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) +...
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, Kamataka, 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=2) to available form of nitrogen (NH₃) 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.
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 1^{st} 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 1^{st} 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} through 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.+ ) 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, leaf 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, internodal length and petiole length total leaf number and projected yield of betelvine

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaf length(cm)</th>
<th>Leaf breadth(cm)</th>
<th>Internodal length(cm)</th>
<th>Petiole length(cm)</th>
<th>Leaf number vine(^{-1}) year(^{-1})</th>
<th>Projected leaf yield (Lakh ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_1</td>
<td>14.24^a</td>
<td>12.44&quot;</td>
<td>4.91^a</td>
<td>7.06^a</td>
<td>54.52^a</td>
<td>72.52^a</td>
</tr>
<tr>
<td>T_2</td>
<td>14.83^g</td>
<td>12.71^f</td>
<td>5.42^a</td>
<td>7.50^d</td>
<td>59.47^m</td>
<td>79.10^h</td>
</tr>
<tr>
<td>T_3</td>
<td>14.89^e</td>
<td>12.78^d</td>
<td>5.06^d</td>
<td>7.52^c</td>
<td>64.74^e</td>
<td>86.13^e</td>
</tr>
<tr>
<td>T_4</td>
<td>14.87^f</td>
<td>12.72^e</td>
<td>5.16^b</td>
<td>7.53^e</td>
<td>62.99^f</td>
<td>83.79^f</td>
</tr>
<tr>
<td>T_5</td>
<td>14.97^c</td>
<td>12.86^c</td>
<td>5.05^d</td>
<td>7.56^b</td>
<td>68.10^c</td>
<td>90.59^c</td>
</tr>
<tr>
<td>T_6</td>
<td>14.93^g</td>
<td>12.86^c</td>
<td>5.12^c</td>
<td>7.51^d</td>
<td>66.29^g</td>
<td>88.19^d</td>
</tr>
<tr>
<td>T_7</td>
<td>14.78^h</td>
<td>12.60^g</td>
<td>5.02^g</td>
<td>7.30^e</td>
<td>61.24^g</td>
<td>81.47^g</td>
</tr>
<tr>
<td>T_8</td>
<td>15.02^b</td>
<td>12.90^b</td>
<td>4.99^f</td>
<td>7.55^b</td>
<td>70.06^b</td>
<td>93.21^b</td>
</tr>
<tr>
<td>T_9</td>
<td>15.04^a</td>
<td>12.96^a</td>
<td>4.96^g</td>
<td>7.57^a</td>
<td>72.03^a</td>
<td>95.83^a</td>
</tr>
<tr>
<td>S.Em.(+ )</td>
<td>0.006</td>
<td>0.005</td>
<td>0.004</td>
<td>0.006</td>
<td>0.009</td>
<td>0.014</td>
</tr>
<tr>
<td>C.D.(P=0.05)</td>
<td>0.019</td>
<td>0.013</td>
<td>0.011</td>
<td>0.018</td>
<td>0.026</td>
<td>0.039</td>
</tr>
</tbody>
</table>
application of 5 kg ha\(^{-1}\), observations, and reduced the percentage of disease
in the vine length, number and weight of leaves, biofertilizers in betelvine were also reported by
\(100 \text{ kg ha}\(^{-1}\) treatment T\(_1\) of N, P and K was noticed in the vines under
minimum P and K content. The maximum uptake
among the treatments except T\(_1\)par with each other except T\(_9\) treatments under this experiment. Though, the
and organic carbon in soil among the different
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\(_2\)O\(_5\) +100 kg K\(_2\)O 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 this 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
