Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 1722-1726
Received: 07-09-2019
Accepted: 09-10-2019

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Effect of integrated use of bio- inoculants, organic and chemical fertilizers on soil microbial population, soil properties and incidence of pathogens on okra [*Abelmoschus esculentus* (L.) Moench

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Abstract
A Field experiment was conducted at Vegetable Research Farm, Department of Horticulture (Vegetable and Floriculture), Bihar Agricultural University, Sabour during *Kharif* season of 2017 to explore the best integration of microbial inoculant, organic and chemical fertilizers with respect to improving physical and biological properties of soil and yield of okra by minimising yellow vein mosaic virus. The experiment was laid out in randomized block design replicated thrice. The results indicated that the application of 25 % RDN through chemical fertilizer + 75 % RDN through vermicompost + *Azotobacter* + PSB (T_{16}) improved, chemical (p^H, EC, O. C, Available N, P, K) and biological (*Azotobacter, PSB,* Fungi) properties of the soil, reduced the Per cent disease incidence was of YVMV and enhanced the yield of okra.
Thus it can be inferred that the nutrient supply through 75% chemical and 25% through Vermicompost along with the use of biofertilizers (*Azotobacter* + PSB) is beneficial for improving the soil health, minimising the YVMV and increasing the yield of okra.

Keywords: Integrated, bio-inoculants, inorganic, vermicompost, YVMV, yield

Introduction
The okra [*Abelmoschus esculentus* (L.) Moench], which is also known as 'lady's finger' or bhindi belonging to family Malvaceae is a native to Tropical and subtropical Africa, possibly Ethiopia. It is cultivated in tropical, sub-tropical and mild temperate parts of the world for its tender pods, which are cooked and consumed as a vegetable purpose. Pods are good source of carbohydrate, protein, vitamins and minerals and contain 35.0 mg calories, 6.4 mg carbohydrates 13.10 mg vitamin C (100 g^{-1}), 66.0 mg calcium, 0.35 mg iron, 97 mg iodine and 30.0 mg sulphur per 100g of fresh weight (Gopalan *et al*., 2007) [7]. The presence of iodine in the pods of okra makes it a good remedy for goitre. The pod also contains good amount of mucilage and fibre, which is beneficial against chronic dysentery, fever and genitor urinary disorders.
Nitrogen is one of the important essential major nutrients that play a vital role in encouraging vegetative growth, imparting a dark green colour to the leaves and improving the quality of vegetables. It is an essential constituent of protein and chlorophyll and is present in many other compounds of known physiological importance like plant metabolism such as nucleotides, phosphatides, alkaloids, enzyme, vitamins and hormones, etc. Phosphorus is rightly called the 'mineral of life' as it helps in vital energy transformation process. It is a constituent of nucleic acid, phospholipids and co-enzyme NADP, which is constituent of ATP an energy rich bond. It promotes the root, improves quality of the crop and increase resistance to diseases. Deficiency of phosphorus may causes dark to blue green foliage, necrosis of leaves petioles and fruits also. Potassium is the third important major essential element that imparts disease resistance to plant.
Vermicompost is a good source of micro and macro nutrients. It also acts as chelating agent and regulates the availability of metabolic nutrients to the plants and increases the plant growth and yield by providing nutrients in available form. Vermicompost contains 1.2, 0.6 and 0.8 % N, P and K, respectively depending upon materials used.
Recently, the biofertilizers have shown a good promise and have emerged as an important component of integrated plant nutrition system.

These are the carrier based preparations containing beneficial microorganisms in viable state intended for seed or soil application. These microbes help to fix atmospheric nitrogen, solubilize and mobilize phosphorus, translocate minor elements like zinc, copper etc. to the plants, produce plant growth promoting hormones, vitamins and amino acids. They are cost effective and renewable source of plant nutrients to supplement the part of chemical fertilizers.

