Evaluation of N_2-fixing cover legumes as green manures for N substitution in coconut (*Cocos nucifera* Linn.) palm

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A field experiment was conducted in an adult coconut (*Cocos nucifera* Linn.) plantation in an acidic laterite soil type to test the feasibility of partially substituting fertilizer-N with the N contributed by cover legume-*Rhizobium* system when grown as green manures in a coconut basin. The treatments included *in situ* cultivation and incorporation of two leguminous creepers, *Mimosa invisa* and *Calopogonium mucunoides* in a 1.8-m radius basin area surrounding the palm, with inorganic N doses of 250 g and 375 g and a control (devoid of legume) with 500 g N palm^{-1} supplied as urea. *Mimosa invisa* and *C. mucunoides* produced 20.5 kg and 12.8 kg of above-ground wet biomass and 134.8 g N and 70.0 g N basin^{-1}, respectively, when harvested after 140 days growth for incorporation. Higher N_2-fixation efficiency was shown by *M. invisa* as evidenced by nodule biomass and acetylene reduction activity of the nodulated root system. Analysis of the coconut basin soils revealed a trend towards higher N in legumes + 375 g N treatment and K in *Calopogonium* treatments, and microbial biomass and dehydrogenase enzyme activity in integrated treatment of legume biomass and fertilizer-N when compared to that of the control. The yield data of coconut palms collected over a period of four years indicated the possibility of substituting 50% fertilizer-N with the N contributed by the leguminous crops.

Keywords: Coconut palm; Cover legumes; Green manures; Nitrogen substitution

The coconut (*Cocos nucifera* Linn.) palm, one of the important perennial oil crops, is cultivated predominantly in the humid tropics where the major portion of applied inorganic N is lost through leaching (Biddappa *et al.*, 1996). Urea is the recommended N source for the coconut palm at the rate of 500 g N palm^{-1} yr^{-1} (Nelliat, 1972). The deleterious effects of long-term application of inorganic N fertilizers on soil acidification and concomitant erosion of the native nutrient reserves have been reported (Joseph and Wahid, 1997). Hence, appropriate nutrient management strategies need to be developed to achieve sustainable production in a perennial crop like coconut.

Biological nitrogen fixation (BNF) especially those associated with legumes, has great potential to contribute to productive and sustainable agricultural systems for the tropics. Biological N-fixation contribution can be incorporated into viable agricultural systems to increase crop yields and to substitute for fertilizer-N inputs (Boddey *et al.*, 1997). Most research on N contribution from legumes in the tropics has focussed on short-duration legumes grown as green manures and subsequently incorporated before the monsoon rice crop in submerged lowland soils (Ladha *et al.*, 1996).

Leguminous creepers are traditionally grown as cover crops in plantations of commercial crops such as rubber (*Hevea brasiliensis* Muell. Arg.), oil palm (*Elaeis guineensis* Jacq.), coconut, and arecanut (*Areca catechu* Linn.). The potential of leguminous cover crops to generate significant quantities of biomass and N in coconut basins during the monsoon season has been reported (Thomas and Shantaram, 1984). Among the different legume species screened, *Mimosa invisa*, *Calopogonium mucunoides*, and *Pueraria phaseoloides* were superior. The present investigation was undertaken with the objective of studying the feasibility of substituting fertilizer-N with the N contributed by leguminous cover crops when grown and incorporated as green manures in a coconut basin.

Materials and Methods

A coconut plantation cropped with 30-year-old bearing palms of West Coast Tall variety on an acidic (pH 5.5) laterite soil type in a farmer's plot at Muliyar, Kasaragod (12°31′ N, 75° E) in the West Coast of India was selected. The area receives an annual rainfall of 3500 mm during June to October. The soil is low in organic C (0.28%) and N (0.02%). The field experiment was conducted for a period of four years from 1992–96. The coconut palms were cultivated at the recommended spacing of 7.5

