Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 513-517
Received: 13-05-2018
Accepted: 18-06-2018

Shweta Kumari
Department of Soil Science, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

RC Yadav
Department of Microbiology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

MN Jha
Department of Soil Science, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

S Jha
Department of Soil Science, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India
Department of Soil Science, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Correspondence
Shweta Kumari
Department of Soil Science, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Effect of microbial inoculants and nitrogen levels on oil content and fatty acid composition in winged bean [*Psophocarpus tetragonolobus* L. (DC)] seed

Shweta Kumari, RC Yadav, MN Jha and S Jha

Abstract
A field experiment was carried out at research farm of Tirhut College of Agriculture, Dholi, Muzaffarpur, Bihar in year 2015-16 on sandy loam soil to study the effect of inoculation and nitrogen levels on oil content and fatty acid composition in matured seed of winged bean. The experiment was laid out in Randomized Block Design comprising twelve treatments with three replications. Effect of different treatment combinations had significant effect on oil content over control. Seed oil content obtained marked variations with application of PGPR inoculant and a decrease in oil content had been observed with use of higher dose of nitrogen. The maximum oil content (26.41%) was recorded in seed treatment with both *Rhizobium* and PGPR inoculants without use of nitrogen whereas, the maximum free fatty acid content (3.87%) was recorded in treatment where seeds were inoculated with *Rhizobium* and no application of nitrogenous fertilizer followed by 0 kg Nha^{-1} + PGPR inoculation (3.80%) and 20 kg Nha^{-1} + no inoculation (3.74). On the other hand, winged bean oil contained high amount of oleic acid (31.9 to 36.20%), linoleic acid (33.46 to 38.50%) of major unsaturated fatty acid. The highest amount of oleic (monounsaturated fatty acid) and linolenic acid were recorded with treatment of 40 kg Nha^{-1} + no inoculation (38.50%) and 0 kg Nha^{-1} + *Rhizobium* + PGPR inoculation (38.50%) respectively. Maximum saturated fatty acid content (29.51%) was recorded in treatment of 40 kg Nha^{-1} + PGPR, however minimum value (22.61%) was obtained with application of 40 kg Nha^{-1} and without inoculation of either PGPR or *Rhizobium*.

Keywords: winged bean, oil content, free fatty acid, oleic acid, linolenic acid

Introduction
Winged bean [*Psophocarpus tetragonolobus* (L.) DC] commonly called as Goa bean, asparagus bean, four cornered bean, princess bean etc. and in Hindi as "*Chaukonia Sem*". Winged bean pods are four cornered and rectangular or square in cross section, with wings at each corner leading to the popular name of winged bean. The crop is being utilized as a food resource in tropical areas, the various plant parts such as leaves, pods and tubers are edible. It is a source of rich dietary proteins and edible oil, thus have uses for pharmacological purposes (Annon, 1981). Compared to soybean oil, winged bean oil contained more long chain fatty acids and some linolenic acid (Homma *et al*., 1983) [6]. Winged bean seed can therefore be utilized as a source of edible oil and can substitute soybean oil if, it is produced commercially. The acceptability of the oil is expected to be more since, it has a pleasant sweet smell in contrast to bitter beanie flavor of soybean oil. Protein content range from 34.52 to 40.0% in seeds and 15.3 to 24.5% in tubers (Hafiz *et al*., 1984), oil content in seeds on dry weight basis ranges from 16.2 to 20.4% (Wijisundera and Chandra, 1983) [12], while long chain fatty acid content falls in the range of 22.0 to 27.0% of total fatty acids as estimated from gas chromatograph (Berry, 1985) [2]. Ekpenyong and Borchers (1980) [4] have studied physic-chemical properties including the fatty oil of Nigerian winged bean seeds. Major fatty acids were palmitic (10.90%), stearic acid (4.50%), oleic acid (37.10%), linoleic acid (19.0%), eicosenic acid (3.60%), behenic acid (18.50%) and lignoceric acid (4.20%). There is considerable interest in winged bean seeds because of their high nutitional quality mainly in terms of high protein and fatty oil content. Although, some preliminary work on chemical composition of winged bean seeds had been done (Mohanty *et al.*, 2015) [9], but there is no reported work on oil content and fatty acid composition of winged bean seeds of crop raised from seed treated with bio-inoculants and soil application of nitrogenous fertilizer. Because of possibilities of using the matured seeds as a source of edible oil, this study was undertaken to determine the effect of microbial inoculation and soil application of varying N levels on oil content and fatty acid composition in matured seed of winged bean.

