Green Manure Plants Influence Growth, Yield and Curcumin Content of Turmeric (Curcuma longa L.) in Dark-red Soil in Okinawa, Japan

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Abstract Using green manure plants is a common practice for crops in light-texture soil with low organic matter content. The effects of the application of crotalaria (Crotalaria spectabilis L.) and hairy vetch (Vicia villosa R.) green manure plants on the growth and yield of turmeric (Curcuma longa L.) were evaluated in dark-red soil in Okinawa, Japan. Dry green manure of crotalaria or hairy vetch was applied at the rate of 81.43-82.86 g per turmeric plant in field experiment. This rate provided 2.52-2.74 g N, 0.11-0.12 g P and 0.89-0.95 g K per turmeric plant. In glasshouse experiment, 250.00 g dry green manure of crotalaria or hairy vetch per turmeric plant was applied and the rate provided 7.62-8.43 g N, 0.33-0.36 g P and 2.70-2.91 g K. The manures maintained the soil pH at 6.5-7.0 and reduced the soil bulk density by 19%. In the field, turmeric yield (rhizome) increased by 7-10% with the application of green manure plants. In the glasshouse, shoots remained green 30 days longer, and plant height, tiller number, leaf dry matter content, shoot dry matter content and yield increased by 20, 76-165, 157-184, 173-197 and 28-86%, respectively, when turmeric was grown with the application of 3-times higher amount of green manure plants, compared to that in the field experiments. Hairy vetch alone provided 46% higher yield, while crotalaria provided similar yield, compared to the fertilizer treatment. Curcumin concentration (%) in the rhizomes was lower by 4-54%, presumably due to the excessive amount of N supplied by the green manure plants and lower K nutrient content. The current study demonstrated that green manure plants provided nutrients and improved some of the physical and chemical properties of dark-red soil, which significantly enhanced the growth and yield of turmeric.

Key words: Hairy vetch, Crotalaria, Soil physical properties, Turmeric growth, Turmeric quality

Introduction

The application of green manures is recommended for crop production in soils with low organic matter and N contents and with a high bulk density (Miyanmar et al., 2008; Sultani et al., 2007; Sullivan, 2003). Green manure plants add organic matter influence the availability of soil minerals. They also decrease the soil bulk density, increase soil aeration and water-holding capacity and improve the soil texture (Manna et al., 2007; McVay et al. 1989; Sullivan, 2003). Leguminous plants are more effective than other plants because their root nodule bacteria accumulate atmospheric N. Biologically fixed N is environment-friendly, because NH₃ is assimilated into an organic form by the plant, and is released gradually through organic matter decomposition by bacterial mineralization.

Hairy vetch (Vicia villosa R.) is one of the best legume plants for nitrogen source that can produce 6-8 t ha⁻¹ dry matter with N up to 222 kg ha⁻¹, and crotalaria (Crotalaria spectabilis L.) is a fast-growing popular green manure that can produce about 8 t ha⁻¹ dry matter with N up to 183 kg ha⁻¹ in a season in the tropics and subtropics (Choi and Daimon, 2008; Marshall, 2002; Smith et al., 1987).

Turmeric (Curcuma longa L.) is cultivated in tropical and subtropical regions of the world, and is widely used as a regular-spice in Bangladesh, India, Myanmar, Pakistan, Sri Lanka, Nepal, Thailand and other Asian countries (Ahmed et al., 1981; Hossain et al., 2005; Singh et al., 1992; Umate et al., 1984; Yangar et al., 2001). About 98% of total turmeric is used as spice and dye, and 2% is used as herbal medicine and cosmetic in the world. Various types of curcumin in turmeric possess antioxidant, anti-inflammatory, anti-carcinogenic, antibacterial and detox properties (Ammon and Wahl, 1991; Nakamura et al., 1998; Sugiyma et al., 1996). In general, curcumins prevent tumor formation, improve liver and kidney functions, and could be used to alleviate biliary and hepatic disorders (Ammon and Wahl, 1991). Since fertilizers may be associated with food risks (Neera et al., 1999), fertilizers are not usually recommended in turmeric cultivation.

