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The Effects of Biofertilizers, Organic and Inorganic Nutrient Sources on Growth Parameters and Yield of *Piper betle* L. (Betlevine) under Gangetic Alluvial Soil of West Bengal

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Authors' contributions

This work was carried out in collaboration among all authors. Author BD *designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors* AKB *and* SS *managed the analyses of the study. Authors* AM, BCR and GD *managed the literature searches. All authors read and approved the final manuscript.*

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ABSTRACT

An experiment was carried out during two consecutive years of 2012-13 and 2013-14 at the experimental site of AICRP on MAP and Betelvine, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal. The experiment was laid out in a randomized block design (RBD) replicated thrice with 9 treatments comprising of different combinations of organics (*Azotobacter*, *Phosphobacter* and mustard oil cake) and inorganic (urea and SSP) sources of nutrients. The maximum vine elongation, basal girth, leaf size, leaf yield and other yield attributing characters were found significantly better under application of *Azotobacter* @ 10 Kg + 140 kg N (MOC) +

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Phosphobacter @ 5 Kg + 50 Kg P +100 Kg K $ha^{-1}year^{-1}$. However, the treatment was statistically at par with *Azotobacter* @ 5 Kg + 170 Kg N (MOC) + *Phosphobacter* @ 5 Kg + 50 Kg P + 100 Kg K $ha^{-1}year^{-1}$ in respect of dry matter production. Hence, the treatment with *Azotobacter* @ 10 kg + 140 kg N (MOC) + *Phosphobacter* @ 5 Kg + 50 Kg P +100 Kg K $ha^{-1}year^{-1}$ application can be used as a best sustainable and eco friendly integrated nutrient management approach for better yield of betelvine under the gangetic alluvial zone of West Bengal.

Keywords: Betelvine; biofertilizer; organic sources; azotobacter, phosphobacter.

1. INTRODUCTION

Betelvine (*Piper betle* Linn.) is a perennial creeper belongs to the family Piperaceae. It has been very intimately connected with the ancient Indian history and culture. Consumption of leaves of betelvine has some beneficial effect on health like it helps in digestion and tends to remove the bad smell of the mouth. The fresh crushed leaves are used as an antiseptic for cut and wounds [1]. The vine was probably originated from Malaysia and it was later spread to Madagascar and East Africa. Other than India, it is grown in Bangladesh, Pakistan, Mayanmar and Srilanka and to a smaller extent in Burma and New Guinea [2]. The betelvine growing regions in India are West Bengal, Orissa, Assam, Andhra Pradesh, Bihar, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Tripura and Uttar Pradesh. In India, it is claimed that the betel leaves which are consumed by about 15-20 million people in the country [3]. In order to meet the domestic requirement for betel leaves, focus should be given to adoption of improved cultural practices such as manuring, irrigation and sanitation practices is imperative [4] . Beside other major nutrients, nitrogen is an important one for its vigorous vine growth, branching and leaf production since the green leaves constitute the economic part of the plant [5]. Though, the various forms of nitrogen have different effect on vines. It has been found that excess use of chemical fertilizers deteriorate the keeping quality of leaves by aggravating foliage diseases [6,7]. Beside this, inorganic fertilizers particularly nitrogen is lost easily in the soil which is a limiting factor for getting good yield. In this context, Integrated Nutrient Management (INM) plays an important role in maintaining the environment, crop productivity and nutrient balance in the soil. Nitrogen fixation is the process of making the atmospheric dinitrogen (N=N) to available form of nitrogen (NH_3) to the plants, viz., nitrate and ammonia. Biological nitrogen fixation is carried out by symbiotic or non-symbiotic enzymic processes or through beneficial effects of associative nitrogen fixation. Both *Azotobacter* and *Azospirillum* are nitrogen fixing free living aerobic bacteria is known to secrete some growth promoting and antifungal substances [8]. On the other hand, Phosphate solubilizing bacteria can solubilise the available soil phosphorous due to secretion of organic acids and formation of stable complex of phosphorous with cations by applying with both organic and inorganic biofertilizers [9]. The present experiment had been carried out to study the individual or combined effect of these sources of nutrients on growth and leaf yield of betelvine.

