Plant Growth Promoting Activity of Two Plant Extracts Combined with Chitosan on Chilli Seedlings

Tarntip Rattana*, Thanakorn Sangsanga

[1]Environmental Science Program, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima 30000, Thailand

**Corresponding author: rattana.tarntip@gmail.com*

Abstract

Plant extracts can enhance and promote plant growth by a wide variety of mechanisms. Some of these aqueous plant extracts were screened in vitro for their plant growth promoting trait such as the production of amylase, ammonia, acetoin and indole acetic acid (IAA). All the aqueous tests produced amylase, ammonia and IAA, whereas the production of acetoin was only possible with 10% papaya pericarp plus effective microorganisms (EM), and with 10% papaya pericarp in EM with chitosan: 200, 400 and 600 ppm. Many treatments showed two times efficiency, much better than the control treatment at 192 h for chilli seedlings. Treatments with EM 1% extract and 1% papaya leaf + pineapple leaf in EM with chitosan 600 ppm presented the highest chilli seed germination index vigor for seedlings at 137% and 1056, respectively.

Keywords: plant extract, effective microorganisms (EM), chitosan, plant growth promoting, chili

Introduction

At present, microorganisms play an important role in organic agriculture systems, especially the group of plant growth promoting microorganisms (PGPM). Plant growth promoting activity can improve the extent or quality of plant growth directly and indirectly. The potential use of PGPM has proved to be useful in plant growth promotion due to their role in nutrient cycling and disease control [1]. A microbial inoculant, containing various kinds of naturally occurring useful microorganisms called “Effective Microorganisms” (EM), has

been used broadly in organic agriculture. The utilization of EM can improve soil and irrigation water. It can be used in seed treatment and phytopathogenic microorganism inhibition [2]. Chitosan have many biological functions, for example, antimicrobial activity, suppression of plant pathogens and induction of systemic resistance. Application of chitosan is easy to use in agriculture and in aqueous solutions at various concentrations. This application has been successful in Thai agriculture especially due to its activity in self-protection systems of plants and as a growth promoter to enhance crop production [3].

Chilli is one of the vital ingredients of Thai food, as well as for foods in every country in the world. Therefore, it would be valuable for crop farming systems (including chilli production) to develop efficient EM systems, such as involving an aqueous extract of pineapple and papaya combined with EM and chitosan. In this study, the germination and growth rate of chilli seeds were tested using various effective aqueous extracts for their multiple plant growth promoting activities.

Material and methods

Seed material

Chilli seeds of Best hot F-1 from OK seeds company were tested to study the effects of chitosan extracts on chilli growth and resistance to anthracnose disease.

Preparation of chitosan solution

Chitosan powder from shrimp shells was used to make chitosan solutions with 200, 400 and 600 ppm concentrations of chitosan in 1% acetic acid. The molecular weight of chitosan was 30,000 Daltons.

Preparation of aqueous plant extracts

Forty grams (40 g) of fresh and clean leaves and unripe fruit of *Ananus comosus* (pineapple) and *Carica papaya* (papaya) were cut into smaller pieces and placed in a 200 ml reagent bottle with 100 mL of distilled water. EM reagent was added to it. The prepared products of

chitosan solutions with 200, 400 and 600 ppm concentrations were added as different doses for the various plant extracts. It was then kept for 72 hours at room temperature. After 72 hours the aqueous slurry was filtered though Whatman filter paper No.1 and was placed in another 200 ml bottle. All aqueous plant extracts were not sterilized by autoclave.

Plant growth promoting of the various plant extracts

Amylase (starch hydrolysis) activity

The one percent of aqueous tests was inoculated on nutrient broth and incubated at 30ºC for 24 h. 10 µl of the incubated supernatant from the aqueous were spot on starch agar and incubated at 30ºC for 48 h. After incubation, the plates were flooded with iodine solution, kept for 15-30 minutes and then poured off. The appearance of a blue color in the media showed the presence of starch. Therefore, the production of amylase [4] was indicated by the loss of blue color or a clear zone around the spot where the aqueous extract had been placed [4].

Phosphate solubilizing activity

Evaluation of tricalcium phosphate solubilization of aqueous tests was assessed using Pikovskaya's agar [5]. Each aqueous test solution from the 24-hour incubated culture was spotted in the centre of the plate. After incubation for 48 h at 30ºC, a clear zone surrounding the spot indicated inorganic phosphate solubilization.

Ammonia production

Aqueous test cultures were assessed for the production of ammonia in peptone broth. 0.5 ml of the cultures were added to 5 ml peptone broth and incubated for 48 h at 30ºC. 0.25 ml of Nesseler's reagent was added to each tube. The color change from brown to yellow is a positive reaction for ammonia production [6].

Acetoin production

Aqueous test cultures were assessed for the production of acetoin in 2 ml of MR VP broth. These tubes were incubated for 48 h at 30ºC. At the end of incubation, 1ml of the aqueous tests were added αnaphthol (5%) solution in ethanol, followed by 1 ml of 40% KOH. Development of yellow to red was a positive test for acetoin production.