Materials and Methods

A field experiment was carried out during 2015-16 in the experimental plots located in the research farm of Tirhut Collage of Agriculture, Dholi, Muzaffarpur, Bihar. It lies in Agro-climatic zone-I of North Bihar and is situated at 25º59' 38.81" N latitude, 85º35' 50.12" E longitude at an altitude of 52.0 metres above the mean sea level. The experiment was laid out in Randomized Block Design comprising twelve treatments *i.e.* T_1- Absolute Control (N_0I_0), T_2- 20kg Nha^{-1} + No inoculation (N_1I_0), T_3- 40kg Nha^{-1} + No inoculation (N_2I_0), T_4- 0kg Nha^{-1} + *Rhizobium* inoculation (N_0I_1), T_5- 20kg Nha^{-1} + *Rhizobium* inoculation (N_1I_1), T_6- 40kg Nha^{-1} + *Rhizobium* inoculation (N_2I_1), T_7- 0kg Nha^{-1} + PGPR inoculation (N_0I_2), T_8- 20kg Nha^{-1} + PGPR inoculation (N_1I_2), T_9- 40kg Nha^{-1} + PGPR inoculation (N_2I_2), T_{10}- 0kg Nha^{-1} + *Rhizobium* + PGPR inoculation (N_0I_3), T_{11}- 20kg Nha^{-1} + *Rhizobium* + PGPR inoculation (N_1I_3), T_{12}- 40kg N ha^{-1} + *Rhizobium* + PGPR inoculation (N_2I_3) with three replications. The tested crop was winged bean germplasm, IC 17002.

Soxhlet distillation technique (Sadasivam and Manickam, 1992) [10] was employed for the determination of oil content. Hexane was used as a solvent for extraction of oil and oil content was calculated on dry weight basis and reported as per cent. The protein content was calculated by multiplying the total nitrogen content of seed by conversion factor (6.25).

The oil samples were taken for methyl ester preparations using boron trifloride, methanol and alkali and separated in GLC (IUPAC, 1979) [8]. The oil samples were taken for esterification by adding 6 ml of 0.5 N methanolic sodium hydroxide solutions. Then, one ml from the top layer of heptane solution was transferred after esterification into a test tube with a round glass neck. A pinch of sodium chloride was added to avoid frothing during shaking. A clear separate heptane layer appears at the top of the mixture and a micro mL volume from thin layer was used for oil profiling using Gas Liquid Chromatograph (DGHS, Gov. of India, 2005). Gas- Chromatograph (NUCON GC 5765) fitted with Flame Ionization Detector (FID), stainless steel column (1/8'' x 2m, *i.e.*, 2 mm, mess size 100-120) was used for analysis. Pure nitrogen was used as a carrier gas at a flow rate of 20 mL min^{-1}. Injector and detector temperature was adjusted to 230^{0}C and 240^{0}C, respectively while the oven temperature was programmed: initial temperature 120^{0}C which gradually increased at the rate 5^{0}C min^{-1} to attend final temperature 210^{0}C within 18 minutes. Peaks were identified on the basis of retention time against reference standard. Per cent fatty acid quantity was obtained by computer software integrated with the GLC. Free fatty acid content in oil was analyzed by the procedure described by Cox and Pearson, 1962. The acid value was calculated by multiplying free fatly content by a factor 1.99 and expressed in percentage.

Results and Discussion

Effect of different treatment combinations had significant effect on hexane extracted oil content over control showed in Fig. 1. Seed oil content showed marked variations with application of PGPR inoculant and the decreased in oil content had been observed with use of higher dose of nitrogen. The maximum oil content (26.41%) was recorded in seeds obtained from treatment of seed inoculation with both *Rhizobium* and PGPR inoculants and without use of nitrogen. The results clearly indicated that the increased quantity of winged bean seed oil was obtained with application of PGPR either alone or in combination of *Rhizobium*. The oil content of winged bean seed ranged from 16.03 to 26.41 per cent and the lowest seed oil content was recorded in control. Application of 0kg Nha^{-1} + *Rhizobium* + PGPR inoculation, maximum 64.75% increment in oil content was amounted over control. Crop fertilized with reduced quantity of nitrogen which affects the quantity of oil.