2. MATERIALS AND METHODS

The experiment was conducted at experimental site of AICRP on MAP and betelvine, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal during the year 2012-13 and 2013-14. 'Simurali Bhabna', local cultivar of Bangla type betelvine was selected for this experiment with bio-inoculants (*Azotobacter, Azospirillum* and *Phosphobacterium* (*Pseudomonas sp.*) each @ 5 kg ha^{-1}), used in combination with farmyard manure (FYM @ 100 kg ha^{-1}) before planting of the cuttings. The plots were single rowed and spaced at 60 cm with plant distance of 10 cm. All the recommended agronomic practices were followed timely to raise a good crop. The vines were treated with oil-cake @ 200 kg N ha^{-1} was maintained as the control. The experiment was laid out in a Randomized Block Design (RBD) replicated thrice with 9 treatments of different nutrient sources combinations. The investigation comprises of the treatments as T_1 = NPK kg ha^{-1} $year^{-1}$ 200 (MOC) : 0 : 0 (Farmers' practice *i.e* control), T_2 = 200 kg N (MOC+Urea 1:1) + 100 kg P_2O_5 + 100 kg K_2O ha^{-1} $year^{-1}$ (RDF), T_3 = *Azotobacter* 5 kg + 170 kg N (MOC 75% + Urea 25%) + 100 kg P_2O_5+100 kg K_2O ha^{-1} $year^{-1}$,T_4 = *Azotobacter* 5 kg + 170 kg N (MOC 50 % + Urea 50%) + 100 kg P_2O_5+100 kg K_2O ha^{-1} $year^{1}$, T_5 = *Azotobacter* 10 kg + 140 kg N (MOC 75% + Urea 25%) + 100 kg P_2O_5 +100 kg K_2O $ha^{-1}year^{-1}$, T_6 = *Azotobacter* 10 kg + 140 kg N (MOC 50% + Urea 50%) + 100 kg P_2O_5 +100 kg K_2O ha^{-1} $year^{-1}$, T_7= *Phosphobacter* 5 kg + 50 kg P_2O_5 + 200 kg N (MOC) + 100 kg K_2O ha^{-1} $year^{1}$,

T_8 = *Azotobacter* 5 kg + 170 kg N (MOC) + *Phosphobacter* 5 kg + 50 kg P_2O_5 + 100 kg K_2O ha^{-1} $year^{-1}$, T_9= *Azotobacter* 10 kg + 140 kg N (MOC) + *Phosphobacter* 5 kg + 50 kg P_2O_5 +100 kg K_2O ha^{-1} $year^{-1}$. *Azotobacter* is an aerobic free living bacteria and fixes atmospheric nitrogen [10,11]. The most abundant species is *Azotobacter chroococcum* and was taken for the experiment. The inoculums were applied @ 5 and 10 kg ha^{-1} in the form of slurry along with 1st split of MOC at the base (rhizosphere) of the plant. Full dose of P_2O_5 and K_2O were applied 15 days after *Azotobacter* application. *Phosphobacter spp.* helps in solubilising phosphorus that are immobilized and fixed in soil to utilizable form and helps in easy uptake [12]. The inoculums of this microorganism were applied @ 5 kg ha^{-1} in the form of slurry along with 1st split of MOC at the base (rhizosphere) of the plant. In farmer's practice (control) NPK 200: 0: 0 kg ha^{-1} $year^{-1}$ (MOC) was applied in four equal splits at 3 months interval. In other treatments, N @ 200 kg ha^{-1} $year^{-1}$ and both P_2O_5 and K_2O were applied @ 100 kg $ha^{-1}year^{-1}$ throughs single super phosphate (SSP) and muriate of potash (MOP) respectively. MOC + Urea (1:1) were applied @ 200 kg N ha^{-1} $year^{-1}$ in four equal splits at an interval of 3 months and P_2O_5 and K_2O were applied along with first application of nitrogenous fertilizer. Data were recorded on ten randomly taken vines from each row using standard procedures for vine elongation(cm), leaf length(cm), leaf breadth (cm), leaf area(sq. cm.), girth of vine(cm), length of internodes(cm), length of petiole(cm), leaf yield per vine at four months interval, leaf yield per vine per year, projected leaf yield per ha per year (Lakh), fresh weight of 100 leaves (g) and dry weight of 100 leaves (g) etc. The data collected from the field were analyzed statistically in the randomized block design following the procedure laid out by anonymous [13]. The significance among the treatments was tested by Fisher and Snedecor's 'F' test at probability level of 0.05 for appropriate degree of freedom. For determination of standard error of mean (S.Em.$\pm$) and the value of critical difference (C.D.) between the treatment means at 5% level of significance, the statistical table formulated by anonymous [14].

