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Research Article

Synergetic Effect of Microbially Fermented Soybean and NPK Fertilizer on Plant Growth of Grand Naine Banana

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Abstract

Soybeans (*Glycine max* L.) have been a significant source of plant origin proteins for both the livestock feed and humans for many years. For agriculture, organic farming is more effective tool that be done by using natural sources like soybean seeds which helps in the supplement of Nitrogen. Present study was aimed to find the combined effect of microbial fermented soy meal and NPK fertilizers. *Bacillus subtilis* isolated from soil was used for the fermentation of soy bean meal. The fermented soy bean meal was used for the growth of grand naine banana along with NPK fertilizer. The findings showed increased growth observed (17cm) in fermented soy bean along with NPK fertilizers when compared to the normal (12 cm) and NPK fertilizer (15 cm). Further, there was no change in the pH of the soil and no deficiency was noted in the nutrients. It may be concluded that *Bacillus subtilis* fermented Soy bean may be used for organic forming along with NPK fertilizers for the growth of grand naine banana.

Keywords: Soybeans, organic farming, *Bacillus subtilis*, grand naine banana, nitrogen content.

Introduction

Organic agriculture is one of the fastest growing segments of agriculture today. In 2015, domestic sales of organic products topped $7.8 billion, with fresh produce the top-selling organic category. The growing popularity of organically grown foods has generated new market opportunities for both wholesale and direct-market organic produce farmers. Soybean (*Glycine max* L.) is one of the most important pulse crops in the world. It has a dual benefit of supplying about 43.3% protein and 19.5% oil hence termed as "Miracle bean". Soybean is indigenous to China and was introduced to India in 1950's (Mukherjee *et al.*, 2016). In India, the area and productivity have been rapidly increasing over the recent years. In Tamil Nadu, it is cultivated as an irrigated crop in an area of 31,000 ha with the annual production crop of 8000 metric tonnes. Soya protein is the only vegetable source of complete protein comparable to meat and egg, which contains all the essential amino acids required by human, animals and plants. Nitrogen is one of the major important nutrients essential for plant growth. The economic and environment importance of legume crops is largely due to their ability to fix atmospheric nitrogen in a symbiosis with specific bacteria *Rhizobium*. Like most legumes, soybeans performs N_2 fixation by establishing a symbiotic relationship with the rhizobia.

Bradyrhizobium japonicum is a slow growing root nodule symbiont, which is widely used as an inoculant in soybean fields throughout the world. Generally, soybean inoculated with *B. japonicum* forms highly effective nodules and frequently increased soybean yields, especially in fields where soybeans are cultivated for the first time (Chen *et al.*, 2002). Soybean meal is the most popular protein source in the animal feed industry because of its high protein content and wide availability (Baker, 2000). Unfortunately, the use of soybean meal in animal diets was primarily limited to adult animals due to the inefficient digestibility of soy proteins by young animals and the susceptibility of young animals to anti-nutritional compounds in soybeans that are either not properly processed or undercooked. These anti-nutritional compounds include trypsin inhibitors, lectins, flatulence producing compounds, and many other allergenic proteins. Fermented soybean meal can successfully replace animal-derived protein sources such as plasma protein and dried skim milk in piglet nursery diets without adversely affecting the growth performance of the piglets (Kim *et al.*, 2009). Fermented soybeans are not only highly digestible and but also nutritious contain calcium, vitamin A and B. Since fermentation can vastly improve the palatability of soy proteins along with increasing its digestibility, it is a very promising processing method for the industry.

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Fermented soybean meal also contains live microorganisms that are beneficial (Kim *et al*., 2009). Fermentation of soybean meal using several *Bacillus* spp. has increased digestibility of soy proteins as well (Kiers *et al*., 2003). Kiers *et al*. (2003) also found that complete breakdown of 3 subunits from β-conglycinin and both polypeptides from glycinin occurred after fermentation with *Bacillus subtilis*. Feng *et al*. (2007) also found that fermentation improved the nutritional value of soybean meal and reduced or eliminated some important anti-nutritional factors, such as glycinin and β-conglycinin. Active trypsin inhibitors have been shown to be liberated from a heat-resistant, inactive, bound form during fermentation by *R. oligosporus* proteases; however, this trypsin inhibitor was readily inactivated by heat. Soybean is a well-known nitrogen fixer and has been a model plant for the study of biological nitrogen fixation (BNF). Its importance in BNF led to the genome sequencing of soybean; details of the soybean genome are available at soybase.org (*G. max* and *G. soja* sequences are available at NCBI as well). Keeping the above facts in view, this study was aimed to find the combined effect of microbial fermented soy meal and NPK fertilizers.

