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# Effects of rhizobacteria on seed germination of water spinach (*Ipomoea aquatica*)

*Nguyen Thi Huynh Nhu[1*] and Nuntavun Riddech[2]*

*[1]Graduate school of Biological Science program, Faculty of Science, Khon Kaen University, Thailand. 40002*
*[2]Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand. 40002*
*[*]Corresponding author: nthnhu123@gmail.com*

## Abstract

Rhizobacteria are considered as the most useful microorganism based on their properties in nitrogen fixing, phosphate and potassium solubilizing, IAA and other plant hormones which improve the germination of seed. However, seed germination varied depending on stimulator such as bacterial strains, bacterial growing media. This investigation focused on testing the germination of *Ipomoea aquatica* vegetable by using three rhizobacteria isolates including H1-702, C1-112, P1-5071 and their growing media. Percentage of seed germination, shoot length, root length and number of root hairs of *I. aquatica* were lowest found in nutrient media treatments without bacteria (50%, 1.67 cm, 1.47 cm, and 1.47, respectively) while the highest numbers were obtained from the treatment of soaked seed with bacterial suspension which gave rise 90%, 4.88 cm, 3.01 cm and 19.26, respectively. Among all treatments, percentage of seed germination was highest (83%) in the single inoculums treatments C1-112 and H1-702. In day 5, the highest root length (2.4 cm) and root hair number (22.05) was showed in H1-702 treatment while C1-112 treatment revealed the highest shoot length (4.52 cm). The combination of two bacterial isolates did not stimulate the germination of *I. aquatica*, especially, P-H treatment which got the lowest number in all observation data.

**Keywords :** *Rhizobacteria, Ipomoea aquatica, seed germination.*

## 1. Introduction

*Ipomoea aquatica*, or water spinach, is an aquatic plant, fast-growing, economic and high nutrient (Khamwan et al, 2013), it provides calcium, potassium, iron, vitamins and proteins with high levels. Wargovich (2000) reported that vegetables play an important role in human health and nutrition, particularly as sources of vitamin C, thiamine, niacin, pyridoxine, folic acid, minerals and dietary fiber. According to Jampeetong (2012), water spinach had been widely applied to purify eutrophic water and microorganisms played a main duty in the removal mechanism of nitrate, nitrogen and ammonia nitrogen in water spinach (Zhang et al., 2104). It is cultivated in the old world tropics and role as a weed of more than 20 crops in many regions of 60 countries, called by people in the Southeast Asia as water cabbage or water spinach. According to the advantage of *I. aquatica* as mention at above, there were very necessary to improve the *I. aquatica* production, especially, the germination of seed. However, according to Vrbničanin (2011), seed germination varied depending on bacterial media and germination was inhibited by bacterial treatments.

In addition, one of the most useful microorganism in agricultural systems is Plant Growth Promoting Rhizobacteria (PGPR). Rhizobacteria can be able to excrete plant growth promoting substances such as vitamins, kinetin and gibberellins and will also improve the vigor and productivity of the crop (Karthikeyan et al., 2008). Rhizobacteria could also stimulate the germination of *Strigahermonthica* (Babalola, 2007), the number of root hairs and root laterals response to IAA production by rhizobacteria (Shahab et al., 2009).

Furthermore, overusing chemical fertilizers in developing the crop of *I. aquatica* has been causing ecosystem change, soil nutrient decrease, human health effect and numerous money lost, so chemical fertilizers should be reduced.

Currently, there are few reports documented on the roles of rhizobacteria applying in *Ipomoea* aquatica vegetable, the new strategy in agricultural habitats should be applied for good qualities ofwater spinach and sustainable agriculture.

In this research,bacterial growing media and the three rhizobacteria isolates were chosen for testing plant growth promoting properties percentage of germination (PG) in *I. aquatica*.

## 2. Materials and methods

### 2.1 Materials

Three rhizobacterial strains H1-702, P1-5071, C1-112 (Sritongon, 2015) were received from Microbial Fertilizer Laboratory group, Department of Microbiology, Faculty of Science, KhonKaen University, Thailand. Nutrient agar media, Gram stain chemicals, fitter paper.