The effect of treatment combination on variation of protein content in seed is reflected in Fig. 1. The protein content in seed was ranged from 31.88 to 38.44 per cent. However, the higher protein content (38.44%) was recorded in seeds of treatment *viz*., soil application of 20kg Nha^{-1} + seed inoculation with *Rhizobium* + PGPR inoculation and it was found *at par* with treatment of 20kg Nha^{-1} + *Rhizobium* inoculation (37.37%) followed by 40kg Nha^{-1} + *Rhizobium* inoculation (36.75%) and 20kg Nha^{-1} + PGPR inoculation (36.32%). Protein content in seed was noticed to increase about 20 per cent in treatment of 20kg Nha^{-1} + *Rhizobium* + PGPR inoculation over control.

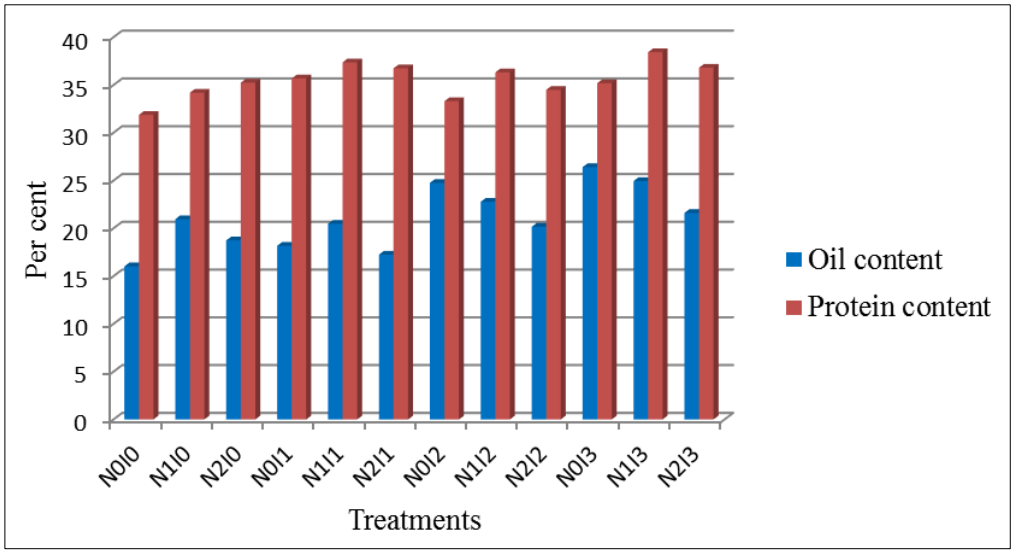

Fig 1: Effect of inoculants and nitrogen levels on oil and protein content in winged bean seed

Free fatty acid is an important quality parameter of edible oil and it has expressed in per cent of free fatty acids calculated as oleic acid. International specifications for top grade edible oil usually are set at 3 per cent free fatty acids for human consumption. The content of free fatty acid always is an increase in acidity with time during transport and storage

(Syam *et al*., 2009). Variations in free fatty acid content in percentage as influenced by effect of seed inoculation with different microbial inoculants and varying levels of nitrogen application is showed in Fig. 2.

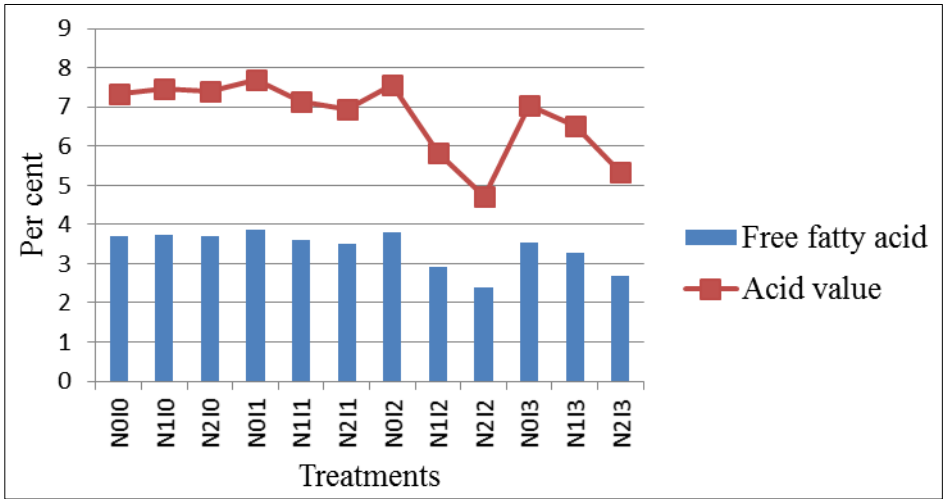

Fig 2: Effect of inoculants and nitrogen levels on free fatty acid and acid value content in winged bean seed