3. RESULTS AND DISCUSSION

From the experiment data clearly showed that regarding vine growth, the treatment T_9 (*Azotobacter* @ 10 kg + 140 kg N (MOC) + *Phosphobacter* @ 5 kg + 50 kg P_2O_5 +100 kg K_2O ha^{-1} $year^{-1}$) was significantly superior in vine elongation during all the three seasons *i.e* summer, rainy and winter. Though rainy season's vine exhibit highest increment of vine length. Among the other growth parameters, leaf length, leaf breadth, leaf area and petiole length, leaf number $vine^{-1}$ in different seasons, leaf number $vine^{-1}$ $year^{-1}$ and projected leaf yield and fresh weight and dry weight of 100 leaves of vines were also recorded higher with T_9. Regarding major leaf diseases, minimum leaf spot and leaf rot disease incidence was recorded in the vines grown under treatment T_7 (*Phosphobacter* 5 kg + 50 kg P_2O_5 + 200 kg N (MOC) + 100 kg K_2O ha^{-1} $year^{-1}$).

Table 1. Effect of bio-fertilizer with organic manure and inorganic fertilizers application on leaf length, leaf breadth, intermodal length and petiole length total leaf number and projected yield of betelvine

Treatments	Leaf length(cm)	Leaf breadth(cm)	Internodal length(cm)	Petiole length(cm)	Leaf number $vine^{-1}$ $year^{-1}$	Projected leaf yield (Lakh ha^{-1} $year^{-1}$)
T_1	14.24^i	12.44^h	4.91^h	7.06^f	54.52^i	72.52^i
T_2	14.83^g	12.71^f	5.42^a	7.50^d	59.47^h	79.10^h
T_3	14.89^e	12.78^d	5.06^d	7.52^c	64.74^e	86.13^e
T_4	14.87^f	12.72^e	5.16^b	7.53^c	62.99^f	83.79^f
T_5	14.97^c	12.86^c	5.05^d	7.56^{ab}	68.10^c	90.59^c
T_6	14.93^d	12.86^c	5.12^c	7.51^d	66.29^d	88.19^d
T_7	14.78^h	12.60^g	5.02^e	7.30^e	61.24^g	81.47^g
T_8	15.02^b	12.90^b	4.99^f	7.55^b	70.06^b	93.21^b
T_9	15.04^a	12.96^a	4.96^g	7.57^a	72.03^a	95.83^a
S.Em.($\pm$)	0.006	0.005	0.004	0.006	0.009	0.014
C.D.(P=0.05)	0.019	0.013	0.011	0.018	0.026	0.039

Table 2. Effect of bio-fertilizer with organic manure and inorganic fertilizers on soil pH, organic carbon (%), total N (%), availableP (kg ha^{-1}) and available K (kg ha^{-1}) in experimental field of betelvine

Treatment	Soil pH	Organic carbon (%)	Total N (%)	Available P (kg ha^{-1})	Available K (kg ha^{-1})
T_1	6.72^{c}	0.79^{abc}	0.072^{c}	32.28^{h}	116.77^{g}
T_2	6.74^{ab}	0.79^{bc}	0.076^{a}	35.69^{c}	133.56^{e}
T_3	6.72^{bc}	0.80^{abc}	0.075^{abc}	35.60^{d}	133.51^{f}
T_4	6.74^{a}	0.79^{abc}	0.076^{ab}	35.78^{b}	133.74^{b}
T_5	6.73^{abc}	0.80^{ab}	0.074^{abc}	35.59^{d}	133.68^{c}
T_6	6.73^{abc}	0.79^{abc}	0.076^{abc}	35.83^{a}	133.63^{d}
T_7	6.73^{abc}	0.78^{c}	0.072^{bc}	35.55^{e}	133.78^{a}
T_8	6.73^{abc}	0.79^{abc}	0.075^{abc}	35.52^{f}	133.64^{d}
T_9	6.73^{abc}	0.80^{a}	0.074^{abc}	35.46^{g}	133.66^{c}
S.Em.(±)	0.003	0.003	0.000	0.007	0.008
C.D.(P=0.05)	NS	NS	0.001	0.021	0.022

Table 3. Effect of bio-fertilizer with organic manure and inorganic fertilizers on nutrient content in plant (%) after completion of experiment