### 2.2 Characterization of the rhizobacteria

The cell biological properties and Gram staining were observed under microscope at magnification 1000 X.

These isolates were grown in Nutrient agar medium for 2 days and determined colony morphology on the basis of shape, color and diameter of colonies.

Three isolates were grown on nutrient medium (20% inoculation size) in incubator with shaking (150 rpm). The optical density at 600 nm was used for measuring the bacterial density in every 2 hours until 24 hours.

**2.3 Evaluation *I. aquatica seed* germination by using bacterial growing media**

Seeds were sterilized with 1% (v/v) sodium hypochlorite solution for 10 minutes, then rinsed three times with sterile distilled water. Ten seeds of *I. aquatica* were transferred to petridish and treated with 3 ml of supernatant solution. Pure sterilized water was used for the control. Three replications used for each treatment were placed in an incubator under dark condition (28 - 30°C) (Vrbničanin et al., 2011).

After 5 days, the shoot length, root length and number of root hairs were collected and calculated for the percentage of germination (PG) as described by Kham-iam et al.(2005) using the following equation:

$$PG\% = \frac{number\ of\ seeds\ germinated}{number\ of\ initial\ seeds} \times 100$$

Seed was grown on the following treatments*;C: control, M: Media, S: supernatant SS: soaked seed*, when Control (C) was 3 ml pure sterilize water, Media (M) is 3 ml fresh media containing peptone and beef extract, Supernatant (S) was 3 ml bacterial supernatant after centrifuging at 600 rpm in 20 minutes, and soaked seed (SS) was soaked the seeds in the bacterial supernatant (centrifuged at 600 rpm in 20 minutes) in 60 minutes before placing in the fitter paper in petri dish and added 3 ml of pure water.

**2.4 Evaluation *I. aquatic* seed germination using rhizobacteria lisolates**

Seven treatments including:Plants inoculated with single of each bacterial isolateH1-702, P1-5071, and C1-112; Combined two isolates: H-P (H1-702 + P1-5071), H-C (H1-702 + C1-112), C-P (C1-112 + P1-5-71); and combined three isolates C-H-P (C1-112 + H1-702 + P1-5071) All treatments of bacteria were inoculated in the dark incubator with shaking (150 rpm) until the number of bacteria reach $10^8$ CFU/ml.

Seeds were sterilized with 1% (v/v) sodium hypochlorite solution in 10 minutes, and then rinsed three times with distilled water.

The shoot length, root length and number of root hair were collected and calculated for the percentage of germination in day 3, 4 and 5. Complete randomized design and triplicates in each treatment was designed for the experiments.

**2.5 Data analysis method**

Data were subjected to statistical analysis by IBM SPSS Statistics 19 software; least significant difference test (LSD) at 5% level was use for comparing means of the treatments with three replications by complete randomized design. Microsoft Office Excel 2013 was used for data presentation.