Materials and methods

Collection of damaged soyabean seeds: The damaged soybeans were collected in the local departmental store, Vaniyambadi, Tamil Nadu, India. The collected soybeans were light brown in colour.

Sample preparation: The sample preparation of collected damaged soybeans was done by two methods.

1. Roasted soybeans (RS): The damaged soybeans were roasted for Nitrogen estimation.
2. Soaked soybeans (SS): The damaged soybeans were soaked overnight for Nitrogen estimation.

Nitrogen estimation was done by Microkjeldahl method.

Isolation of Bacillus sp.: The soil sample was aseptically collected in a sterile container from Tirupattur, Vellore district, Tamil Nadu, India. Both dilution plate and enrichment method were used for isolation of *Bacillus* sp. from soil sample. For the enrichment method, 1 mL of soil sample was subjected to heat treatment for 10 minutes at 80°C in a water bath in order to kill most of the vegetative cells and thus to eliminate non-spore forming bacteria (Mora *et al.*, 1998). After heat treatment, the samples were transferred into 100 mL of skim milk agar medium. Incubation was performed in a rotary shaker at 50°C until turbidity obtained. Then, 500 µL of the broth was platted on skim milk agar medium. For the dilution plate method, 1 g of sample was transferred in 9 mL of 0.85% saline water. After pasteurization at 80°C for 10 minutes, 1 mL aliquot from each of the samples was transferred in 9 mL of 0.85% saline water and 6 fold dilutions were prepared. One mL of dilutions was platted on Skim milk agar plates and incubated for 48-72 h at 37°C. Single colonies with different morphologies were picked and purified using streak plate method. The *Bacillus* isolates were observed under the microscope, the colony morphology was noted with respect to colour, size, shape and nature of colony. Identification of the *Bacillus* strain were carried out by the routine bacteriological methods by examining the colony morphology, Gram staining, motility test and biochemical tests.

Fermentation of soybean by Bacillus: The soybean which had higher nitrogen content was selected for the fermentation process using *Bacillus* for a period of 10 d. The soaked soybean were directly taken for fermentation while some dried soybeans were ground into powder form after soaking. They were covered, sealed and kept undisturbed for 10 d. Post-fermentation was done after 10 d, the sealed covers were opened and the fermented mixture were tested for the nitrogen content by Micro Kjeldahl method as before.

Experiment with grand naine banana plants using Bacillus fermented soybean as organic fertilizer: Ten banana plants were selected from green house in Genewin Biotech and categorized as Control (c1-10), Standard (s1-10) and Test (T1-10). Initial readings such as length and number of leaves were noted for each plant and the application of fertilizers were done twice a week for a period of a month and the parameters were noted. Control plants were treated with water alone; standard plants were treated with inorganic chemicals whereas test plants were treated with organic fertilizer (soybean) and observed for the difference. The nitrogen content of the leaves of banana plant treated with NPK fertilizer and *Bacillus* sp. fermented soybean after treatment was also determined.

Results and discussion

Estimation of nitrogen content in damaged soybean: Nitrogen content in solid (roasted soybean) sample was 8.68% whereas, nitrogen content in liquid (soaked soybean) sample was 17.64%. It was observed that the nitrogen content was maximum in the soaked soybean when compared to roasted soybean. The reduction of nitrogen percentage in roasted soybean may be due to the heat since heating may interfere with the nitrogen content in the soybean. Increased nitrogen content of soaked soybean may be due to the interaction of soybean with the moisture content due to the presence of water or automatic fermentation by the normal microbial flora. The characteristic of isolated *Bacillus subtilis* strain is shown in Table 1.