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m × 7.5 m, accommodating 175 palms ha^{-1}. The experiment was laid out in a completely randomised design with five treatments: T_1, 500 g fertilizer-N; T_2, *M. invisa* + 250 g fertilizer-N; T_3, *M. invisa* + 375 g fertilizer-N; T_4, *C. mucunoides* + 250 g fertilizer-N; and T_5, *C. mucunoides* + 375 g fertilizer-N. There were 16 single-palm replications for each treatment. The fertilizer-N rates of 250 g, 375 g, and 500 g corresponded to 50%, 75%, and 100% of recommended fertilizer-N for a coconut palm annually. All the palms in the experiment received uniformly recommended doses of P and K at the rate of 320 g P_2O_5 $palm^{-1}$ and 1200 g K_2O $palm^{-1}$ yr^{-1} (Nelliat, 1972). One-third dose of fertilizer was applied with the onset of the South-West monsoon by the end of May and the other two-thirds were applied in October. The palms did not receive the recommended dose of fertilizers during the pre-experimental period. Fifty grams of seeds of legumes were sown in a 1.8-m radius basin area from the bole of the palm at the beginning of June. Scarification of seeds was done before sowing, by soaking in concentrated sulphuric acid for 15 min.

The legumes were allowed to grow in basins and surrounding areas during the monsoon period. For nodule biomass estimation, three replications were randomly selected from treatments T_2–T_5. Three plants were uprooted from each replication at 130 days growth during each year of the experiment, the nodules collected, oven-dried at 65°C for 48 h, and weighed. Nitrogen-fixing activity of root portions with intact nodules was determined by acetylene (C_2H_2) reduction assay (ARA) (Hardy *et al.*, 1968). The nodulated root system of each of five plants was collected at 130 days growth from the basin of a palm during the third year and eight replications were maintained for each legume. The samples were incubated in 500-mL capacity air-tight glass containers fitted with subba seals in the presence of 10% C_2H_2 in air. Gas samples were withdrawn after 2 h and analysed for C_2H_4 production using an AIMIL gas chromatograph fitted with porapak N column and FID. Nitrogen was used as the carrier gas and temperatures of column, detector, and injection port were maintained at 80°C, 110°C, and 110°C, respectively.

The biomass generated by the two legumes was harvested at 140 days, weighed, and incorporated in respective basins with the second dose of fertilizers during the four years of the experiment. Samples of legume biomass were dried, powdered, and analysed for N content by the Kjeldahl method. Soil samples were collected from representative coconut basins in each treatment at a distance of 1 m from the bole of the palm at the 0–25 cm soil depth. The samples were analysed for total N by the Kjeldahl method, available P by the Bray I method, and K by flame photometry. Leaf samples were collected from the 14th leaf of the crown of coconut during the fourth year, washed with detergent solution, rinsed in water, oven-dried at 65°C for 72 h, and powdered in a cyclotech mill. The powdered samples were analysed for N, P, and K contents.

Soil samples of 0–25 cm depth collected from the basins at a lateral distance of 1 m from the bole of the palm obtained in the fourth year of the experiment were analysed for biological properties. Soil microbial biomass was determined by the chloroform fumigation technique (Jenkinson and Powlson, 1976). A modified 2,3,5 triphenyl tetrazolium chloride (TTC) reduction procedure (Casida *et al.*, 1964) was used to determine dehydrogenase enzyme activity in the soil.

The field experiment involving cultivation and incorporation of legumes and differential fertilizer application was continued for a period of four years. The yield data of coconut palms were recorded as the number of nuts in each harvest throughout the experimental period. The number of nuts harvested from a coconut palm from July to June was calculated as the average nuts per year. The number of nuts in the palm at the initiation of the experiment was taken as the pre-treatment yield. The mean yield of the third and fourth years of the experiment was taken as the post-treatment yield as the effect of agronomic treatments are manifested in coconut from the third year of treatment. Cumulative yield was calculated as the total number of nuts from a palm during the four years of the experiment. The weight of copra was recorded after drying the kernel in copra dryers. The copra output per palm during the third and fourth year was calculated based on the copra content of the nut and the number of nuts harvested.