Turmeric has been growing for more than 600 years in Okinawa where soils are not suitable for cultivation. Dark-red soil, one of the major soils in Okinawa, is better than other soils for turmeric cultivation (Hossain and Ishimine, 2005), although the bulk density is high and the soil organic-mater content is low. Green manure
plants could be used to supply nutrients and organic matters for improving physical and chemical properties of the soil, and increasing growth and yield of turmeric.

Field experiments are important to evaluate effects of manures, fertilizers, etc. on the soil properties and growth and yield of turmeric for applied research. However, it is considered that soil nutrients move from plot to plot by heavy rainfall which contaminates the treatment elements. It is also considered that a portion of nutrients runs out with heavy rainfall, which affects results of study. Therefore, glasshouse experiments are necessary to evaluate the actual effects of the application of green manure plants on the soil properties and turmeric cultivation.

Previous studies had revealed significant differences in growth, yield and quality of turmeric depending on the soil types, planting times, sunlight levels, planting depths, seed sizes, planting patterns, application of farm yard manures, fertilizers and harvest times in Okinawa (Ishimine et al., 2003, 2004; Hossain et al., 2005a, 2005b; Hossain and Ishimine, 2005, 2007; Akamine et al., 2007; Hossain et al., 2009; Hossain, 2010). The objectives of the present studies were as follows: (i) evaluate the effects of the application of two green manure plants on some of the chemical and physical properties of dark-red soil in Okinawa, (ii) evaluate the effects of the application of green manure plants on the growth and yield of turmeric in dark-red soil, and (iii) evaluate the effects of the application of green manure plants on the cucurmin content of turmeric.

**Materials and Methods**

**Field experiment**

The experiment was conducted on a dark-red soil field at the Subtropical field Science Center, University of the Ryukyus, Okinawa, Japan. Soil pH and bulk density (g cm⁻³) were 7.2 and 1.01, respectively. Total nitrogen (N), water-soluble phosphorus (P) and water-soluble potassium (K) contents were 1361.7, 8.3 and 39.4 mg kg⁻¹ soil, respectively. The land was plowed up to a depth of 35 cm, and 9 plots were prepared (plot size: 15 m², 2.0 m × 7.5 m). The experiment was conducted according to a randomized complete block design. Three treatments with three replications of the experiment included (1) a control (bare land), (2) application of crotalaria and (3) application of hairy vetch. Seeds of hairy vetch (70 kg ha⁻²) and crotalaria (100 kg ha⁻¹) were sown on February 25, and the plants were grown up to May 8, 2010. Hairy vetch and crotalaria green manure plants produced 5.8 ton ha⁻¹ (0.58 kg m⁻² dry, 3.77 kg m⁻² fresh) and 5.7 ton ha⁻¹ (0.57 kg m⁻² dry, 2.62 kg m⁻² fresh) of shoot dry matter, respectively, within 70 days after seed sowing. The fresh plants were chopped at the flowering stage (around 80% of plants had flowers) and incorporated into soil using a rototiller. Each plot consisted of two ridges 1 m wide, and 100 seed rhizomes (30±3 g each) of turmeric (cv. Ryudai gold) were planted in a 30-cm triangular pattern at a depth of 10 cm on May 13, 2010, according to the methods reported previously by Hossain et al. (2005a, 2005b) and Ishimine et al. (2003). Seven turmeric plants were grown per m² and therefore, each turmeric plant received 82.86 g (dry matter) of hairy vetch or 81.43 g of crotalaria. Irrigation and weeding were performed as required, but fertilizer was not applied in this experiment to evaluate the actual effects of the green manure plants on growth, yield and quality of turmeric in dark-red soil.