## 3. Results and discussion

**Morphology, Gram stain and Growth curve**

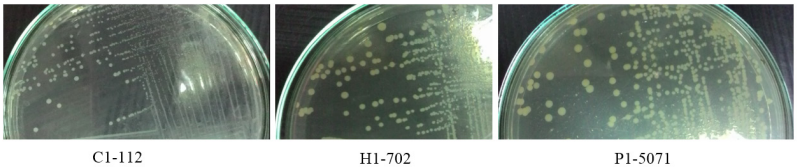


**Figure 1.** Colony of C1-112, H1-702 and P1-5071 in nutrient agar.

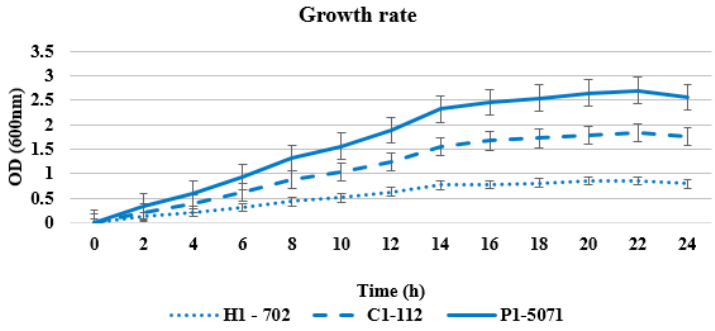


**Figure 2**. Growth curve of three rhizobacterial isolates.

The colony shape of three rhizobacterial isolates were circular, convex, and entire with white color and colony diameter was varied from 0.7 to 1.5 mm) (figure 1), and similar growth rate and the maximum growth rate at 22 hours (figure 2) were observed. H1-701 was rod shape and gram negative while others were coccus and gram positive bacteria (table 1).

**Table 1.** Properties of three rhizobacterialisolates.

| Isolates | Colony Shape | Colony color | Colony diameter | Bacterial shape | Gram stain | Sampling location |
|---|---|---|---|---|---|---|
| H1-702 | Circular Convex Entire | white | 1.5 mm | Rod | - | Pak Chong-Nakhon Ratchasima-Thailand |
| C1-112 | Circular Convex Entire | white | 1.2 mm | Coccus | + | Chulapbron Dam-Thailand |
| P1-5071 | Circular Convex Entire | white | 0.7 mm | Coccus | + | Muang- Nakhon Ratchasima-Thailand |

### 3.2 Seed germination method

The length of root, shoot, and number of root hairs and percentage of germination in seed soaking treatments were significantly higher than bacterial nutrient media and bacterial supernatant treatment. It can be concluded that bacterial nutrient media inhibited the seed germination of *I. aquatica* (Figure 3).

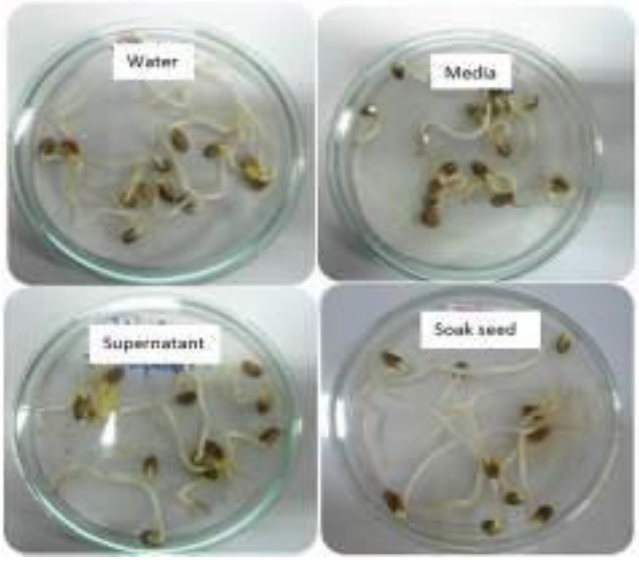


**Figure 3.** Seed germination treated with water, culture media, supernatant and soaked in bacterial supernatant.

The length of root in soaked seed was 4.88 cm comparing to water 4.49 cm, there was no significant difference among these two treatment. However, it was twice higher than those from the treatment of bacterial media (with just 2.14 cm) and 1.6 times higher than the treatment supernatant (2.96 cm) (Figure 4).