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Table 1. Characterization of *Bacillus subtilis* isolated from soil sample.

Test	Results
Gram staining	Gram positive, thick, short rods
Endospore	Central spores present
Motility	Non-motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Large, circular, white, adherent, colonies with membranous growth
MacConkey agar	Non-lactose fermenting colonies
Glucose fermentation	Acid produced
Mannitol fermentation	Acid produced
Sucrose fermentation	Not fermented
Dextrose fermentation	Not fermented
Indole	Negative
Methyl red test	Negative
Voges Proskauer test	Positive
Nitrate reduction	Positive
Gelatin hydrolysis	Positive
Starch hydrolysis	Positive
Urease	Negative

Members of the genus *Bacillus* are heterogeneous and they are very versatile in their adaptability to the environment. The colony morphology of isolated *Bacillus subtilis* strain is shown in Fig. 1 and its microscopic characteristics are shown in Fig. 2. There are various factors that influence the nature of their metabolic processes and enzymes produced. The most important factor in having a product ideal for fermentation is the heat treatment given to soy proteins at any stage during its preparation before inoculation with the organisms for fermentation at 100°C for 20 minutes (Patel *et al*., 1980). Fermentation of soybean meal using several *Bacillus* spp. has increased digestibility of soy proteins as well (Kiers *et al*., 2003). Kiers *et al*. (2003) also found that complete breakdown of 3 subunits from β-conglycinin and both polypeptides from glycinin occurred after fermentation with *Bacillus subtilis*. Feng *et al*. (2007) also found that fermentation improved the nutritional value of soybean meal and reduced or eliminated some important anti-nutritional factors, such as glycinin and β-conglycinin.

Number of leaves and height of Naine banana plant in Control treatment during initial stage: The effect of Control treatment (without fertilizer) on number of leaves and height of Naine banana plants (10 plants) were studied and the results were furnished in Table 2. It was observed that the maximum number of leaves (5 leaves) and height (13.2 cm) was recorded in C1. Least number of leaves (3 leaves) and plant height (10 cm) was recorded in the C2, C6 and C8.

Number of leaves and height of Naine banana plant in Standard (NPK) treatment during initial stage: The effect of Standard treatment (NPK fertilizer) on number of leaves and height of Naine banana plants (10 plants) were tested and the findings were presented in Table 3.

Fig. 1. Colony morphology of isolated *Bacillus subtilis* in nutrient agar.

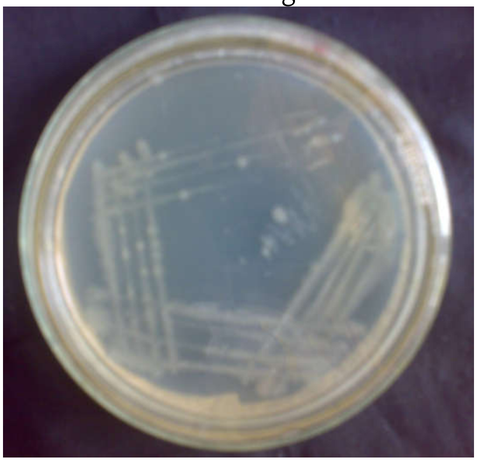

Fig. 2. *Bacillus subtilis* showing gram positive violet rods in Gram staining.

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Maximum number of leaves (7 leaves) and height (15 cm) was noticed in S1. Least number of leaves (3 leaves) and plant height (12.3 cm) was recorded in the S2 and S3.

Number of leaves and height of Naine banana plant in Test (Bacillus subtilis fermented soybean) treatment during initial stage: The effect of Test treatment (*Bacillus subtilis* fermented soybean) on number of leaves and height of Naine banana plants (10 plants) was studied and the results were given in Table 4. It was observed that the maximum number of leaves (7 leaves) and height (14 cm) was recorded in T1. Least number of leaves (3 leaves) and plant height (12 cm) was recorded in the T2, T6, T7 and T10.

Number of leaves and height of Naine banana plant in Control treatment after 10 d: The effect of Control treatment (Without fertilizer) on number of leaves and height of Naine banana plants (10 plants) after 10 d was tested and the findings were showed in Table 5. Maximum number of leaves (8 leaves and new shoot) and height (15 cm) was noticed in C1. Least number of leaves (3 leaves) and plant height (12 cm) was recorded in C6, C7 and C8.