**Glasshouse experiment**

This experiment was conducted in a glasshouse located in the Subtropical Field Science Center, University of the Ryukyus. Six treatments were applied as follows: (1) control (Con), (2) fertilizer (CF), (3) crotalaria (Cro), (4) Cro+CF, (5) hairy vetch (HV) and (6) HV+CF. The treatments were replicated six times in a randomized complete block design. The CF, Cro+CF and HV+CF treatments were included in this experiment to compare the effects of the application of fertilizer, green manure plants, and green manure plants plus fertilizer on turmeric growth. The green manure plants and soil samples (upper 15 cm layer) for the green manure treatments were collected from the respective green manure plots. The soil sample for other treatments were collected from bare plots (no green manure plants) of the field experiment, as described above. Twelve kilogram (12 kg) soil and 1.13 kg fresh (250 g dry, 7.62 g N, 2.70 g K and 0.33 g P) crotalaria plants (chopped) or 1.66 kg fresh (250 g dry, 8.43 g N, 2.91 g K and 0.36 g P) hairy vetch plants (chopped) were mixed and placed in a Wagner pot (25 cm diameter × 30 cm height; 0.05 m³) for the green manure treatments. The other treatments consisted of 12 kg soil in a pot. Hairy vetch or crotalaria green manure plants produced about 5.8 ton ha⁻¹ dry shoot within 70 days after seed sowing in the field experiment. The plants may produce a large amount of dry-shoots with a longer growth period in a favorable environment. In addition, turmeric roots distributed into soil at more than 1 m depth and a single plant could obtain nutrients (N, P, K, etc.) from about 70 kg of field soil (data not published); whereas in a pot, a single plant obtained nutrients from only 12 kg of soil in this experiment.
Turmeric is a plant with a long duration of growth (≥8 months) and a high dry matter production (about 200 g shoot and 200 g rhizome per plant) that may require a large amount of nutrients for maximize growth. Based on the above facts, a quantity of green manure plants about 3-times per turmeric plant was taken in a pot, compared to that in the field to evaluate the maximum effect of the green manure plants on growth, yield and quality of turmeric. One seed-rhizome (30±1 g) of turmeric (cv. Ryudai gold) was planted per pot at an 8 cm depth on May 7, 2010. Fertilizers (granular form) with 1.13 g N (H₂NCONH₂), 1.13 g P (P₂O₅; CaH₂(PO₄)₂H₂O) and 1.13 g K (K₂O; KCl) were applied per pot in the CF, Cro+CF and HV+CF treatments at 120 and 153 days after planting (DAP). Water was applied to the plants as required and corks were used to prevent water leaching.

Data collection
In the field experiments, plant height, number of leaves and tillers, and weight of dry leaf, stem and yield (rhizome) were determined at 108, 129, 150, 171 and 192 DAP. Largest leaf area and total leaf area per plant were determined at 108, 129, 150 and 171 DAP, and stem diameter was determined at 108, 129 and 150 DAP. Dry weight of shoot and rhizome was measured again at 255 DAP when the shoots dried-up completely. These measurements were recorded from six plants (two plants from each plot) for each treatment. Soil samples were collected by the core sampling method from the layer up to a 15 cm depth of ridge at 0, 66 and 132 DAP, and the contents of N, carbon (C), P and K in soil were determined. The contents of N, C, P and K in the rhizomes were determined at 108, 129, 150, 171, 192 and 255 DAP. Curcumin content in the rhizomes was analyzed at 150, 171, 192 and 255 DAP.

In the glasshouse experiment, plant height, leaf number and tiller number were measured at 55, 80, 101, 122, 143, 164 and 185 DAP, and the main stem diameter and largest leaf area were measured at 164 DAP. The SPAD value (chlorophyll-meter reading) of two fully expanded top leaves from each main shoot was measured at 80, 101, 122, 143, 164 and 185 DAP using a SPAD meter (SPAD-502, Minolta Co. Ltd.). Total leaf area, and dry leaf, dry stem and dry rhizome weights were measured at 218 DAP. Contents of N, P and K in the leaf, stem and rhizome, and of curcumin in the rhizome were measured at 218 DAP. Soil samples were collected at 0 DAP (beginning of turmeric planting) and 218 DAP (after turmeric harvest) from the mixed soil, and at 167 DAP by the core sampling method from a 10 cm depth.

Contents of available NO₃-N, total N and C, and watersoluble P and K in soil were determined at 0, 167 and 218 DAP. Soil bulk density and soil pH were determined at 218 DAP. Chopped leaves and stems of green manure plants and sliced rhizomes of turmeric were dried at 80 °C for 48 h for dry weight measurement in both experiments.

