STUDY OF PLANT PATHOGEN SUPPRESSION THE SYNERGISTIC EFFECT BETWEEN BIOFERTILIZER AND IRRADIATED OLIGOCHITOSAN OF TOMATO

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ABSTRACT

Evaluation of the synergy effect between bio-fertilizer and irradiated oligochitosan was conducted to determine the growth and yield of the test plants. Study for synergistic effect of biofertilizer and irradiated oligochitosan, some positive effect such as plant growth promoter and pathogen suppression in tomato yield in the green house pot experiment. It was guessed that oligocitosan induced resistance for seedling treated. Data showed synergistic effect using disease control intensities, in this case, synergistic effect between biofertilizer and oligochitosan is clearly positive.

KEY WORDS: Rhizobacterial bio-fertilizer, fruit, fruit weight, pathogen, resistance

INTRODUCTION

In order to supply food to increasing population, agricultural production should be increased and a large amount of fertilizer is required. Most of fertilizers are chemical fertilizers, however oil and natural gas are needed to produce chemical fertilizers. Furthermore, improper use of chemical fertilizers and other agrochemicals damage agroenvironment, for example nitrate pollution in ground water.

Using inorganic fertilizers farmers can increase the yield of crops but the soil pollution is also increased with this day by day. Fertility of the soil increases due to the continuous use of the inorganic fertilizers but it also reduces the crop productivity. Soil pollution is caused due to the use of inorganic fertilizers, pesticides, and other chemicals etc (Badoni, 2006).

Martinez et al. (1993) Cuba, reported that soil inoculation with Azotobacter increased tomato seed germination by 33-46 per cent, shortened the period between sowing and transplanting by 5-7 days, increased the yield by 38-60 per cent.

Bio-fertilizers are the carrier-based preparations containing mainly effective strains of microorganisms in sufficient number, which are useful for nitrogen fixation.

If they are used in association with macronutrients the expected yields per unit area may be much higher. Amongst these nutrients, nitrogen is the only nutrient, which play major role in synthesis of chlorophyll, amino acids and protein building blocks, which is ultimately responsible for higher source to sink ratio.

The main advantage of bio-fertilizer is that it does not pollute the soil and also does not show any negative effect to environment and human health [5].

Plant growth promoting rhizobacteria (PGPR) are free living bacteria (Azospirillum, Azotobacter, Azoarcus) commonly found in soil and in association with plant roots, including important agricultural crops such as wheat. Some bacteria convert atmospheric nitrogen into soil nitrogen and some bacteria help in the solubilization of insoluble phosphates, improving P uptake from soil.

In 2001 the institute successfully launched its biofertilizer product under the trade name of Rhizobacterial fertilizer, which reduces the input cost of fertilizers and increases crop yield. The Rhizobacterial fertilizer is a low cost and environment friendly product and can be used to enhance the yield of all crops as well as soil fertility.

Oligochitosan is a low molecular weight chitosan and it can be obtained by γ-ray irradiation to chitosan. It has the effect of promoting the growth of plants such as rice, barley and soybean. In order to evaluate the synergy effect between biofertilizer and irradiated oligochitosan on plant pathogen suppression, tomato seedlings, which were inoculated by Pseudomonas fluorescens strain

FPH9601, were transplanted to the infected field by tomato bacterial wilt and irradiated oligochitosan solution was sprayed. By the application of irradiated oligochitosan, suppression effect against the pathogen by *Pseudomonas fluorescens* strain FPH9601 was enhanced, but it became lower in higher infected field.

Oligochitosan have been reported to increase growth and several crops. The seed yield was higher in 100 ppm chitosan might be due to increase number of seeds. Fusarium diseases widely distributed in soil is known as a plant pathogen[9].

Fusarium crown and root rot is an important soilborne disease, with the potential to limit productivity in glasshouse and field tomato crops. The causal agent, Fusarium oxysporum and f. sp. Increased early injury to the roots and collar of tomato plants caused by Forl was also observed in Tunisia [3], where yield losses were reported to range between 20 and 60%. Fungicides are of little use on most Fusarium diseases widely distributed in soil is known as a plant pathogen.