Number of leaves and height of Naine banana plant in NPK fertilizer treatment after 10 d: The effect of NPK fertilizer treatment at 19:19:19 ratio on number of leaves and height of Naine banana plants (10 plants) was tested and the findings were tabulated in Table 6. Maximum number of leaves (10 leaves and new shoot) and height (17.7 cm) was noticed in S1. Least number of leaves (4 leaves) and plant height (12.2 cm) was recorded in the T2, T4 and T6.

Number of leaves and height of plant in Bacillus subtilis fermented Soybean and NPK fertilizer treatment after 10 d: The effect of *Bacillus subtilis* fermented Soybean and Phosphorous, Potassium fertilizer treatment (0:19:19) on number of leaves and height of Naine banana plants (10 plants) was tested and the findings were furnished in Table 7. Maximum number of leaves (8 leaves and new shoot) and height (16.5 cm) was noticed in T1. Least number of leaves (4 leaves) and plant height (11.8 cm) was recorded in the T2, T5 and T7.

Nitrogen estimation of the leaves of banana plant treated with NPK fertilizer and Bacillus subtilis fermented soybean: The nitrogen content of the leaves of banana plant treated with NPK fertilizer and *Bacillus subtilis* fermented soybean after treatment was determined. Nitrogen content in leaves of the *Bacillus subtilis* fermented Soybean treated plants recorded 14.28% whereas nitrogen content in leaves of the NPK treated plants was 9.94%. Thus, the nitrogen content of the plants treated with *Bacillus subtilis* fermented Soybean was higher than the plants treated with chemical fertilizers.

Table 2. Number of leaves and height of Naine banana plants in Control treatment during initial stage.

Control plant	Number of leaves	Height of the plant (cm)
C1	5	13.2
C2	3	10
C3	4	12.2
C4	4	12.4
C5	4	12.3
C6	3	10
C7	4	12.2
C8	4	10
C9	3	12.4
C10	4	12.3

Table 3. Number of leaves and height of Naine banana plants in Standard treatment (NPK) during initial stage.

Control plant	Number of leaves	Height of the plant (cm)
S1	7	15
S2	3	12.3
S3	3	12.3
S4	4	12.4
S5	5	12.6
S6	4	12.5
S7	4	12.4
S8	5	12.7
S9	4	12.5
S10	5	12.6

Table 4. No. of leaves and height of Naine banana plants in Test (*B. subtilis* fermented soybean) treatment during initial stage.

Control plant	Number of leaves	Height of the plant (cm)
T1	7	14
T2	4	12.8
T3	4	12.9
T4	4	12.8
T5	4	12.8
T6	3	12.0
T7	3	12.0
T8	4	12.6
T9	4	12.8
T10	3	12.0

Table 5. Number of leaves and height of Naine banana plants in Control treatment after 10 d.

Control plant	Number of leaves	Height of the plant (cm)
C1	8 + new shoot	15
C2	4	12.3
C3	5	12.7
C4	4	12.5
C5	5	12.4
C6	3	12
C7	3	12
C8	3	12
C9	5	12.9
C10	4 + new shoot	12.6

*Corresponding author

Table 6. Number of leaves and height of Naine banana plants in Standard treatment (NPK) after 10 d.

Control plant	Number of leaves	Height of the plant (cm)
S1	10 + new shoot	17.7
S2	4	12.2
S3	5	12.9
S4	4	12.2
S5	5	13.2
S6	4	12.2
S7	5	13.2
S8	5	13.3
S9	5	13.5
S10	5	12.9

Table 7. No. of leaves and height of Naine banana plants in Test (*B. subtilis* fermented soybean) treatment after 10 d.