IAA production

Nutrient broth medium, incorporating 3 µg/l L-tryptophan, was inoculated with the aqueous tests. They were incubated for 48 h at 30^{o}C on an incubator shaker. Cultures were centrifuged at 10,000 rpm for 10 min. The supernatant (1ml) was added with 1ml of Salkowski's reagent and incubated in the dark for 30 min. A pink color will be positive test for IAA production. These tubes were measured for concentration change at 530 nm in a spectrophotometer [7].

Growth of chilli crop

Laboratory experiments in Petri dishes were used to observe the germination percentage of the chilli seeds; also observed were shoot growth, root growth, plant height, etc. Fourteen different test extracts were used with chilli seeds in the following combinations:

- control (untreated seeds)
- EM control dosed at 1% and 10%
- chitosan solutions: 200, 400, 600 ppm
- 10% papaya pericarp as EM control
- 1% papaya leaf+ pineapple leaf as EM control
- 10% papaya pericarp as EM with chitosan: 200, 400 and 600 ppm
- 1% papaya leaf + pineapple leaf as EM with chitosan: 200, 400 and 600 ppm

Petri dishes with various sheets of tissue paper were used. For the investigation of germination percentage, growth and development of chilli seeds, 15 ml of each tested extract was put in each Petri dish. As a control, distilled water was used and the amount of distilled water was also same. Each Petri dish, containing twenty-five seeds of each chilli crop, was kept on room temperature and each treatment was replicated four times. 5 ml of deionized water was added to moisten per day per Petri dish. Petri dishes were incubated at room for 192 h. IRSA germination index [8] was calculated using the following equation:

$$GI\ (\%) = \frac{\text{RL treatment x GS treatment}}{\text{RL control x GS control}}$$

where GI is germination index, RL is the root length, and GS is the number of germinated seeds. When the germination index exceeded 60%, the extract is considered not phytotoxic and was selected for further analyses.

Results and discussion

In vitro screening of aqueous extracts for their plant growth promoting activities

Screening results of plant growth promoting traits are showed in Table 1. All the aqueous tests showed amylase activity, ammonia production and IAA production. The highest IAA production was provided by 1% papaya leaf + pineapple leaf in the EM control at 21.31 mg/L. Phosphate solubilization was not detected in any aqueous extract tests. Production of acetoin was detected with some aqueous tests: by 10% papaya pericarp in EM control and 10% papaya pericarp in EM with chitosan: 200, 400 and 600 ppm. Various plant growth promoters were detected in this study for example, amylase activity, ammonia production and IAA production.

Effect of seed treatment with aqueous plant extracts on seed germination

Among fourteen aqueous extracts screened, only seven test extracts revealed enhanced germination to varying degrees (Table 2). Among the chitosan 600 ppm treatments recorded, the highest seed germination was 149.63%, followed by Chitosan 400 ppm and Chitosan 200 ppm treatment recorded 135.31 and 130.19% germination respectively. The GI results increased following the enhancing of chitosan concentration in chitosan treatment and 10% papaya pericarp in EM with chitosan treatment but not in 1% papaya leaf + pineapple leaf in EM with chitosan treatment.

Table 1. Plant growth promoting characteristics of aqueous plant extracts

Treatment	Amylase activity (cm)	Phosphate solubilization	Ammonia production	Acetoin production	Indole acetic acid (mg/L)
EM control in dose 10%	2.5	-	+	-	13.02
10% papaya pericarp in EM control	4.66	-	+	+	11.78
Chitosan 200 ppm	4.55	-	+	-	8.44
Chitosan 400 ppm	2.95	-	+	-	13.17
Chitosan 600 ppm	4.62	-	+	-	5.19
10% papaya pericarp in EM with chitosan: 200 ppm	4.63	-	+	+	13.25
10% papaya pericarp in EM with chitosan 400 ppm	4.15	-	+	+	6.35
10% papaya pericarp in EM with chitosan 600 ppm	3.48	-	+	+	7.51
EM control in dose 1%	4.8	-	+	-	6.12
1% papaya leaf+ pineapple leaf in EM control	5.83	-	+	-	21.31
1% papaya leaf+ pineapple leaf in EM with chitosan 200 ppm	6.84	-	+	-	3.02
1% papaya leaf+ pineapple leaf in EM with chitosan 400 ppm	5.76	-	+	-	2.48
1% papaya leaf+ pineapple leaf in EM with chitosan 600 ppm	5.76	-	+	-	6.12

Effect of seed treatment with aqueous plant extracts on seed germination and seedling vigor

Table 3 shows the shoot length, root length, germination indexes (GI) and seedling vigor after 8 days of incubation using different aqueous extract tests. Following the IRSA method [8], a GI index below 60% is a symptom of phytotoxic effect, while GI over 60%, is considered not phytotoxic, with no damage for plant growth. The greatest GI in aqueous tests was presented with EM control in dose 1% treatment followed by Chitosan 400 ppm treatment at 136.93 and 131.41 % respectively. The least GI was depicted in Chitosan 600 ppm treatment at 50.97 %. Maximum seedling vigor was noticed at 1055.18 and 989.46g with 1% papaya leaf + pineapple leaf in EM with chitosan 600 ppm treatment followed by 10% papaya pericarp in EM with chitosan 400 ppm treatment.