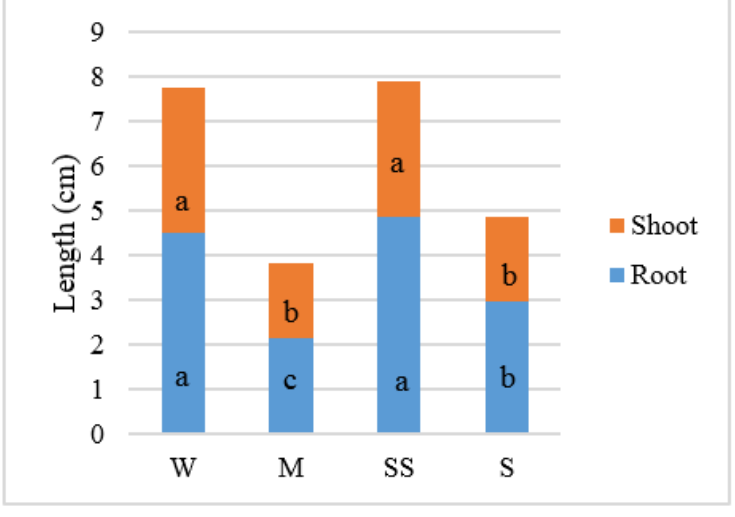


**Figure 4.** Root and shoot length after 3 days germination. C: control, M: Media, S: supernatant, SS: soaked seed.

The shoot length and number of root hair were the highest in soaked seed treatment with 3.01 and 19.26 cm, respectively, and the lowest number was found in bacterial media treatment with shoot length of 1.67 cm and root hair number of 1.47 (Figure 5).

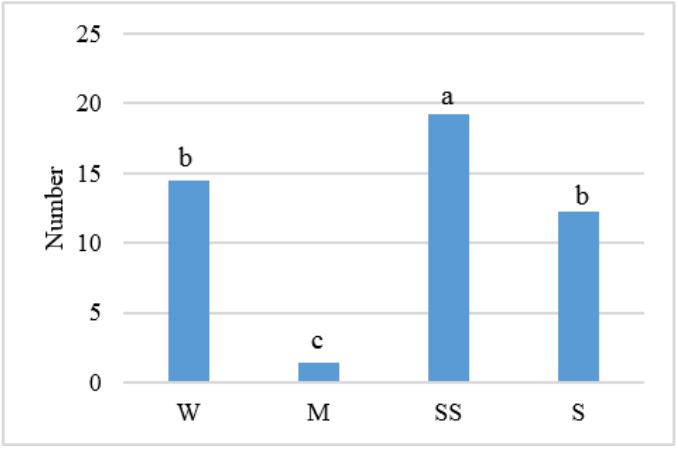


**Figure 5.** Number of root hair after 3 days germination.C: control, M: Media, S: supernatant, SS: soaked seed.

PG value was variety among the treatment, soaked seed got the highest PG with 90%, following by water (76.7%) and supernatant 70 % while the lowest PG belonged to media treatment with 50% (Figure 6).

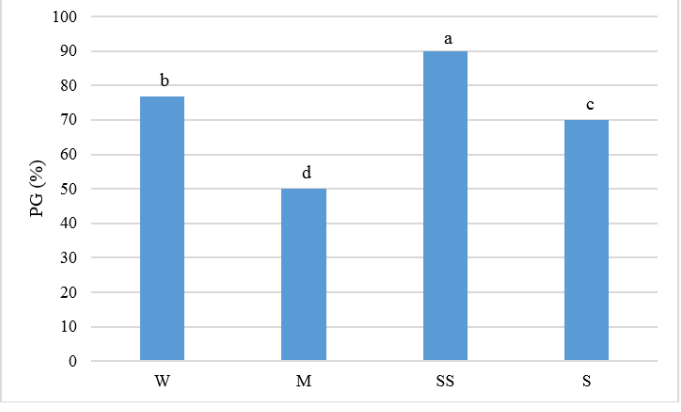


**Figure 6.** Percentage of germination (PG) of *I. aquatica* after 3 days. C: control, M: Media, S: supernatant, SS: soaked seed.

According to Roychowdhury (2012), germination percentage of floricultural was decreased when the used plant growth regulators increasing concentrations because the lower concentration of growth regulators favors the increased enzymatic activity which leads to the favorable environment for the germination as well as the growth of the radical and plumule.

This result was similar to the study of Vrbničanin (2011), seed germination varied depending on bacterial media, germination was inhibited by bacterial treatments Pseudomonas fluorescens and

*Bacillus licheniformis*. Nutrient media comprises Soya Peptone and Beef extract containing nitrogen in inorganic form as $NO_3^-$ or $NH_4^+$ and organic form as polypeptides or amino acids. Dijk and Eck (1995) reported that some species cannot use inorganic nitrogen, when the requirements for amino compounds are most probably satisfied by transport of these substances with a symbiotic fungus. Moreover, most amino acids act as inhibitor or do not increase the growth of seedling, only arginine and aspartic acid support growth (Mitra, 1989). Therefore *I. aquatic* must be soaked 60 minutes in the bacterial supernatant to absorb the stimulation hormone such as auxin from bacteria secretion.