Analysis of variance was done to compare the treatments. Since the treatments were a set of factorial combinations of two legumes and two fertilizer levels, the treatment sum of squares was split into variation due to each factor and their interaction. Analysis of variance was done to compare cover crops with no cover crops (control) and factorial combinations of cover crops, fertilizer levels, and their interaction. Yield data of coconut palms were analysed using co-variance analysis with pre-treatment values as ancillary variable.

Results and Discussion

The above-ground wet biomass (WB) yield of *M. invisa* and *C. mucunoides* during the four experimental years are presented in Table 1. There was significant variation in the WB yield of the two legumes. The average WB yield over the four-year period was 20.8 kg and 12.8 kg $basin^{-1}$ by *M. invisa* and *C. mucunoides*, respectively. The performance of both legumes was poor in the first year of the experiment. The relatively high WB yield recorded in the third year could be due to the

Table 1 Wet biomass yield (kg basin^{-1}) of green manure legumes (*Mimosa invisa* and *Calopogonium mucunoides*) in coconut basins

Legume	Inorganic N (g palm^{-1})	Year 1	Year 2	Year 3	Year 4	Mean
M. invisa	250	8.36	20.85	31.38	23.56	21.04
M. invisa	375	6.06	20.28	29.83	23.44	19.90
C. mucunoides	250	3.09	13.68	16.56	14.97	12.08
C. mucunoides	375	4.29	16.39	18.16	14.94	13.45
Mean						
M. invisa		7.21	20.41	30.61	23.50	20.43
C. mucunoides		3.69	15.13	17.36	14.95	12.78
250 g N		5.72	17.36	23.97	19.27	16.58
375 g N		5.17	18.18	23.99	19.19	16.63
SE plot^{-1}		2.18	6.36	7.48	3.56	
CV (%)		40.05	35.80	31.21	18.53	
$LSD_{0.05}$						
Legumes		1.09	3.18	3.74	1.78	
N levels		ns	ns	ns	ns	
Legumes × N levels		1.54	ns	ns	ns	

SE, Standard Error; CV, Coefficient of Variation; LSD, Least Significant Difference
ns, Not significant

better rainfall distribution during the year. There was not much variation in the WB production by the two legumes except during the first year. During the first year, *M. invisa* recorded significantly higher biomass yield at 250 g fertilizer-N treatment than at 375 g N level.

Nitrogen accumulation in the above-ground WB of legumes showed a similar pattern to the WB yields (Table 2). *Mimosa invisa* and *C. mucunoides* contributed approximately 134.8 g N_2 and 70.0 g N_2 basin^{-1} from the above-ground legume biomass, respectively. Variation in the genetic capacity of different species of green manures to grow and fix N under the same environmental conditions has been reported (Ghai *et al.*, 1985). The difference in N yield due to the legumes and the interaction of legumes with the doses of N was found to be significant during the first year. *Mimosa invisa* produced a significantly higher quantity of nitrogen than *C. mucunoides* at both doses of fertilizer-N. It was also seen that the nitrogen yield from *M. invisa* was significantly greater at 250 g N than at 375 g N application. *Calopogonium mucunoides* showed no significant yield differences at either of the two doses of N application. During the second and third years, the nitrogen yield did not differ significantly in the two doses of fertilizer-N treatments. However, in the fourth year, the differences due to the legumes, doses of N, and their interaction were found to be significant.

The BNF inputs by legumes depend on the symbiotic efficiency of legume–*Rhizobium* asso-

Table 2 Nitrogen contribution (g basin^{-1}) of green manure legumes (*Mimosa invisa* and *Calopogonium mucunoides*) in coconut basins