Treatment	Nutrient content in plant (%)			Nutrient uptake by plants (kg ha^{-1})		
	N	P	K	N	P	K
T_1	1.15^{f}	0.08^{d}	1.02d	43.37^{g}	3.02^{e}	38.41^{e}
T_2	1.26^{d}	0.10^{bc}	1.18c	57.85^{e}	4.74^{d}	53.90^{d}
T_3	1.30^{b}	0.10^{bc}	1.19bc	59.94^{c}	$4.81^{C.D.}$	55.09^{b}
T_4	1.28^{c}	0.10^{c}	1.20ab	59.09^{d}	4.68^{d}	55.18^{b}
T_5	1.31^{b}	0.10^{bc}	1.20ab	59.38^{d}	4.57^{d}	$53.97^{C.D.}$
T_6	1.29^{b}	0.10^{c}	1.20ab	60.19^{c}	4.60^{d}	54.86^{bc}
T_7	1.23^{e}	0.11^{abc}	1.18c	52.72^{f}	5.06^{bc}	$54.14^{C.D.}$
T_8	1.33^{a}	0.11^{ab}	1.21a	61.70^{b}	5.11^{b}	$54.14^{C.D.}$
T_9	1.33^{a}	0.12^{a}	1.21a	62.22^{a}	5.63^{a}	56.43^{a}
S.Em.(±)	0.004	0.003	0.003	0.171	0.093	0.311
C.D.(P=0.05)	0.010	0.009	0.009	0.493	0.266	0.897

There was no significant difference in soil pH and organic carbon in soil among the different treatments under this experiment. Though, the treatments showed total soil N was statistically at par with each other except T_1 (N @ 200 Kg ha^{-1}year^{-1} as MOC). The P and K content in betelvine showed no significant difference among the treatments except T_1 which recorded minimum P and K content. The maximum uptake of N, P and K was noticed in the vines under treatment T_9 (*Azotobacter* @10 kg + 140 kg N (MOC) + *Phosphobacter* @ 5 kg + 50 kg P_2O_5 +100 kg K_2O ha^{-1} year^{-1}). The positive impact of biofertilizers in betelvine were also reported by anonymous [15] where they concluded that the use of bioinoculants, along with FYM increased the vine length, number and weight of leaves, and reduced the percentage of disease incidence in both the years. In another observations, anonymous [16] showed that application of 5 kg ha^{-1} *Phosphobacteria* + 200 kg ha^{-1} N + 100 kg ha^{-1} K resulted in the highest average fresh weight of 100 leaves (263.75 g) and leaf yield (45.41 lakh ha^{-1}). Though, the results revealed that vine elongation was not significantly affected by the treatments, but the inoculation of bacteria along with the application of P and K fertilizers increased the net return and cost benefit ratio over the application of vermin compost and oilseed cake + urea. The application of organic fertilizer helps the soil micro-organisms to produce the polysaccharides and thus a better soil structure due to N fixation and P solubilisation which improve microbial activity [17]. On the other hand, anonymous [18] also showed from their experiment that minimum shelf life of betel leaves (days to 50% rotting) and higher disease incidence were noticed significantly with NPK: 200 (MOC: Inorganic 1:1):100:100 kg ha^{-1}. It has been also observed that application of biodynamics+ biofertilizers (*Azotobacter* and *Phosphobacter* at 10 kg ha^{-1} each) with N @ 100 kg ha^{-1} as FYM recorded significantly higher crop growth parameters, marketable leaves (lakh/ha), shelf life of leaves, nutrient uptake by plants, soil nutrient status and economics than all other treatments. Increase in leaf yield could be due to properly colonized roots, increased mineral and water uptake from the soil and biological nitrogen fixation [19]. It could also be attributed to the production of indole acetic acid, gibberellins and cytokinins like substances produced by the bacterium [20].

4. CONCLUSION

Based on two consecutive years data it can be concluded that the application of *Azotobacter* @ 10 kg + 140 kg N (MOC) + *Phosphobacter* @ 5 kg + 50 kg P_2O_5 +100 kg K_2O ha^{-1} $year^{-1}$ resulted in the better growth, improved soil fertility and nutrient uptake by crop as well as the highest productivity and profitability of betelvine. So, to reduce the use of urea (inorganic source of nitrogen) in the betlevine crop gradually and to change the trend of using conventional nutrient inputs, the use of low-cost, eco-friendly bio-organisms along with the inorganic sources will results in better management of g soil and plant health.

DISCLAIMER

Some parts of the manuscript has been presented in the following Conference- International Conference on Agriculture, Food Science, Natural Resource Management and Environmental Dynamics: The Technology, People and Sustainable Development. 13th to 14th August 2016 Mohanpur, Nadia, West Bengal, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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