Soil, turmeric and green manure sample preparation for chemical analysis
Soil was dried at room temperature (20-30 °C) as required, and ground finely and sieved (2 mm mesh). Chopped leaves and stems and sliced rhizomes of turmeric were dried at 60 °C for 48 h; and they were separately ground and sieved (2 mm mesh). Green manure samples were similarly prepared.

Determination of soil bulk density, pH and available NO₃-N
Soil samples were dried at 110 °C for 5 h and the soil bulk density was calculated according to the standard methods (Nakano et al., 1995). Dried soil (20 g) was diluted with 50 ml distilled water and the solution was shaken using an electrical shaker at 170 rpm (Neoshaker, NSA-SNH, As One Corp. Ltd.) for 1 h. The solution was filtered using paper No. 2, and then centrifuged for 90 min (Table Top Centrifuge 4000, Kubota Co. Ltd.) at 3500 rpm. Soil pH was determined with a Horiba pH meter.

Dried soil (10 g) from each replication was diluted with 100 ml distilled water, and the mixture was shaken for 1 h using the shaker adjusted to 170 rpm. Two ml of soil solution was centrifuged at 15000 rpm for 10 min, and the amount of available NO₃-N was measured by using RQFlex 10 and Relectoquant, Nitrat-Test (Merck Co. Ltd.), according to the method of Ando et al. (2004).

Determination of N, P, K and C contents in soil, green manure and turmeric plants
Soil samples (10 g) and distilled water (50 ml) were taken into a beaker, and the solution was shaken for 1 h at 170 rpm. The solution was filtered with paper no. 2, and then centrifuged for 90 min at 3500 rpm, followed by filtering with a disposable syringe filter of 0.45 μm (Advantec Co. Ltd.). Plant powder (0.25 g) was taken into a 50 ml beaker filled with 0.5% nitric acid (HNO₃). For extracting elements, the beakers were kept into a water bath adjusted to 80°C for 24 h, and the solution was then filtered sequentially with paper No. 2 and disposable syringe filter (0.45 μm). The plant and soil solution was diluted as necessary by the addition of deionized water
for determining the contents of mineral elements. The
contents of P and K in soil and plant were determined by
using a Multiple Inductively Coupled Plasma Emission
Spectrometer (ICPE-9000, Shimadzu Co. Ltd.), and the
total C and N contents were determined by using a
Shimadzu gas chromatograph (NC-220F). N, P, K and C
contents in soil, green manure plant and turmeric plant
were determined from six replications for each treatment
in the field and glasshouse experiments, but from three
replications at 255 DAP for turmeric in field experiment.
Uptake (accumulation) of N, P or K by turmeric plants
was calculated by the following formula.

\[
\text{N, P or K uptake (accumulation)} = \text{dry weight per plant} \times \text{concentration (\%) of N, P or K}
\]

**Determination of curcumin content in turmeric rhizome**

Turmeric powder (0.1g) was taken into a 100 ml
beaker, and 40 ml of ethyl alcohol (99.5%) was added.
Extraction was completed by supersonic wave for 10 min
and the solution was then filtered with paper No. 5A.
Residues on filter paper were re-extracted three times fol-
lowing the same procedure, and ethyl alcohol was added
up to a 250 ml solution. The solution was filtered with
the disposable syringe filter (0.45 μm) and the curcumin
content was determined by HPLC (Shimadzu Co. Ltd.).
Column (Intact Cadenza CD-C18 100×3.0 mm, 3 μm)
was run at 40 °C, and the acetonitrile and 1% phosphoric
acid solvents were used at the ratio of 43:57. The 5 μL
solution was supplied at 0.5mL min⁻¹, and analyzed for
11 min. The contents of curcumin, demethoxy curcumin
and bis-demethoxy curcumin were determined from six
replications for each treatment at the wavelengths of
424, 420 and 416 nm, respectively.

**Statistical analysis**

All the data were subjected to analysis of variance.
A one-way ANOVA was used for all the parameters.
Means were separated by the Fisher’s protected least
significance difference (LSD) test at \( p<0.05 \).