Yellow vein mosaic virus is a serious pest of okra that causes great loss in marketable fruits. An application of RDF 75% + 25% Neem oil cake reduced the incidence of YVMV from 30.87 to 18.01% (Mishra *et al*. 2019) [11].

Integrated Nutrient Management includes the integrated use of organic, inorganic and biological resources, which sustain optimum yield, improve or maintain the soil physical and chemical properties and provide crop nutrition packages, which are technically sound, economically attractive, practically feasible and environmentally safe (Tandon, 1990) [14]. It helps to improve various physical, chemical and biological properties of soil leading to improved soil fertility and also to increase fertilizer use efficiency (Dick and Greegorich, 2009) [5]. Keeping these facts in to consideration an experiment was conducted to meet out the best integration of organic, chemical and bio fertilizers on eco-friendly nutrient use as well as YVMV management in okra.

Materials and methods

The field experiment was carried out at Vegetable Research Farm, Department of Horticulture (Vegetable and Floriculture), Bihar Agricultural University, Sabour during *kharif* season of 2017. The sixteen treatments VIZ T_1- Recommended dose of fertilizers (RDF), T_2 - (75% of RDN through chemical fertilizer + 25% RDN through Vermicompost), T_3 - (50% of RDN through chemical fertilizer + 50% RDN through Vermicompost), T_4 - (25% of RDN through chemical fertilizer + 75% RDN through Vermicompost), T_5 - (T_1 + *Azotobacter*), T_6 - (T_2 + *Azotobacter*), T_7 - (T_3 + *Azotobacter*), T_8 - (T_4 + *Azotobacter*), T_9- (T_1 + PSB), T_{10} - (T_2 + PSB), T_{11}-(T_3+ PSB), T_{12}-(T_4 + PSB), T_{13} - (T_1 + PSB + *Azotobacter*), T_{14} -(T_2+ PSB + *Azotobacter*), T_{15}- (T_3 + PSB + *Azotobacter*) and T_{16} - (T_4 + PSB + *Azotobacter*) were allocated randomly in to the plots in such a way that the each and every treatments got once in a replication. The field was thoroughly prepared and experimental plots of 3.0m x 2.25m size were made. Inorganic fertilizers (NPK) and vermicompost were applied as per the treatment. Seeds were treated with *Azotobacter* and PSB and two healthy seeds were dibbled at 50 x 45 cm row to row and plant to plant spacing on 16 July, 2017. Composite soil samples from five spots were collected with the help of soil auger from 0 - 20 cm depth before the start of the experiment and subjected to analysis. Enumeration of *Azotobacter*, PSB, and Fungi was done on Nutrient Jensen's medium, Pikovasya's medium and Rose Bengal agar medium respectively, using serial dilution spread plate technique. The media were prepared and sterilized in an autoclave at 15 psi pressure and 121°C temperature for 20 minutes. Ten grams of the fresh soil was transferred to Erlenmeyer flask (150 ml) containing 90 ml sterile distilled water and was shaken at 120 rpm for 15 minutes to make homogenous solution. Serial dilutions (up to 10-8) were made by pipetting 1 ml of the soil suspension into 9ml of sterile water blank. Finally, 0.1 ml aliquot of the diluted soil suspension was uniformly spread with the help of sterilized spreader on solidified petriplates with respective medium. The petriplates were incubated for 2 to 6 days at 28 ± 2°C in an inverted position. After incubation, the number of colonies appearing on dilution plates were counted to find the number of cells per gram of soil sample:

CFU/g soil = Number (average of 3 replicates) of colonies × Dilution factor

The observations were recorded on chemical properties of soil (P^H, EC, O.C, N, P, K) biological (A*zotobacter*, PSB, fungi count) yield. Per cent disease incidence was calculated using following formula

PDI (%) = Number of diseased plants / Total number of plant observed × 100

The data were collected and subjected to statistically analysis using analysis of variance (ANOVA) under Randomized Block Design as described by Panse and Sukhatme (1985). The critical difference was also calculated to draw the valid conclusion.