Table 2. Effect of aqueous plants extracts on chilli-seed germination at 192 hours

Treatment	Germination index (%) after 8 days
Control	100a
EM control in dose 1%	102a
EM control in dose 10%	111b
10% papaya pericarp in EM control	70c
Chitosan 200 ppm	130d
Chitosan 400 ppm	135d
Chitosan 600 ppm	150e
10% papaya pericarp in EM with chitosan: 200 ppm	87f
10% papaya pericarp in EM with chitosan 400 ppm	96a
10% papaya pericarp in EM with chitosan 600 ppm	98a
1% papaya leaf + pineapple leaf in EM with chitosan 200 ppm	89f
1% papaya leaf + pineapple leaf in EM with chitosan 400 ppm	122g
1% papaya leaf + pineapple leaf in EM with chitosan 600 ppm	96a
1% papaya leaf + pineapple leaf in EM control	120b

The value of each column followed by a different letter indicates significant differences ($P<0.05$) according to LSD test.

Table 3. Effect of aqueous plants extracts on seed germination and vigor on 192 h

Treatment	Shoot length (cm)	Root length (cm)	GI (%)	Vigor index
Control	2.37a	5.43a	100a	412a
EM control in dose 1%	3.81b	4.78b	137b	840b
EM control in dose 10%	3.88c	6.64c	117c	418a
10% papaya pericarp in EM control	3.09a	3.35d	101a	813c
Chitosan 200 ppm	2.81a	3.84e	124b	926d
Chitosan 400 ppm	4.01d	4.24f	131b	925d
Chitosan 600 ppm	2.6a	2.89g	51d	394e
10% papaya pericarp in EM with chitosan: 200 ppm	2.88a	5.79h	75e	270f
10% papaya pericarp in EM with chitosan 400 ppm	3.16a	2.3i	78e	989g
10% papaya pericarp in EM with chitosan 600 ppm	2.84a	4.35j	126c	838b
1% papaya leaf+ pineapple leaf in EM with chitosan 200 ppm	2.34a	2.61k	62f	503h
1% papaya leaf+ pineapple leaf in EM with chitosan 400 ppm	2.97a	3.57l	107a	813c
1% papaya leaf+ pineapple leaf in EM with chitosan 600 ppm	3.82e	3.75m	130b	1055i
1% papaya leaf+ pineapple leaf in EM control	4.08f	2.54n	75e	748j

The value of each column followed by a different letter indicates significant differences ($P<0.05$) according to the LSD test.

Discussion

In the present study, four aqueous extracts affected plant growth promoting activities viz., amylase, ammonia, IAA and acetoin production. All the treatments promoted the production of ammonia that indirectly influences plant growth. This compound, as a secondary metabolite product of the plant growth promoting bacteria, has an important role for the biological N cycle (Kennedy et al., 2004). The amylase enzyme was present in all test extracts. This enzyme promotes initial germination and enhances the availability of starch utilization [9]. Acetoin is an important volatile substance utilizable for plant growth

promotion [10]. Indole acetic acid (IAA) is one of the various plant hormones. IAA plays a key role in stimulating both a rapid response and a long-term response [11].

On 8 days of seed treatment, the maximum germination of 150% was observed in the Chitosan 600 ppm treatment. Chitosan has been used to stimulate growth, seed germination acceleration and crop product improvement in various crop species [12]. After 192 hours, the EM control, with a dose of 1% treatment, presented the highest germination index at 137%. The maximum vigor was detected with 1% papaya leaf + pineapple leaf in EM with chitosan 600 ppm treatment at 1056 seedling vigor. Both treatments were combined with 1% EM. Arshad and Rukhsana [13] reported that shoot biomass was significantly increased in farmyard manure, ½ NPK and NPK amendments respectively. These treatments were combined with dilute EM solution. A significant increase was observed in nitrogen, phosphorus and potassium changes in soil of the mung bean plants both at the flowering and maturity stages when using EM application. Hussain et al. [14] reported that the major microorganisms in EM cultures are many species of photosynthetic bacteria, lactobacilli, yeasts and actinomycetes. These microorganisms enhance crop quality and crop production by enhancing photosynthesis, producing bioactive compounds like hormones and enzymes. The metabolites produced by EM microbes are absorbed directly by plants.

Conclusion

This study interestingly showed that applied plant extracts, chitosan with EM have potential as bioactive compounds to stimulate field-crop development. Therefore, we suggest that the use of 1% papaya leaf + pineapple leaf in EM with chitosan 600 ppm and 10% papaya pericarp in EM control plant extracts as effective plant growth promoters as being beneficial for chilli cultivation.

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