**Results and Discussion**

**Effects of application of green manure plants on physical
and chemical properties of soil**

Application of green manure plants changed the
soil pH values significantly (Fig. 1), which is in agree-
ment with the results reported previously (Bokhtiar
and Sakurai, 2005; Dutta et al., 2003; Manna et al., 2007).
Application of chemical fertilizer alone or in combina-
tion with green manure plants reduced the soil pH values
due to the N inputs from urea (Ferguson et al. 1984).
Soil bulk density decreased by 19% (calculated from
Fig. 1(B)) with the green manure plant treatments, and
increased slightly with the application of chemical fertil-
izer. Similar results were reported by other researchers
(Sultani et al., 2007; Goto and Nagata, 2000).

The green manure plants provided 2.52-2.74 g N,
0.11-0.12 g P and 0.89-0.95 g K in the field experiment,
and 7.62-8.43 g N, 0.33-0.36 g P and 2.70-2.91 g K in
the glasshouse experiment for a single turmeric plant
(Table 1). The control treatment contained less than 5
mg NO₃-N kg⁻¹ soil (not detected). The HV+CF treatment
showed the highest level of NO₃-N, followed by the
Cro+CF and HV treatments at 167 DAP (Table 2). Hairy
vetch treatment showed a higher NO₃-N value than cro-
talaria, which is in agreement with the results reported
in another study (Ochini et al., 2008). The CF treatment

![Fig. 1. Effects of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) application on soil pH (A) and soil bulk density (B) in soil of glasshouse experiment. Data are means ± SD of 3 replications for soil pH and 6 for bulk density. Bars with the same letter are not significantly different, as determined by Fisher’s Protected LSD test at \( p<0.05 \).](image-url)
Table 1. Amounts of N, P and K supplied by hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) per turmeric plant (g plant⁻¹) in field and glasshouse experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Field Experiment</th>
<th>Glasshouse Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (g plant⁻¹)</td>
<td>P (g plant⁻¹)</td>
</tr>
<tr>
<td>CF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cro</td>
<td>2.52</td>
<td>0.11</td>
</tr>
<tr>
<td>HV</td>
<td>2.74</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Note: -, not supplied. Data are means ± SD of six replications.

Table 2. Effects of application of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) on NO₃-N content in soil at 0, 167 and 218 days after planting in glasshouse experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NO₃-N content in soil (mg kg⁻¹)</th>
<th>Days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Con</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CF</td>
<td>261±39dc</td>
<td>258±51a</td>
</tr>
<tr>
<td>Cro</td>
<td>247±4d</td>
<td>67±7d</td>
</tr>
<tr>
<td>Cro+CF</td>
<td>367±12b</td>
<td>139±35c</td>
</tr>
<tr>
<td>HV</td>
<td>282±11c</td>
<td>83±7cd</td>
</tr>
<tr>
<td>HV+CF</td>
<td>463±64a</td>
<td>210±32b</td>
</tr>
</tbody>
</table>

Note: ND, not detected (NO₃-N, less than 5 mg kg⁻¹ soil). Data are means ± SD of six replications. Data with the same letter within each column are not significantly different, as determined by Fisher’s Protected LSD test at P<0.05.

showed the highest NO₃-N value 218 DAP, presumably due to the lower turmeric growth, whereas the HV+CF and Cro+CF treatments showed a lower NO₃-N value due to the higher turmeric growth.

Effects of application of green manure plants on growth parameters, SPAD value and yield of turmeric

Plant height, stem diameter and tiller number increased slightly in the field, but significantly in the glasshouse with the application of green manure plants. This may be due to the fact that the application of green manure plants per turmeric plant was 3-times higher in the glasshouse than in the field experiments (Fig. 2). In the glasshouse, plant height increased by 20% and tiller number increased by 76-165% with the application of green manure plants (Fig. 2 (B and F)). Plant height was almost similar with the application of hairy vetch and crotalaria, although the tiller number was highest with hairy vetch application in both field and glasshouse experiments. Chemical fertilizer did not affect significantly the plant height and stem diameter.