Results and Discussion

The data (table 1) clearly revealed that the various treatments under study did not bring any tangible impact towards pH of the soil after harvest of okra crop and varied in the range of 7.24 to 7.00 with maximum in T_1 (RDF: 120:60:60kg NPK ha^{-1}) and minimum in the plots receiving 25 % of recommended dose of N through chemical fertilizer + 75% N through Vermicompost + *Azotobacter* + PSB (T_{16}). The lowering of pH with addition of organic manures may be attributed to the presence of organic acids in the manure that played an important role in buffering the soil, which in turn lowered the pH towards acidic. Microbial inoculants might have also increased the content of organic acids through their secretions and helped in lowering the soil pH. The results were in accordance with the findings of Gopinath *et al.* (2009) and Jaipaul *et al.* (2011) [8]. The electrical conductivity did not touch the level of significant due to various integrated nutrient management options. However, it slightly decreased to 0.270 with the application of 25 % of recommended dose of N through chemical fertilizer + 75% N through Vermicompost + *Azotobacter* + PSB (T_{16}) as compare to RDF, which recorded maximum EC (0.290 ds/m). It may be due to the production of acids or acid forming compounds through the decomposition of organic materials, that reacted with the sparingly soluble salts already present in the soil or either converted them into soluble salts thereby increasing their solubility. The results were in accordance with the findings of Bulluck *et al.* (2002) [3] and Khanday and Ali (2012) [9]. The organic carbon content of the soil did not vary significantly due to various integrated nutrient management options. However, it varied from 0.40 % under control to 0.48 % under the treatment receiving 25 % of recommended dose of N through chemical fertilizer + 75 % N through Vermicompost + *Azotobacter* + PSB (T_{16}). High microbial biomass production and high rhizodeposits of carbonaceous materials through root exudates may be one of the reasons for higher organic carbon in organically treated soils (Franzluebbers *et al.* 1995 [6] and. Varalakshmi *et al*., (2005) [15] also reported the similar findings.

The data presented in table 1 showed that the integrated application of vermicompost, bio inoculants and chemical fertilizer resulted in significant increase in available nitrogen after harvest of the crop. The maximum available nitrogen (221.67 kg ha^{-1}) was analysed where the plot received 75 % of recommended dose of N through chemical fertilizer + 25% N through Vermicompost + *Azotobacter*+ PSB (T_{14}), which was at par with T_{15} and T_{16}. The data indicate that there was least available nitrogen (197.30 kg ha^{-1}) with the recommended dose of NPK @ 120:60:60 kg ha^{-1}. This might be due to the fact that the decomposition and/or mineralization of organic manures might have released the considerable quantities of carbon dioxide, which when dissolved in the water formed carbonic acid capable of weathering certain primary minerals. Besides, organic matter might have also helped in the multiplication of microbes for the conversion of organically bound N to inorganic forms. Better efficacy of increasing N availability by addition of organic manures (cow dung manure, Neem cake and vermicompost) was reported by several workers Reddy *et al*. (1988a) [13], Rajendra *et al*. (1990) [12] and Chandra *et al*. (2008) [4].

The data (table 1) revealed that the available phosphorus of the soil after harvest of okra varied insignificantly from 13.67 to 18.17 kg ha^{-1} with maximum in the plots receiving 75 % of recommended dose of N through chemical fertilizer + 25% N through Vermicompost + *Azotobacter* + PSB (T_{14}) and minimum in the control plot (T_1). Build-up of available phosphorus through application of chemical fertilizers with Bio-inoculants and vermicompost might be due to the release of organic acids during decomposition, which in turn helped in releasing phosphorus through solubilizing action of native phosphorus in the soil. Besides, organic matter also formed a cover on sesquioxides and made them inactive and thus reduced the phosphate fixing capacity of the soil, which ultimately, helped in release of ample quantity of phosphorus as reported by Bhardwaj *et al.* (2010) [2].