The maximum free fatty acid content (3.87%) recorded in treatment of seed inoculation with *Rhizobium* and soil application of 0kg Nha^{-1} followed by 0 kg Nha^{-1} + seed inoculation with PGPR (3.80%) and 20kg Nha^{-1} + no inoculation (3.74%) respectively. The lowest quantity of free fatty acid was noticed in 40kg Nha^{-1} + PGPR inoculation. Treatments *viz*., soil application of 40kg Nha^{-1} + PGPR, 40kg Nha^{-1} + *Rhizobium* + PGPR and 20kg Nha^{-1} + PGPR were recorded with less than 3 per cent free fatty acid indicating suitable for human consumption. Whereas, remaining treatments yielded more than 3 per cent free fatty acid making the oil unfit for human health point of view. Similar trend of results was also obtained with respect to acid value (%) in different treatment combinations.

Effect of inoculation and nitrogen levels on fatty acid composition (%) of wined bean seed oil is depicted in Fig. 3, 4 & 5. The winged bean seed oil contained behenic acid as a major saturated fatty acid, followed by palmitic, stearic, lignoceric and behenic acid. The content of palmitic and stearic acid in winged bean oil ranged between 9.72 to 7.50 per cent and 4.91 to 3.71 per cent respectively. Similar results have also been reported by Ekpenyong and Borchers (1980) [4]. The winged bean oil is nutritionally of slightly lower quality than soybean oil and it also contains higher amount of long chain saturated fatty acids. The neutral lipid separated from hexane extracted oil content has poor percentage of palmitic acid ranging from 7.5 to 9.7 per cent with the mean value 8.61 per cent (Fig. 3)**.** Lignoceric acid was found rather low in concentration than other saturated fatty acid.

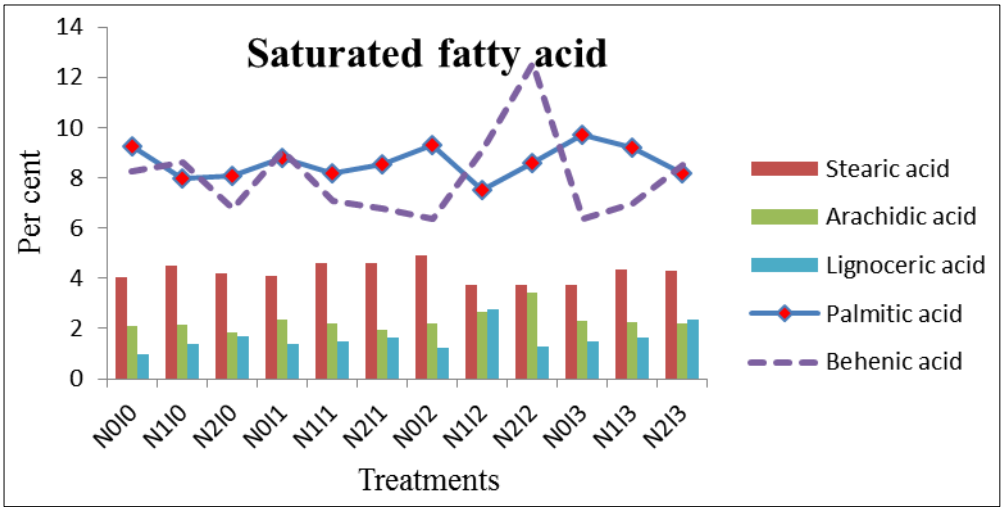

Fig 3. Effect of inoculants and nitrogen levels on saturated fatty acid in winged bean seed