Biological control of Fusarium wilts, in the form of natural microbial populations in soils, has been recognized for over 70 years.

MATERIALS AND METHODS

Synergy effect of oligochitosan and biofertilizer on growth of tomato are tested in the pot test in green house.

Experimental design was RCBD with 4 replications. Treatments as fallow;

• Oligochitosan 7 (every week) T-1

T-2

- Oligochitosan 14 (every 2 weeks)
- Rhizobacterial biofertilizer + Oligo 7 T-3
- Rhizobacterial biofertilizer + Oligo 14 T-4
- Rhizobacterial biofertilizer T-5
- Control T-6

Pot contained 20 kg of sterile soil. Pathogenic fungus is *Fusarium* sp. For rhizobacterial biofertilizer inoculation treatment seed were coated at sowing. Cultivate pathogenic fungus and mix them into sterile soil were planting the seedlings before one week. The performances of tomatoes seedling were spraying the oligochitosan solution (100ppm) before 1 day transplanting. In conclusion the effect of integrated use of Oligochitosan, with

Fusarium on tomato seedling were investigated in this experiment. The performances of tomatoes seedling were spraying the oligochitosan solution (100ppm) every week and every two weeks during the 2 months.

Identified *Fuzarium* spp observed pathogenic fungal culture morphology, mycelium and spores Dr. Katsuhiko Ando's "Identification of microscopic Fungi" book[6].

Biocontrolled Fusarium spp fungi: Mixing bacteria of PGPR and Oligochitosan from Japan, as were transferred on PDA with fauzarium spp fungi. Observed growth of mycelium and control.

The analysis of variance and interpretation of data were done as per procedures given by Fisher and Yates (1963), Panse and Sukhatme (1967) and Gomez and Gomez (1984). Level of significance used in 'F' test was P=0.05 critical difference (CD) values were calculated only wherever the 'F; test was found significant.

RESULTS

Concerning synergistic effects among biofertilizers and oligochitosan, we have investigated two targets: 1st, plant growth promotion activities and 2nd, disease control intensities.

1) Plant growth promotion activities:

Survey tomato biofertilizer effect on the growth of the highest measured at 40 days after planting. Effect of treatment and biofertilizer+Oligo 14 on number of fruits per plant was found significant (Table 1, Fig. 1).

In biofertilizers' application the higher number of fruits per plant was noticed (23) over without

biofertilizers application (17). Among the treatments significantly higher number of fruits per plant was noticed in biofertilizer with oligochitosan every two week (26).

The significant differences in fruit yield per plant was noticed in treatment and biofertilizers, where the interaction effect was also found to be significant. Significantly higher fruit yield per plant was noticed in biofertilizer with oligochitosan every two week application (1252 g) as compared to without biofertilizers application (575 g).

Table 1

Effect of oligochitosan and biofertlizer on tomato growth

_	Plant height, cm			Number of fruits per plant			Fruit weight, g/plant		
Variants	2013	2014	Mean	2013	2014	Mean	2013	2014	Mean
T 1	57.8	77	67.4	24	13	19	735	875	805
T 2	57.8	76.8	67.3	25	11	18	839	785	812
T 3	58.8	77.8	68.3	16	11	14	968	609	789
T 4	57.5	78	67.8	39	13	26	1169	1335	1252
T 5	60.3	75.6	68.0	29	17	23	1032	1065	1049
T 6	49	75.8	62.4	23	10	17	675	475	575
LSD5%			7.6			5.6			72.3

It is well known that oligochitosan related materials induces plant defense mechanisms to plant pathogens. Data showed synergistic effect using

disease control intensities, in this case, synergistic effect between biofertilizer and oligochitosan is clearly positive.

Table 2 Synergistic effect between biofertilizer and irradiated oligochitosan of yield under contaminated soil by *Fuzarium sp*

Parameters	Rhizobacterial biofertilizer	Rhizobacterial biofertilizer + Oligochitosan 14	(Rhizobacterial biofertilizer + Oligochitosan 14)/(Rhizobacterial biofertilizer)	
Height of plant, cm	67.9	67.9	-	
Number of fruit per plant	23	26	113	
Weight of fruit per plant	1048	1252	119	

However, concerning synergistic effect using plant growth parameters, there are very difficult to distinguish efficacy of oligochitosan to plant growth from those of biofertilizer tested.