Legume	Inorganic N (g palm^{-1})	Year 1	Year 2	Year 3	Year 4	Mean
M. invisa	250	45.78	154.02	201.28	188.92	147.50
M. invisa	375	32.54	135.54	176.57	144.18	122.05
C. mucunoides	250	11.63	92.52	85.76	81.51	67.86
C. mucunoides	375	18.37	99.01	93.12	77.66	72.04
Mean						
M. invisa		39.16	144.78	188.93	166.55	134.86
C. mucunoides		15.00	95.76	89.44	79.58	69.95
250 g N		28.71	123.27	143.52	135.22	107.68
375 g N		25.46	117.28	134.85	110.92	97.15
SE plot^{-1}		11.19	43.66	44.87	23.17	
CV (%)		41.33	36.30	32.24	18.82	
$LSD_{0.05}$						
Legumes		5.60	21.83	22.44	11.58	
N levels		ns	ns	ns	11.58	
Legumes × N levels		7.92	ns	ns	16.38	

SE, Standard Error; CV, Coefficient of Variation; LSD, Least Significant Difference
ns, Not significant

ciation. A higher nodule biomass was observed in *M. invisa* than in *C. mucunoides* (Table 3). There was no significant change in nodule biomass in *C. mucunoides* over the years. But in *M. invisa*, the nodule biomass increased significantly from the second year onwards. A higher ARA was observed in the nodulated root system of *M. invisa* and indicated better symbiotic effectiveness of the legume. The profuse nodulation and effective N_2 fixing association enabled *M. invisa* to accumulate more biomass and nitrogen than *C. mucunoides*. A high level of biomass and nitrogen contribution was recorded in *M. invisa* and *C. mucunoides* at 140 days of growth under the rainfall distribution pattern prevalent in this area. Reliance on N_2 fixation for growth and the total amount of legume nitrogen accumulated are important factors determining the contribution of legume N_2 fixation to the N economy in any ecosystem. Nitrogen fixation to the extent of 91 kg N_2 ha^{-1} was reported in *Macroptilium atropurpureum* in a growth period of 190–195 days under tropical conditions (Ladha *et al.*, 1996). Reynolds (1982) calculated inputs from N_2 fixation by *C. mucunoides* in the range of 136–182 kg N ha^{-1} during a period of one year in the grass–legume mixture. In the present experiment, the contribution of 137.8 g and 70 g N by *M. invisa* and *C. mucunoides* was from an area of 10.17 m in the root zone of coconut under partially shaded conditions during a growth period of 140 days. It may amount to a contribution of 135.45 kg and 68.81 kg N by the respective legumes when estimated on a per hectare basis for comparative purpose.

The level of major nutrients in the coconut basin soil did not differ significantly under the different treatments (Table 4). A trend towards higher nitrogen content in the treatments of legume biomass plus 75% fertilizer N rates and higher soil K in the *C. mucunoides* treatment was observed in the basin soil at 0–25 cm depth. The level of major nutrients in the coconut leaves also did not differ significantly under the different treatments. Fertilizer-N inputs were reduced up to 50% of the recommended dose, but their effects were not reflected in the soil nutrient status and foliar levels due to the substitution by legume biomass. The soil N pool benefited from BNF by the N_2 released from decomposition of legume biomass after incorporation. Snapp *et al.* (1996) reported that high quality residues of perennial legumes were most effective at supplying N in the short- to medium-term. A study on four management systems under rubber plantations in Malaysia by Broughton (1977) revealed greater nutrient returns by leguminous crops such as *Pueraria*, *Calopogonium*, and *Centrosema* when compared to other management systems. Diekmann *et al.* (1993) reported that incorporation of leguminous green manure with urea reduced gaseous N losses from urea when measured by ^{15}N and non-isotope techniques.

Ladha *et al.* (1996) reported significant contribution of below-ground residue of legumes to the rice crop. Buresh *et al.* (1993) showed that a sesbania crop reduced soil N loss by assimilating NO_3 and recycling it through incorporation into the legume green manure. The green manure legumes are considered as NO_3 catch crops capable of returning N in an organic form that is less susceptible to loss. Agboola (1974) reported the capability of green manure legumes to recycle leached plant nutrients by absorbing the nutrients from lower depths and translocating them to the leaves.