Control plant	Number of leaves	Height of the plant (cm)
T1	8 + new shoot	16.5
T2	4	11.8
T3	5	14.1
T4	5 + new shoot	13.5
T5	4	11.8
T6	5	13.7
T7	4	11.8
T8	5 + new shoot	14.5
T9	5	13.8
T10	5 + new shoot	14.6

Conclusion

For agriculture, organic farming is more effective which can be done by usage of natural sources like soybean seeds which helps in the supplement of nitrogen source to plants. We have proven that there is a sharp increase in the growth of the plants when compared to the treatment using chemical fertilizers. Organic farming doesn't alter the pH of the soil, does not cause any deficiency in the nutrients and also avoids the pest attack. Organic fertilizers are easily absorbed by the plants compared to chemical fertilizers. It is a very cheap source and environmental friendly technique helps in increasing the yield of the plants. From this study, it may be concluded that the nitrogen content of the plants treated with *Bacillus subtilis* fermented Soybean was higher than the plants treated with Chemical fertilizers.

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References

1. Baker, D.H. 2000. Nutritional constraints to use of soy products by animal. Soy in Animal Nutrition. Drackley, J. K., eds. Federation Animal Science Society, Savory, IL, pp.1-12.
2. Chen, L.S., Figueredo, A, Villani, H., Michajluk, J. and Hungria, M. 2002. Diversity and symbiotic effectiveness of rhizobia isolated from field-grown soybean nodules in Paraguay. *Biol. Fert. Soil.* 35: 448-457.
3. Duvick, D.N. 2005. The contribution of breeding to yield advances in maize (*Zea mays* L.). *Adv. Agron.* 86: 83-145.
4. Economic Research Service. 2011. Fertilizer use and price. Retrieved from: Economic Research Service.
5. Feng, J., Liu, X., Xu, Z.R., Lu, Y.P. and Liu, Y.Y. 2007. The effect of *Aspergillus oryzae* fermented soybean meal on growth performance, digestibility of dietary components and activities of intestinal enzymes in weaned piglets. *Anim. Feed Sci. Tech.* 134: 295-303.
6. Grassini, P., Specht, J.E., Ciampitti, M. and Cassman, I.K.G. 2015. High-yield maize B soybean cropping systems in the US corn belt, in: Sadras, V.O., Calderini, D.F. (Eds), Cropphysiology-applications for genetic improvement and agronomy (2nd edition), *Elsevier, The Netherlands,* pp.17-41.
7. Jamtgard, S., Nasholm, T. and Huss-Danell, K. 2010. Nitrogen compounds in soil solutions of agricultural land. *Soil Biol. Chem.* 42: 2325-2330.
8. Kiers, J.L., Meijer, J.C., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. and Meulen, J. 2003. Effect of fermented soya beans on diarrheoa and feed efficiency in weaned piglets. *J. Appl. Micro.* 95: 545-555.
9. Kiers, J.L., Meijer, J.C., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. and Meulen, J. 2003. Effect of fermented soya beans on diarrheoa and feed efficiency in weaned piglets. *J. Appl. Microbiol.* 95: 545-555.
10. Kim, S.W., Van Heugten, E., Ji, F., Lee, C.H. and Mateo, R.D. 2009. Fermented soybean meal as a vegetable protein source for nursery pigs: I. Effects on growth and performance of nursery pigs. *J. Animal Sci. 87: (in press).*
11. Mora, D., Fortina, M.G., Nicastro, G., Parini, C. and Manachini, P.L. 1998. Genotypic characterization of thermophilic bacilli: A study on new soil isolates and several reference strains. *Res. Microbiol.* 149: 711-722.
12. Mukherjee, R., Chakraborty, R. and Dutta, A. 2016. Role of fermentation in improving nutritional quality of soybean meal—A Review. *Asian-Australas J. Anim. Sci.* 29(11): 1523-1529.
13. Patel, A.A., Waghmare, W.M. and Gupta, S.K. 1980. Lactic fermentation of soymilk: a review. *Proc. Biochem.* 15(7): 9-13.
14. Vinall, K., Schmidt, S., Brackin, R., Lakshmanan, P. and Robinson, N. 2012. Amino acids are a nitrogen source for sugarcane. *Funct. Plant Biol.* 39: 503-511.
15. Xu, G., Fan, X. and Miller, A. 2012. Plant nitrogen assimilation and use efficiency. *Ann. Rev. Plant Biol.* 63: 153-182.

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