Fig.1. Pot experiment in green house of tomato

2) Results of disease control intensities:

Oligochitosan 100 ppm and 1 μ l-added to potato dextrose medium fungus infected. Also control to Oligochitosan-potato dextrose medium without fungus infects 28 $^{\circ}$ C 7 days later when grown in an incubator Oligochitosan was resistant to Fusarium

soil testing was conducted from 14 days after the spraying by Oligochitosan. According to these microscopic fungus Oligochitosan 7 and control were spores detected. In other spores were low activity and opaque (fig 2,3).

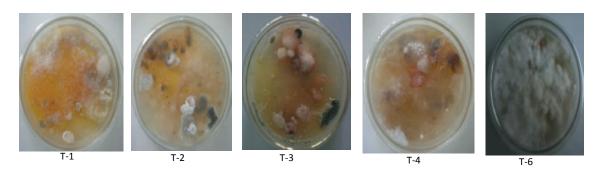


Fig.2. Soil testing contaminated by Fuzarium sp

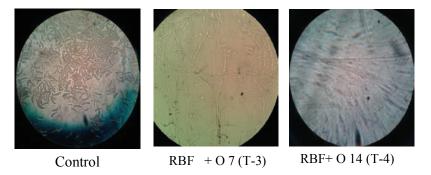


Fig.3. Observation of spores by electron microscope

Plant growth promoting rhizobacteria (PGPR) belongs to *Pseudomonas* spp. are being exploited commercially for plant protection to induce systemic resistance against various pests and diseases. Mixtures of different PGPR strains have resulted in increased efficacy by inducing systemic resistance

against several pathogens attacking the same crop. Seed-treatment with PGPR causes cell wall structural modifications and biochemical/physiological changes leading to the synthesis of proteins and chemicals involved in plant defense mechanisms [7].

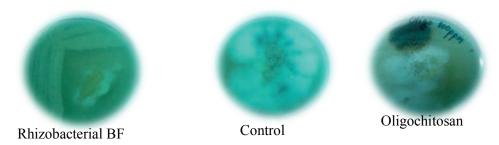


Fig.4. Antifungal effects

In this research also studied mixed culture of rhizobacterial fertilizer (PGPR) wasn't as effective as we've hoped against entomopathogenic *Fuzarium* spp (fig 4).

DISSCUSSION

Plant growth promoting rhizobacteria (PGPR) belonging to *Pseudomonas* spp. are being exploited commercially for plant protection to induce systemic resistance against various pests and diseases. Mixtures of different PGPR strains have resulted in increased efficacy by inducing systemic resistance against several pathogens attacking the same crop. The performance of PGPR has been successful against certain pathogens, insect and nematode pests under field conditions [9].

We conclude that the two PGPR strains induced resistance systematically in cucumber against Fuzarium wilt. In addition to the cytological modifications, induction of resistance can result from a biochemical reaction. In this case, addition

of the non-pathogenic strain of *F. oxysporum* (Fo47) to tomato plants before inoculation with *Fusarium oxysporum* f. sp. *lycopersici* increased the chitinase, glucanase, and glucosidase activities in treated plants [7].

The results indicated that the utilization of biofertilizer or humic fertilizer in fertilization practices can lead to higher yield of greenhouse tomato with improved market quality.[8]

Significantly higher fruit yield per plant was noticed in Rhizobacterial biofertilizer with oligochitosan every two week application as compared to without biofertilizers application.

CONCLUSION

- 1. The use of biofertilizers play a key role for sustainable agriculture and environmental degradation through reduction of chemical fertilizer usage.
- 2. Synergy application biofertilizer and irradiated oligochitosan, some positive effect such as plant growth promoter and pathogen suppression on tomato yield in green house pot experiment.
- 3. In this research also studied mixed culture of rhizobacterial fertilizer (PGPR) wasn't as effective as we've hoped against entomopathogenic Fuzarium spp.

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