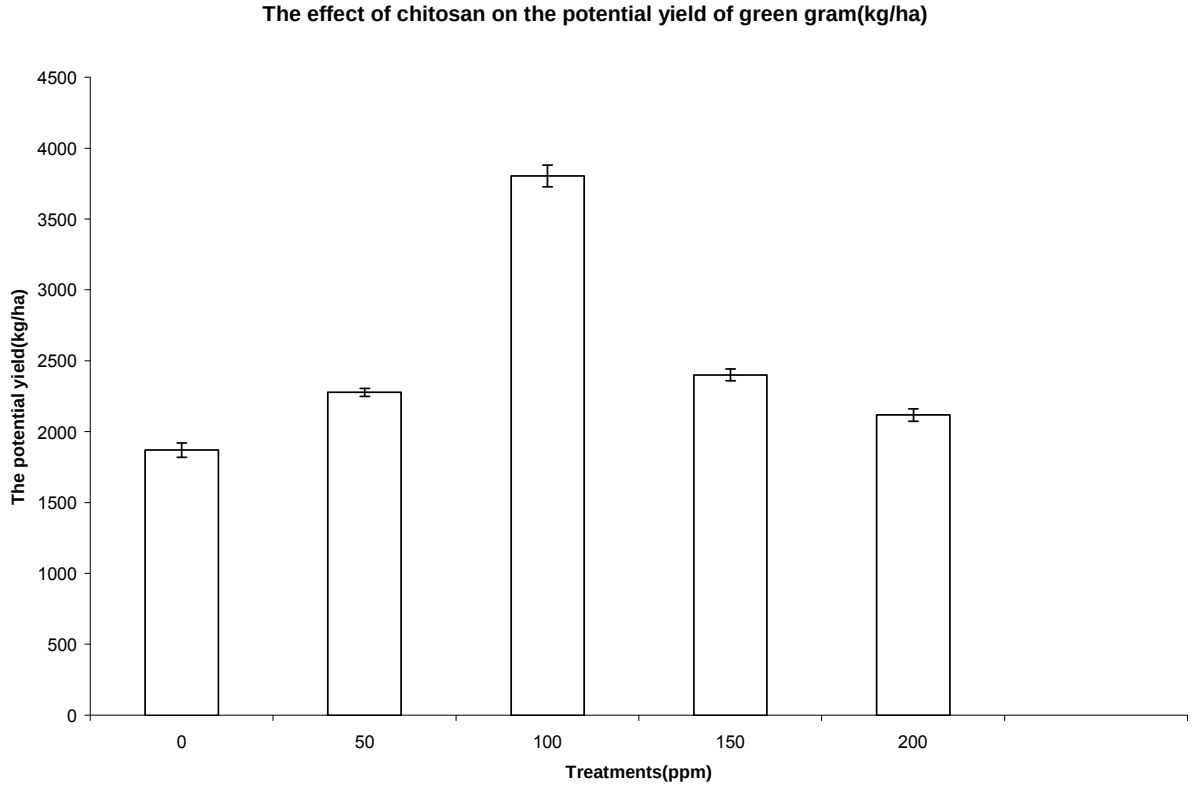


Figure 1. Effect of chitosan on the number of pods per plant (A), number of seeds per pod (B) and production yield of green gram bean kg per ha (C)

A comparison was made between the effects of chitosan solutions concentration 0-200 ppm on the spraying to the plants. Number of pod per plant, number of seed per pod and production yield was increased by spraying with 100 ppm chitosan solution to the green gram bean field. However the other chitosan concentrations did not support to increase the production yield of green gram plant. Therefore the best method for the high yield production of green gram bean is soaking the green gram seeds with 20 ppm chitosan solution and spraying with 100 ppm chitosan solution to the green gram bean field. The production yield of green gram was two times per ha higher than that of control field.

Conclusion

Different chitosan preparations were investigated in green gram bean production. Fungal chitosan 100 ppm is most effective on production yield of green gram bean. Therefore chitosan from fungus can be used for biostimulation and increase yield of crops. In addition the chitosan acts as antifunges component. The unique combination of plant stimulation and plant protection makes chitosan to very useful biocontrol agent with a large perspective for horticulture in general.

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