The SPAD value was higher in turmeric leaves due to the additional amount of N supplied by the green manure plants (Table 1, Fig. 3). Higher SPAD value probably contributed to a higher photosynthesis and resulted in higher growth and yield of turmeric. Similarly, Sarker et al. (2002) reported the increase of the SPAD value with the increase of N fertilizer application, which resulted in higher growth and yield in rice plant. Addition of chemical fertilizer to green manure plants did not increase the SPAD value in turmeric leaf, indicating that the amount of N provided by green manure plants was sufficient to maximize the SPAD value (Fig. 3 and Table 1).

Turmeric grown with hairy vetch developed the largest number of leaves in both experiments, which was significantly higher from 122 DAP with green manure plants in the glasshouse (Fig. 4 (A and B)). Largest values of leaf and total leaf area were observed with the application of green manure plants (Fig. 4 (C, D, E and F)). Green manure plants supplied N that led to a larger number of leaves and leaf area of turmeric in both experiments, which agreed with the results reported by Akamine et al. (2007). Chemical fertilizer application alone did not affect significantly the parameters.

Leaf and stem weight of turmeric increased by 7-25% in the field and 157-211% in the glasshouse with green manure plant application, while chemical fertilizer did not affect these parameters (Fig. 5 (A, B, C and D)). The application of green manure plants per turmeric plant was 3-times higher in the glasshouse than in the field, which may have resulted in a 157-184% higher leaf weight and 173-197% higher shoot (leaf + stem) weight of turmeric plants in the glasshouse (calculated from Fig. 5 (B and D)).

The green manure plants increased the turmeric yield by 7-10% in the field (Fig. 5 (G)) and 28-86% in the glasshouse experiments (Fig. 5 (F)). Both hairy vetch and crotalaria plants exhibited a similar effect on turmeric yield in the field but the use of hairy vetch resulted in a higher yield than that of crotalaria in the glasshouse (Fig. 5 (F and G)). The green manure plants probably improved the physical and chemical properties, water-holding capacity, aeration, mineralization
Fig. 2. Effects of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) application on plant height (A, B), stem diameter (C, D) and tiller number (E, F) of turmeric in the field (A, C, E) and glasshouse (B, D, F) experiments. Main stem diameter of turmeric shoot was measured at 164 days after planting in the glasshouse experiment. Data are means ± SD of six replications. Data with the same letter are not significantly different, as determined by Fisher’s Protected LSD test at p<0.05.
and nutrient digestion in soil, which contributed to the increase of shoot (leaf and stem) weight and yield of turmeric (Sullivan, 2003). Goto and Nagata (2000) reported that crotalaria manure improved the soil chemical and physical properties that resulted in higher crop yield. Application of crotalaria alone resulted in a similar yield to that by chemical fertilizer and the application of hairy vetch alone resulted in a higher yield than that by chemical fertilizer. Chemical fertilizer showed a slightly positive effect on turmeric yield when combined with crotalaria, but a negative effect when combined with hairy vetch. It is considered that the nutrient amounts supplied by chemical fertilizer and hairy vetch were not balanced for turmeric growth, which adversely affected yield. Turmeric plants grown with both green manure plants showed a higher tiller number with greener leaves and survived 30 days longer (Fig. 6), which generally contributed to higher photosynthesis and resulted in higher yield. Similar findings were reported previously (Ishimine et al., 2004; Sarker et al., 2002).

Effects of application of green manure plants on N, P and K contents in leaf, stem, shoot and rhizome of turmeric

In the glasshouse, the percentage of N increased in turmeric leaf in the treatments with both green manure plants and chemical fertilizer, while in the stem, the percentage increased with the CF, HV and HV+CF treatments (Table 3). The percentage of P did not increase in leaf, but increased in stem with the application of green manure plants. Potassium percentage in leaf and stem increased with the application of green manure plants (Table 3). In the field, the percentage of N and P increased significantly or slightly, while the percentage of K decreased significantly in turmeric shoot with the application of green manure plants (Table 3).