The data displayed in table 1 showed that the potassium content of the soil varied from 180.93 kg ha $^{-1}$ under control to 187.0 kg ha $^{-1}$ under the treatment receiving 75 % of recommended dose of N through chemical fertilizer + 25 % N through Vermicompost + *Azotobacter* + PSB (T_{14}). However, it was remained unaffected due to various treatment under study. the increase in K content due to integration of organic, chemical and bioinoculants was might be due to addition of vermicompost that ascribed to release of potassium by reducing potassium fixation due to interaction of organic matter with clay, besides the direct potassium addition to the pool of soil. Such increase in the content of available potassium with the use of organics with chemical fertilizers was also reported by Bhardwaj *et al.* (2010) [2].

Table 1: Integrated effect of bio-inoculants, organic and chemical fertilizers on pH, EC, organic carbon and available NPK of soil

Symbol	pH	EC (ds m^{-1})	O.C (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
T_1	7.24	0.290	0.40	197.30	13.67	180.93
T_2	7.23	0.285	0.42	203.10	14.46	183.70
T_3	7.22	0.283	0.43	201.70	13.80	182.63
T_4	7.17	0.278	0.43	200.83	13.20	178.33
T_5	7.20	0.288	0.42	206.67	14.00	182.67
T_6	7.16	0.283	0.44	210.13	15.03	185.83
T_7	7.10	0.281	0.45	209.80	14.40	184.60
T_8	7.02	0.276	0.45	207.13	13.13	180.67
T_9	7.23	0.287	0.42	212.03	15.87	182.33
T_{10}	7.20	0.283	0.45	209.47	16.97	184.13
T_{11}	7.14	0.280	0.45	208.90	15.93	183.33
T_{12}	7.09	0.274	0.45	215.80	14.60	180.00
T_{13}	7.20	0.286	0.43	212.17	17.03	184.33
T_{14}	7.14	0.281	0.46	221.67	18.17	187.00
T_{15}	7.00	0.279	0.48	219.33	17.23	186.33
T_{16}	7.00	0.270	0.48	211.67	16.13	182.67
SEm ±	0.063	0.009	0.019	2.89	1.22	2.59
CD at 5 %	NS	NS	NS	8.34	NS	NS

The data presented in table 2 clearly revealed that the *Azotobacter* population varied significantly due to the various treatments of integrated nutrient management. The highest *Azotobacter* count (20.27 × 10^5 CFU g^{-1} soil) was observed in soil samples under treatment T_{16} (25 % of recommended dose of N through chemical fertilizer + 75 % N through Vermicompost + *Azotobacter* + PSB), which was statistically at par with all the treatments receiving *Azotobacter* and significantly higher than all other treatments. The minimum bacterial population (12.67 ×10^5 CFUg^{-1}soil) was observed in soil samples having treatment T_1 (RDF). It may be due to the fact that the solid manures might have introduced a high amount of beneficial microflora and phytohormones in the soil, which might have enhanced the organic matter content and water-air relationships in the soil thereby stimulated the growth of bacterial strains. These results are in accordance with the works of Mandal *et al.* (2007) [10] and Zhong *et al.* (2010) [16].

An examination of data presented in table 2 clearly established that the different treatment brought significant variation in number of PSB. Maximum PSB count (36.57 ×10^5 CFUg^{-1}soil) was observed in treatment T_{16} (25 % of recommended dose of N through chemical fertilizer + 75 % N through Vermicompost + *Azotobacter* + PSB) which was statistically at par with PSB population in treatment T_{15} and T_{14}. The minimum PSB count (17 ×10^5 CFUg^{-1}soil) was observed in treatment T_1 (RDF). Organically treated soils had significantly higher PSB population than treatments containing inorganic sources of phosphorus. This may be due to the fact that the application of organic manure might have increased the organic carbon content in soil and thereby survival of phosphate solubilizing bacteria in the rhizosphere.