On the other hand, winged bean oil contained high amount of oleic acid (31.9 to 36.20%), linoleic acid (33.46 to 38.50%) of major unsaturated fatty acid. The highest amount of oleic (monounsaturated fatty acid) and linolenic acid was recorded with treatment of soil application of 40kg Nha^{-1} + no inoculation (38.50%) and 0kg Nha^{-1} + *Rhizobium* + PGPR inoculation (38.50%). The linoleic acid content represented the large amount among all fatty acid profile as shown in Fig. 4 and 5. Comparing to the soybean oil, the winged bean oil has a very small amount of linolenic acid but has large amount of long chain fatty acid such as behenic acid. The major unsaturated fatty acid *viz*., behenic acid was found with wide differences in concentration ranging from 6.36 (0kg Nha^{-1} + PGPR inoculation) to 12.53 per cent (20kg Nha^{-1} + *Rhizobium* inoculation) with average value 8.04 per cent (Fig. 3).

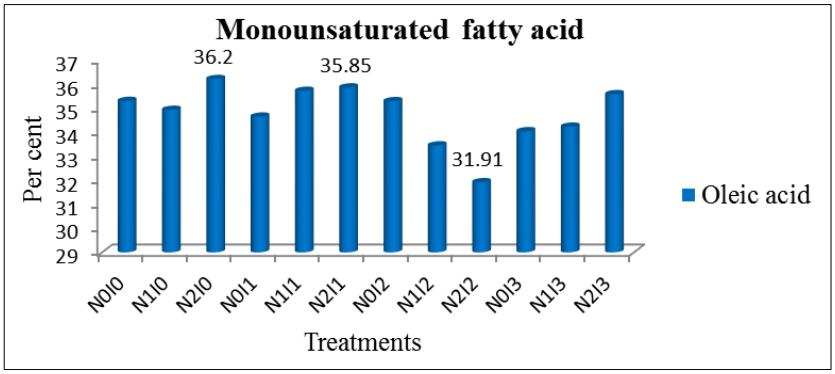

Fig 4: Effect of inoculants and nitrogen levels on monounsaturated fatty acid in winged bean seed

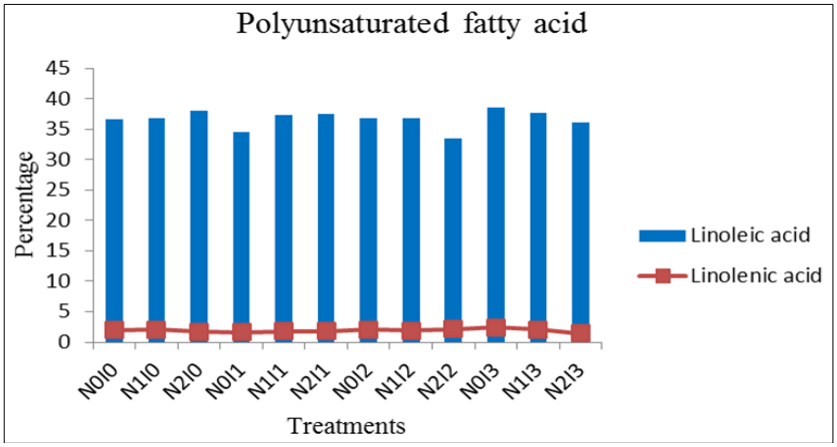

Fig 5: Effect of inoculants and nitrogen levels on polyunsaturated fatty acid in winged bean seed

The concentration of stearic and arachidic acid was found in range of 3.71 to 4.91 per cent. The amount of palmitic and stearic acid was observed to decrease. However, the level of behenic acid increases in matured winged bean seed (Hun-TeikKhor 1985). The high content of behenic acid in winged bean seed may reduce its digestibility besides to have all other desirable effects. The winged bean oil also have little amount of long chain fatty acids such as arachadic and lignoceric acid with average value of 2.30 and 1.61 per cent respectively as shown in Fig 3. In the present study, the erucic acid also was detected in few of the treatment samples of winged bean in fractional quantity as reported by Homma *et al*., 1983 [6] in soybean oil in small amount. Oleic acid was found highest (36.20%) followed by 35.85 per cent in winged bean seeds of treatment, soil application of 40kg Nha^{-1} + no seed inoculation and treatment, 40kg Nha^{-1} + *Rhizobium* inoculation. Lowest oleic acid (31.91%) was found in seeds of treatment, 40kg Nha^{-1} + PGPR inoculation (Fig. 4). Oleic acid content increased while linolenic acid decreased in the matured seeds of winged bean (Hun-TeikKhor, 1985). Considering the polyunsaturated fatty acid, the maximum quantity of linoleic acid (38.50%) was recorded in treatment of soil application of 0kg Nha^{-1} + *Rhizobium* + PGPR inoculation whereas, minimum content (33.46%) was found in 40kg Nha^{-1} + PGPR inoculation (Fig. 5). The winged bean seed oil contains approximately 73 per cent of unsaturated fatty acids (Mohanty *et al.*, 2015) [9]. The mean value of fatty acid concentration is shown in Fig. 6. Polyunsaturated fatty acid of winged bean seed irrespective of treatment contained highest amount of linoleic acid with average value of 36.71 per cent. However, the content of linolenic acid was quite low and it ranged from 1.48 to 2.38 per cent with mean value of 1.91 per cent.