1:1:1:100:100 kg ha\(^{-1}\) each) with N @ 100 kg ha\(^{-1}\) as FYM recorded
significantly higher crop growth parameters,

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil pH</th>
<th>Organic carbon (%)</th>
<th>Total N (%)</th>
<th>Available P (kg ha(^{-1}))</th>
<th>Available K (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(_1)</td>
<td>6.72(^{c})</td>
<td>0.79(^{abc})</td>
<td>0.072(^{c})</td>
<td>32.28(^{i})</td>
<td>116.77(^{g})</td>
</tr>
<tr>
<td>T(_2)</td>
<td>6.74(^{ab})</td>
<td>0.79(^{bc})</td>
<td>0.076(^{a})</td>
<td>35.69(^{c})</td>
<td>133.56(^{a})</td>
</tr>
<tr>
<td>T(_3)</td>
<td>6.72(^{bc})</td>
<td>0.80(^{abc})</td>
<td>0.075(^{abc})</td>
<td>35.60(^{d})</td>
<td>133.51(^{i})</td>
</tr>
<tr>
<td>T(_4)</td>
<td>6.74(^{a})</td>
<td>0.79(^{abc})</td>
<td>0.076(^{ab})</td>
<td>35.78(^{b})</td>
<td>133.74(^{b})</td>
</tr>
<tr>
<td>T(_5)</td>
<td>6.73(^{abc})</td>
<td>0.80(^{ab})</td>
<td>0.074(^{abc})</td>
<td>35.59(^{d})</td>
<td>133.68(^{c})</td>
</tr>
<tr>
<td>T(_6)</td>
<td>6.73(^{abc})</td>
<td>0.79(^{abc})</td>
<td>0.076(^{abc})</td>
<td>35.83(^{a})</td>
<td>133.63(^{d})</td>
</tr>
<tr>
<td>T(_7)</td>
<td>6.73(^{abc})</td>
<td>0.78(^{c})</td>
<td>0.072(^{bc})</td>
<td>35.55(^{e})</td>
<td>133.78(^{a})</td>
</tr>
<tr>
<td>T(_8)</td>
<td>6.73(^{abc})</td>
<td>0.79(^{abc})</td>
<td>0.075(^{abc})</td>
<td>35.52(^{f})</td>
<td>133.64(^{d})</td>
</tr>
<tr>
<td>T(_9)</td>
<td>6.73(^{abc})</td>
<td>0.80(^{a})</td>
<td>0.074(^{abc})</td>
<td>35.46(^{g})</td>
<td>133.66(^{c})</td>
</tr>
<tr>
<td>S.Em.((\pm))</td>
<td>0.003</td>
<td>0.003</td>
<td>0.000</td>
<td>0.007</td>
<td>0.008</td>
</tr>
<tr>
<td>C.D.((P=0.05))</td>
<td>NS</td>
<td>NS</td>
<td>0.001</td>
<td>0.021</td>
<td>0.022</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nutrient content in plant (%)</th>
<th>Nutrient uptake by plants (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>T(_1)</td>
<td>1.15(^{d})</td>
<td>0.08(^{c})</td>
</tr>
<tr>
<td>T(_2)</td>
<td>1.26(^{d})</td>
<td>0.10(^{bc})</td>
</tr>
<tr>
<td>T(_3)</td>
<td>1.30(^{b})</td>
<td>0.10(^{bc})</td>
</tr>
<tr>
<td>T(_4)</td>
<td>1.28(^{c})</td>
<td>0.10(^{d})</td>
</tr>
<tr>
<td>T(_5)</td>
<td>1.31(^{b})</td>
<td>0.10(^{bc})</td>
</tr>
<tr>
<td>T(_6)</td>
<td>1.29(^{b})</td>
<td>0.10(^{d})</td>
</tr>
<tr>
<td>T(_7)</td>
<td>1.23(^{a})</td>
<td>0.11(^{abc})</td>
</tr>
<tr>
<td>T(_8)</td>
<td>1.33(^{h})</td>
<td>0.11(^{abc})</td>
</tr>
<tr>
<td>T(_9)</td>
<td>1.33(^{a})</td>
<td>0.12(^{c})</td>
</tr>
<tr>
<td>S.Em.((\pm))</td>
<td>0.004</td>
<td>0.003</td>
</tr>
<tr>
<td>C.D.((P=0.05))</td>
<td>0.010</td>
<td>0.009</td>
</tr>
</tbody>
</table>
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 P₂O₅+100 kg K₂O 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 betelvine crop gradually and to change the trend of using conventional nutrient inputs, the use of low-cost, eco-friendly bioorganisms along with the inorganic sources will results in better management of g soil and plant health.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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