The percentage of N in the rhizome increased slightly with the application of green manure plants in the field, but significantly in the glasshouse (Fig. 7 (A and B)). In the glasshouse, the turmeric plants survived longer with the application of both green manure plants, and this may have resulted in a higher N accumulation. Chemical fertilizer increased N accumulation in the rhizome when applied alone or with hairy vetch, but slightly decreased it when applied with crotalaria. The P and K contents in the rhizome were similar in all the treatments in the field (Fig. 7 (C and E)), but decreased significantly in the glasshouse (Fig. 7 (D and F)) when turmeric was grown with green manure plants applied alone or in combination with chemical fertilizer. Chemical fertilizer applied alone increased the P and K contents in the rhizomes. It is assumed that the N amount added by green manure plants was excessive (Table 1) and not suitable for the accumulation of P and K, which resulted in lower P and K contents in the rhizome. On the other hand, it is possible that the P and K contents in the field soil were sufficient for turmeric growth, which caused a similar accumulation of P and K in the rhizomes in all the treatments (Fig. 7 (C and E)).

Total N content in leaf, stem and rhizome of turmeric increased significantly or slightly with the application of green manure plants and chemical fertilizer (Fig. 8 (A)). Total N uptake by turmeric plant was 5-times higher with green manure plants and 3-times higher with chemical fertilizer, compared to the control treatment. Total P content was higher in leaf and stem with green manure treatments, while the highest in the rhizomes with CF and HV, and the lowest in the rhizomes with Cro+CF (Fig. 8 (B)). Total P uptake per plant was highest with HV, followed by Cro. Total K uptake was higher with all the green manure treatments in leaf and stem, and highest with the HV treatment, followed by CF and Cro+CF K in the rhizome. Uptake of K per plant was more than double with the green manure plant application, compared to that with the control treatment (Fig. 8 (C)). Total N, P and K contents per turmeric plant increased (Fig. 8 (A, B and C)) as the total dry matter amount increased (71, 87, 122, 133, 156 and 131 g in Con, CF, Cro, Cro+CF, HV and HV+CF, respectively; data calculated from Fig. 5 (B, D and F)).
Fig. 4. Effects of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) application on leaf number (A, B), largest leaf area (C, D) and total leaf area (E, F) of turmeric in field (A, C, E) and glasshouse (B, D, F) experiments. For the bar graphs, largest leaf area and total leaf area were measured at 164 and 218 days after planting, respectively. Data are means ± SD of six replications. Data with the same letter are not significantly different, as determined by Fisher’s Protected LSD test at p<0.05.
Fig. 5. Effects of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) application on dry leaf weight (A, B), dry stem weight (C, D) and dry yield (E, F, G) of turmeric in the field (A, C, E, G) and glasshouse (B, D, F) experiments. Data for the bar graphs were measured at 218 days after planting in the glasshouse experiment, and at 255 days after planting in field the experiment. Data are means ± SD of six replications. Data with the same letter are not significantly different, as determined by Fisher’s Protected LSD test at $p<0.05$. 
Fig. 6. Effects of crotalaria (Cro), hairy vetch (HV) and chemical fertilizer (CF) application on growth of turmeric at 114 and 181 days after planting (DAP).

Table 3. Contents of N, P and K (% of dry weight) in leaf, stem and shoot of turmeric influenced by the application of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) at 218 days after planting in glasshouse experiment (GH ext) and at 255 days after planting in field experiment (FD ext).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Con</th>
<th>CF</th>
<th>GH Cro</th>
<th>Cro+CF</th>
<th>HV</th>
<th>HV+CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>0.86±0.03c</td>
<td>0.96±0.02b</td>
<td>0.96±0.03c</td>
<td>0.96±0.02b</td>
<td>0.96±0.03c</td>
<td>0.96±0.02b</td>
</tr>
<tr>
<td>P (mg g⁻¹)</td>
<td>5.5±0.1c</td>
<td>6.3±0.1a</td>
<td>5.5±0.1c</td>
<td>6.3±0.1a</td>
<td>5.5±0.1c</td>
<td>6.3±0.1a</td>
</tr>
<tr>
<td>K (mg g⁻¹)</td>
<td>31.2±0.4b</td>
<td>60.4±0.6b</td>
<td>31.2±0.4b</td>
<td>63.5±1.8a</td>
<td>31.2±0.4b</td>
<td>63.5±1.8a</td>
</tr>
</tbody>
</table>

Note: GH ext indicates glasshouse experiment, and FD ext indicates field experiment. ND indicates not determined. Data are means ± SD of six replications for the glasshouse experiment, and three replications for the field experiment. Data with the same letter within each column are not significantly different, as determined by Fisher’s Protected LSD test at p<0.05 for each experiment.