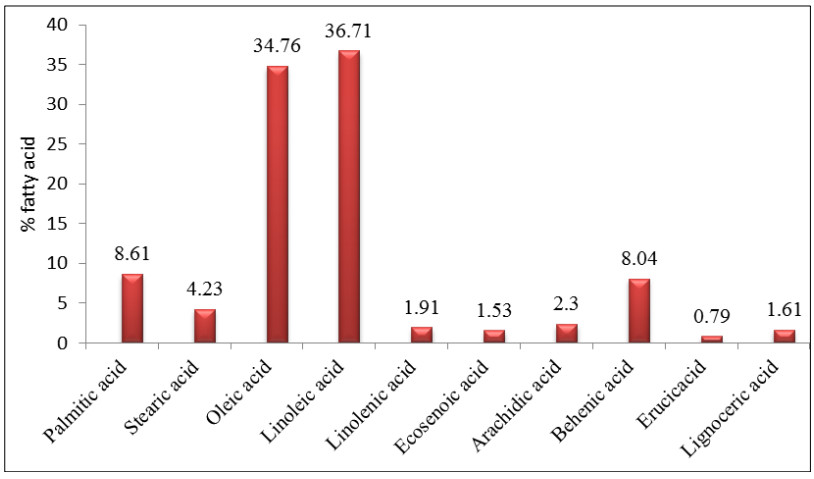

Fig 6: Effect of inoculants and nitrogen levels on average value of fatty acid in winged bean seed

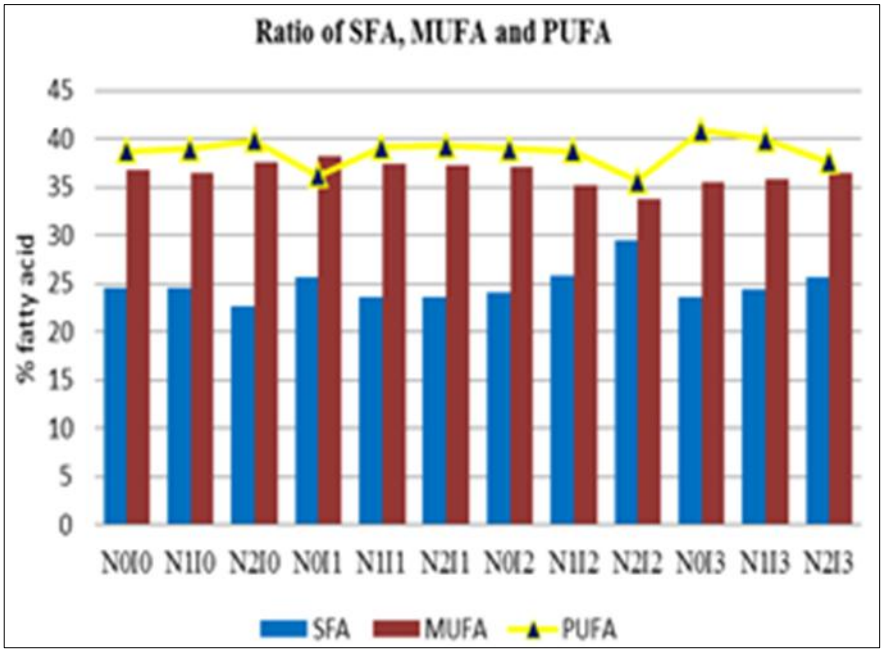

Fig 7: Effect of inoculants and nitrogen levels on ratio of fatty acid in winged bean seed oil

The essential fatty acid in winged bean seed was found rather in reduced quantity in comparison to soybean oil. Winged bean oil not only contains acceptable amount of unsaturated fatty acid but due to presence of low content of linolenic acid, it gives winged bean seed oil the greater stability.