It was also observed that the various treatments brought progressive variation in the fungal population. Maximum fungi count (17.33 ×10^4 CFUg^{-1}soil) was observed in treatment T_{16} (25 % of recommended dose of N through chemical fertilizer + 75 % N through Vermicompost + *Azotobacter* + PSB), which was statistically at par with T_{13}, T_{14} and T_{15}, however, minimum fungi count (9.67 × 10^4 CFUg^{-1}soil) was found in T_1 (RDF). This may be because of the fact that fungi can flourish in environments having low nutrient content which condition is fulfilled by application of organic manures due to slow release of nutrients and, on the contrary, inorganic fertilizers act as rapid sources of nutrients for the growing fungal species. Decrease in fungal population with application of mineral nitrogen may be attributed to the production of toxic metabolites such as nitrosamines and nitrosamides. Similar results have also been reported by Barabasz *et al.* (2002) [1].

Table 2: Integrated effect of bio-inoculants, organic and chemical fertilizers on microbial population of *Azotobacter*, PSB and fungi

Symbol	Azotobacter ×10 ^{5} CFUg^{-1}soil	PSB ×10^{5} CFUg^{-1}soil	Fungi ×10^{4} CFUg^{-1}soil
T_1	12.67	17.00	9.67
T_2	13.69	19.00	12.00
T3	14.03	19.33	12.33
T_4	14.00	21.00	13.33
T_5	17.67	18.33	12.67
T_6	18.00	20.67	13.00
T_7	19.67	21.67	13.33
T_8	20.33	24.33	13.67
T_9	14.67	26.00	13.69
T_{10}	15.33	31.00	14.33
T_{11}	16.00	33.33	15.00
T_{12}	16.33	36.33	16.00
T_{13}	17.43	26.80	15.00
T_{14}	17.80	31.89	16.33
T_{15}	19.40	33.80	17.00
T_{16}	20.27	36.57	17.33
SEm ±	1.02	1.62	0.94
CD at 5 %	2.94	4.69	2.72

Data regarding the incidence of yellow vein mosaic virus (%) was recorded at 30, 60 and 90 DAS and displayed in mean summary table 3. It was observed that the incidence of yellow vein mosaic at initial stage at 30 DAS did not appear, however, it incidence varied significantly with the advancement of crop i.e. at 60 and 90DAS. The incidences at 60 and 90days varied significantly in the range of 2.37 to 5.37 and 9.20 to 14.16 with maximum under control and minimum under T_{16} treatment (25% of RDN through chemical fertilizer + 75% RDN through Vermicompost + *Azotobacter* + PSB), respectively during both the stages. The minimum incidence in T_{16} treatment was mainly due to integration of organic, inorganic and bio inoculants that might have increased the resistance in the plant. However, the maximum incidence in control may be due to the use of chemical fertilizers that might have prompted the incidence of YVMV.

Table 3: Incidence % of yellow vein mosaic virus of okra at 60 and 90 DAS as influenced by the integration of organic, chemical fertilizers and bioinoculants

Symbol	30 DAS	60 DAS	90 DAS
T_1	0	5.37	14.16
T_2	0	5.13	13.97
T_3	0	3.83	12.62
T_4	0	2.70	10.13
T_5	0	5.33	14.10
T_6	0	5.27	13.89
T_7	0	3.70	12.42
T_8	0	2.53	10.07
T_9	0	5.03	14.01
T_{10}	0	5.00	13.77
T_{11}	0	3.67	12.24
T_{12}	0	2.43	9.93
T_{13}	0	5.00	13.91
T_{14}	0	4.97	13.59
T_{15}	0	3.40	12.10
T_{16}	0	2.37	9.20
SEm ±	0	0.32	0.26
CD at 5 %	0	0.44	0.75

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