**3.3. Seed germination of *I. aquatica***

The results showed that three rhizosphere bacterial isolates could stimulate the growth of *I. aquatica* (Figure 7).


**Figure 7.** *I. aquatica* seeds in all treatments after 5 days germination.

The overall strend was that the root length of all treaments incresed slighly from day three to day five of germination. In day three, the root length of P-H (1.40 cm) and C-P (1.50 cm) lower than the control (1.59 cm) while highest root length was in the treatment C-H-P (2.1 cm) and there were no significant difference among other treaments. However, the highest root length in day five was in H1-702 (2.40 cm), following by C1-112 (2.27 cm) and the control treament, 1.72 cm. The root length inceased slighly in H1-702 from 1.8 cm in day three to 2.40 cm in day five (Table 2).

There shoot length was no significantly different in the treatment H1-702 at day three with 3.23 cm comparing to control (3.11 cm). However, in day four and day five, the higest shoot length belonged to C1-112 with 4.12 cm and 4.52 cm, respestively, following by H1- 702 (4.35 cm in day five). The length of shoot of the control treatment in day five was 4.34 cm and there were not significantly diference among all treatments, excepted P-H which the lowest shoot length found from three germination days, 1.9 cm, 2.6 cm, and 2.8 cm in day three, four and five, respectively (Table 2).

**Table 2.** Seed germination of *I. aquatica*

| Treatments | ROOT (cm) | | | SHOOT (cm) | | | ROOT HAIR NUMBER | | | PG (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 3 | Day 4 | Day 5 | Day 3 | Day 4 | Day 5 | Day 3 | Day 4 | Day 5 | |
| Control | $1.59^{ab}$ | $1.65^{bc}$ | $1.73^{cd}$ | $3.11^{ab}$ | $3.57^{abc}$ | $4.34^{a}$ | $12.48^{ab}$ | $16.38^{bc}$ | $17.63^{b}$ | $80^{ns}$ |
| H1-702 | $1.80^{ab}$ | $2.21^{a}$ | $2.40^{a}$ | $3.23^{a}$ | $3.78^{ab}$ | $4.35^{a}$ | $13.45^{ab}$ | $21.19^{a}$ | $22.05^{a}$ | $83^{ns}$ |
| C1-112 | $1.85^{ab}$ | $1.97^{ab}$ | $2.27^{ab}$ | $3.17^{ab}$ | $4.15^{a}$ | $4.52^{a}$ | $16.03^{a}$ | $19.64^{ab}$ | $20.35^{ab}$ | $83^{ns}$ |
| P1-5071 | $1.65^{ab}$ | $1.65^{bc}$ | $1.83^{bcd}$ | $2.65^{ab}$ | $3.64^{abc}$ | $3.88^{a}$ | $12.37^{ab}$ | $18.16^{abc}$ | $20.31^{ab}$ | $80^{ns}$ |
| C-P | $1.50^{b}$ | $1.89^{ab}$ | $1.90^{bcd}$ | $2.30^{cd}$ | $3.10^{bc}$ | $3.41^{ab}$ | $8.60^{b}$ | $18.24^{abc}$ | $19.26^{ab}$ | $83^{ns}$ |
| P-H | $1.40^{b}$ | $1.41^{c}$ | $1.56^{d}$ | $1.91^{d}$ | $2.68^{c}$ | $2.89^{b}$ | $12.35^{ab}$ | $15.63^{c}$ | $19.00^{ab}$ | $86^{ns}$ |
| C-H | $1.82^{ab}$ | $1.98^{ab}$ | $2.00^{abcd}$ | $2.55^{bc}$ | $3.47^{abc}$ | $4.12^{a}$ | $12.75^{ab}$ | $19.99^{a}$ | $21.14^{ab}$ | $76^{ns}$ |
| C_H_P | $2.11^{a}$ | $2.15^{a}$ | $2.20^{abc}$ | $2.91^{ab}$ | $3.78^{ab}$ | $4.03^{a}$ | $14.39^{a}$ | $21.12^{a}$ | $22.10^{a}$ | $70^{ns}$ |

Note. The everage values with the same letter in each column were not significantly different at the 95% confidence level

The number of root hairs increased sharply from day two to day three and increased slower in day five, one typical example, the root hair number in treament H1-702 was 16.03, 21.19 and 22.05 in three continuous observation days. In day five, the root hair numbers of all treatments were significantly higher than the control treament which had 17.63 root hairs (Table 2).