The variations in quantity of saturated fatty acid were noticed in winged bean seeds with different treatment combinations. Maximum saturated fatty acid content (29.51%) was recorded in treatment, where soil application of 40kg Nha^{-1} + PGPR inoculation was done. However, minimum value (22.61%) was obtained with application of 40kg Nha^{-1} without seed inoculation (Fig. 7). Fig. 7 also reflects that about 36 per cent MUFA is present in winged bean seed oil. The range of monounsaturated fatty acid was recorded approximately 31 to 36 per cent in winged bean seed oil. The saturated, monounsaturated and polyunsaturated fatty acid are the important components of oil. The essential fatty acid is an important component of fats and oil found in the form of linoleic and linolenic acid which is considered as a poly unsaturated fatty acid. The range of polyunsaturated fatty acid in winged bean seed oil was found 36.16 to 40.88 per cent. The highest percentage was noticed in the treatment of no soil application of nitrogen and seed inoculation with *Rhizobium* + PGPR. As per recommendation made by World Health Organisation, the ratio of SFA, MUFA and PUFA is 1:1.5:1. In the present study, the balanced ratio of SFA:MUFA was found in seed oil of treatment *viz*., soil application of 20kg Nha^{-1} + seed inoculated with PGPR followed by 0kg Nha^{-1} + *Rhizobium* inoculation; 40kg Nha^{-1} + *Rhizobium* + PGPR inoculation; absolute control and 20kg Nha^{-1} + no inoculation respectively. The lowest (1:1.37) and highest (1:1.66) MUFA ratio was recorded in treatment 20kg Nha^{-1} + PGPR inoculation and 40kg Nha^{-1} + no inoculation respectively. The omega-3 and omega-6 ratio in winged bean seed oil ranged from 16.18 to 34.39 in which low quantity of omega-3 fatty acid was present.

Conclusion

Winged bean seed oil is almost similar in nutritional parameters to that of soybean oil. However, the balanced proportion of various fatty acids could make it better for human consumption.

References

1. Anonymous. The Winged Bean: A high - protein crop for the tropics. National Academy of Sciences, 2nd Edn., Washington, DC, 1981, 48.
2. Berry SK. Long chain fatty acids (C20 to C24) in winged bean (*Psophocarpus tetragonolobus*) oil. Nutrition-Reports-International. 1985; 31(2):265-272.
3. Cox HE, Pearson D. The chemical analysis of foods. Chemical publishing Co. Inc., New York, 1962, 420.
4. Ekpenyong TE, Borchers. The fatty acid composition of the oil of winged bean (*Phophocarpus tetragonolobus*). Journal of the American Oil Chemists Society.1980; 57(5):147-149.
5. Hafez YS, Mohamed AI, Herath W. Nutrient composition of different strains of winged bean seeds and tuber. Nutrition Reports International. 1984; 29(2):253-261.
6. Homma S, Omachi M, Tamura A, Ishak E and Fuzimaki M. Lipid composition of winged bean. Journal of Nutrition Science Vitaminol. 1983; 29:375-380.
7. Hun-TeikKhor. Lipids and fatty acid composition of developing winged bean seeds. Phytochemistry. 1985; 24(4):856-857.
8. IUPAC. Standard methods for the analysis of oils, fats and derivatives. 6th Edition by C. Paquot. 1979, 188.
9. Mohanty CS, Pradhan RC, Singh V, Singh N, Patnayak R, Prakash O *et al*. (2015). Physiochemical analysis of *Psophocarpus tetragonolobus* (L.) DC seeds with fatty acids and total lipid compositions. Journal of Food Science Technology. 2015; 52(6):3660-3670.
10. Sadasivam S, Manickam A. Biochemical methods for agricultural sciences. Wiley Eastern Limited, New Delhi, 1992, 26-27.
11. Syam AM, Yunus R, Ghazi TIM, Yaw TCS. Methanol analysis of jatropha oil in the presence of potassium hydroxide catalyst. Journal of Applied Sciences. 2009; 9:3161-3165.
12. Wijesundera RC Chandra W. Fatty acids of winged bean [*Psophocarpus tetragonolobus* (L.) D.C.]. Journal of the National Science Council of Sri Lanka. 1983; 11(1):143-147.