Effects of application of green manure plants on curcumin content in turmeric rhizome

In the field experiment, curcumin concentration (%) in the turmeric rhizome was lower, while demethoxy curcumin and bis-methoxy curcumin concentrations in the rhizomes did not differ with the application of green manure plants, compared to the values in the control treatment (Fig. 9 (A, B and C)). Total curcumin concentration was lower by 4-15% with the green manure plant treatment (calculated from Fig. 9 (A, B and C)). In the glasshouse, all the curcumin concentrations decreased by 52-54% (calculated from Fig. 9 (D, E and F)) when turmeric was grown with green manure plants or chemical fertilizer. In the field, the rhizomes showed a slightly lower K content and a slightly higher N content (Fig. 7 (A and E), (Table 3)), which probably accounted for the lower curcumin concentration (Fig. 9 (A, B and C)). In the glasshouse, the percentage of N in rhizome was 3-times higher, while those of P and K were lower when turmeric was grown with the green manure plants or chemical fertilizer, which resulted in a significantly lower curcumin concentration. Percentages of N and P in the rhizome were higher which may have resulted in a lower curcumin concentration when turmeric was cultivated with chemical fertilizer applied alone. Similarly, Akamine et al. (2007) reported that K fertilizer applied alone resulted in a higher percentage of curcumin, but combined application of P and K resulted in a lower curcumin percentage in the turmeric rhizome. Yamawaki et al. (2014) did not find differences in the curcumin concentration when turmeric was cultivated with combined application of hairy vetch plant and chemical fertilizers (N, P and K). However, further studies should be carried out to elucidate the effects of green manure plants...
Fig. 7. Effects of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) application on N (A, B), P (C, D) and K (E, F) contents in turmeric rhizome in the field (A, C, E) and glasshouse (B, D, F) experiments. For the bar graphs, all the data were determined at 218 days after planting. Data are means ± SD of six replications. Data with the same letter are not significantly different, as determined by Fisher’s Protected LSD test at p<0.05.

and chemical fertilizers on curcumin accumulation in turmeric.

Conclusion

Crotalaria and hairy vetch plants supplied organic matter, NO₃-N and other nutrients, maintained the soil pH at 6.5-7.0, and reduced the soil bulk density. It is possible that the green manure plants in the soil enhanced aeration, water-holding capacity and mineralization, and made the soil loose, which resulted in higher shoot and rhizome (yield) growth of turmeric. Crotalaria alone provided a similar yield and hairy vetch provided a higher yield, compared to chemical fertilizer alone. The chemical fertilizers did not show a positive effect when combined with green manure plants, which indicated the role of green plants providing necessary nutrients to maximize shoot growth and yield of turmeric. Larger amount of green manure plants resulted in higher N, and lower K and curcumin concentrations in the rhizome. The present study strongly suggests that the application
Fig. 8. Uptake of N (A), P (B) and K (C) by turmeric plant influenced by hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) application in the glasshouse experiment. N, P and K contents were measured at 218 days after planting. Data are means ± SD of six replications. Bars with the same letter within leaf, stem or rhizome are not significantly different, as determined by Fisher's Protected LSD test at p<0.05.

Fig. 9. Effects of hairy vetch (HV), crotalaria (Cro) and chemical fertilizer (CF) application on the concentrations (%) of curcumin (A, D), demethoxy curcumin (B, E) and bis-demethoxy curcumin (C, F) in turmeric rhizome in the field (A, B, C) and glasshouse (D, E, F) experiments. Curcumin concentrations in the rhizome were measured at 255 days after planting (DAP) and 218 DAP for the field experiment and glasshouse experiment, respectively. Data are means ± SD of six replications. Bars with the same letter within each harvest time are not significantly different, as determined by Fisher’s Protected LSD test at p<0.05.
of green plants increased turmeric yield by improving the physical and chemical factors of soil and providing nutrients and organic matter. The cause of the decrease of the curcumin content in turmeric rhizomes with green manure plant application requires further investigation.

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References