In germination percentage, the results showed that three treatments accounting for 83% were H1-702, C1-112 and C-P which were higher than the control (80%). Germination percentage of P1-6071 was also 80%. The highest PG belonged to P-H treatment (86%). Comparing to control treatment, C-H and C-H-P had lower PG, 76% and 70%, respectively (Figure 8).

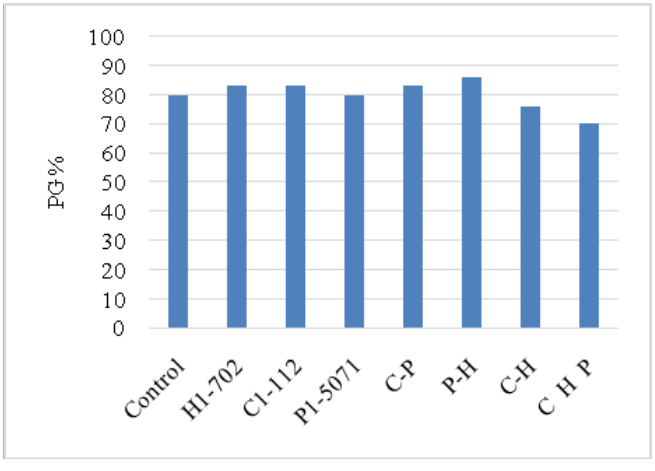


**Figure 8.** Percentage of germination (PG) in *I.* aquatica seed at day 5.

These results was similar to the results of Sachevet et al. (2009), the root and shoot length of wheat seedlings were significantsignificantly increase in the treatmentinoculated with *Klebsiella* supernatant comparing to the control.

Babalola (2007) also demonstrated that there were some potential in certain rhizobacteria could stimulate the germination of Strigahermonthica in vitro experiment. Furthermore, all isolated bacteria could produce IAA and Gibberellic acid which the key regulators to seed germination (Davies, 1987) during their growth.

## 4. Conclusion and suggestion

### 4.1 Conclusion

Three rhizobacterial isolate as single inoculum (H1-702, C1-112 and P1-5071) which produced IAA and plant hormones could stimulate the germination of *I. aquatica* seed, increased the length of root and shoot and the number of root hairs. The treatment co-inoculum of two isolates were low effect on seed germination, especially P-H treatment had the lowest among all investigated data. Seed germination was inhibited by the medium while the soak seed with bacterial suspension treatments were better choice because of its highest stimulation in I. aquaticgermination.

### 4.2. Suggestion

More studies should be done to identify rhizobacteria using analysis of 16s rRNA gene,and the potential of applying these isolates as the biofertilizer are needed for future study in pot experiment.

## 5. Acknowledgement

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## 6. References

1. Babalola, O.O., Berner D.K., and Amusa, N.A. 2007. Evaluation of some bacterial isolates as germination stimulants of Strigahermonthica African Journal of Agricultural Research Vol. 2(1), pp 027-032.
2. Davies, P.J. 1987. The plant hormones: their nature, occurrence, and functions. In: Plant hormones and their role in plant growth and development.MartinusNijhoff Publishes, Dordrecht, pp. 1-11,Springer Netherlands, New York.
3. Dijk, E., Eck, N. 1995. Ammonium toxicity and nitrate response ofaxenically grown Dactylorhizain-carnate seedling. New Phytot 131, pp 361-367
4. Hari, M., Perumal K., 2010. Biofertilizer. Shri AMM MurugappaChettiar Research Centre Taramani. Chiennai-6000113, 4-5.
5. Jampeetong, A., Brix, H., Kantawanichkul, S. 2012. Effects of inorganic nitrogen forms on growth, morphology, nitrogen uptake capacity and nutrient allocation of four tropical aquatic macrophytes *(Salviniacucullata, Ipomoea aquatica, Cyperusinvolu-cratus and Vetiveriazizanioides).* Aquatic Botany 97,pp 10-16.

6. Karthikeyan, B., Abdul J.C., Lakshmanan G.M.A., M. Deiveekasundaram, M. 2008. Studies on rhizosphere microbial diversity of some commercially important medicinal plants. Colloids and Surfaces B: Biointerfaces 62, pp 143-145.
7. Khamwan, K., Akaracharanya, A., Chareonpornwattana, S., Choi, Y.E., Nakamura, T., Yamaguchi, Y., Sano, H., Shinmyo, A. 2003. Genetic transformation of water spinach *(Ipomoea aquatica)*. Plant Biotechnology, 20 (4), pp 335-338.
8. Mitra, G.C. 1989. Biology, conservation, and culture of orchids, in vitro culture of orchid seeds for obtaining seedlings. East – West Press pvt ltd. pp 401-409.
9. Monaliza, M.M.A., Newton, P.S., Carolina, E.R.S., Santos, A.D.S.F., Clayton A.S., Mário A., Lira, J. 2013. Effects of biofertilizer with diazotrophic bacteria and mycorrhizal fungi in soil attribute, cowpea nodulation yield and nutrient uptake in field conditions. Scientia Horticulturae 162, pp374-379.
10. Nantakorn, B., Neung, T., 2010. Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. European Journal of Soil Biology 47, pp 44-54.
11. Piromyou, P., Bancha, B., Piyada, T., Panlada, T., Nantakorn, B., Neung, T., 2010. Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. European Journal of Soil Biology 47, pp 44-54.
12. Roychowdhury, R., Ray, S., Umrao, V. K., and Tah, J. 2012. Comparative study for the effect of gibberellic acid, kinetin and indole 3-acetic acid on seed germination performance of *Dianthus caryophyllus.* Journal of advanced laboratory research in biology Vol 3(3),pp 166-170.
13. Sachev, D.P., Chaudhari, H.G., Kasture, V.M., Dhavale, D.D. and Chopade, B.A. 2009. Isolation and characterization of indole acetic acid (IAA) producing *Klebsiella pneumonia* strains from rhizophere of wheat (*triticumaestivum* and their effect on plant growth. Indian Journal of Experimental Biology, 47, pp 993-1000.
14. Shahab, S., Ahmed, N. and Khan, N.S. 2009. Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. African Journal of Agricultural Research 4 (11), pp 1312-1316.

15. Somkiat Kham-iam, S., Kanalerk, C., Soonthornpat, S., Boonkerd, N., Santadwoo, M. 2005. Compost. National Bureau of Agricultural Commodity and Food Standards Ministry of Agriculture and Cooperatives. ICS 65.080 ISBN 947-403-339.
16. Sritongon, K. 2015. Screening of plant growth promoting rhizobacteria from rhizosphere for improving growth of Jerusalem artichoke (*Helianthus tuberosus* L.). A Thesis, Degree of Master of Science in Microbiology, Khon Kaen Univeristy, Thailand: 105 p
17. Vrbničanin, S., Božić. D., Sarić, M., DanijelaPavlović, D. and Raičević, V. 2011. Effect of Plant Growth Promoting Rhizobacteria on Ambrosia artemisiifolia L. Seed Germination. Pestic. Phytomed. (Belgrade), 26(2), pp 141-146.
18. Wargovich, M.J. 2000. Anticancer properties of fruits and vegetables. Hort Science, 35 (4), pp 573-575.
19. Zhang, Q.,Achal, V., Xu, Y., Xiang, W. N. 2014. Aquaculture wastewater quality improvement by water spinach *(Ipomoea aquatica Forsskal)* floating bed and ecological benefitassessment in ecological agriculture district. Aquacultural Engineering 60: pp 48-55.