Table 3 Nodule biomass and acetylene reduction activity (ARA) of nodulated root system of green manure legumes (*Mimosa invisa* and *Calopogonium mucunoides*)

Legume	Nodule biomass (mg $plant^{-1}$ yr^{-1}) 1	2	3	4	Mean	ARA nM C_2H_4 $plant^{-1}$ h^{-1}
M. invisa	112.01	295.70	396.90	323.50	267.02	416.46
C. mucunoides	21.21	48.73	51.63	32.66	38.54	88.60
Mean	66.61	172.18	194.27	178.06		

Standard Error per plot, 92.94; Coefficient of Variation, 60.84%; Least Significant Difference (5%): Legumes, 54.23; Years, 76.69; Legumes x Years, 108.45

Table 4 Major nutrients [parts per million (ppm)] in coconut basin soil (0–25 cm depth) and leaf N content (%) as influenced by integrated treatment of inorganic and green manure (*Mimosa invisa* and *Calopogonium mucunoides*)

Treatment	Soil nutrient content N	P	K	Leaf N content
500 g N (Control)	213.9	27.9	270	1.76
M. invisa + 250 g N	215.5	31.2	308	1.85
M. invisa + 375 g N	236.2	26.2	310	1.80
C. mucunoides + 250 g N	223.1	32.3	350	1.79
C. mucunoides + 375 g N	235.0	36.6	350	1.80
SE $plot^{-1}$	26.1	8.3	55.9	0.11
CV (%)	11.6	26.7	17.6	6.01
$LSD_{0.05}$	ns	ns	ns	ns

SE, Standard Error; CV, Coefficient of Variation; LSD, Least Significant Difference
ns, Not significant

Biological activity was augmented in basin soils under the integrated treatment as indicated by microbial biomass content and dehydrogenase enzyme activity, when compared to the levels in the control (Table 5). The variation in microbial biomass, the key functional component of microbiota primarily responsible for decomposition, is influenced by the quality and quantity of organic matter inputs to the soil (Wardle, 1992). The microbial biomass (which ranged from 220 μg C to 370 μg C g^{-1} soil) recorded in the present study was within the range of 109 μg C to 390 μg C g^{-1} soil reported in an oil palm plantation in West Malaysia (Haron *et al.*, 1998). Even when grown as cover crops, legumes such as *C. mucunoides, P. phaseoloides,* and *Centrosema pubescens* provided a conducive environment for microbial proliferation, enzyme synthesis, and accumulation in soil matrix by increased C turnover and N availability in soils of a coconut plantation (Dinesh *et al.*, 1999). Addition of legume biomass to soil also enhanced heterotrophic N_2 fixation.

Table 5 Microbial biomass (μg C g^{-1} soil) and dehydrogenase enzyme activity (μg TPF g^{-1} soil h^{-1}) in coconut basin soil (0–25 cm depth) in relation to integrated green manure (*Mimosa invisa* and *Calopogonium mucunoides*) and fertilizer treatments

Treatment	Microbial biomass	Dehydrogenase activity
500 g N (Control)	223.71	2.59
M. invisa + 250 g N	297.18	3.68
M. invisa + 375 g N	249.32	2.62
C. mucunoides + 250 g N	343.52	3.22
C. mucunoides + 375 g N	368.57	3.54
Mean integrated treatment	314.65	3.27
SE $plot^{-1}$	112.21	0.92
CV (%)	37.85	29.32
$LSD_{0.05}$	ns	ns

SE, Standard Error; CV, Coefficient of Variation; LSD, Least Significant Difference
ns, Not significant

The yield of coconut palms showed substantial increase during the post-treatment period when compared to the pre-treatment yield (Table 6). Addition of nutrients either as chemical fertilizers or in the integrated form of chemical fertilizers with legume green manure resulted in increased yield of nuts. As the experimental palms did not receive the recommended dose of fertilizers during the pre-experimental period, the palms responded to the nutrient inputs which resulted in obtaining double the yield in the post-experimental period. Although not statistically significant, the palms which were under the integrated treatment produced more nuts than the palms under inorganic N treatment. The same trend was reflected in the cumulative yield of nuts in the palms during the four years

Table 6 Effect of N substitution by green manure legumes (*Mimosa invisa* and *Calopogonium mucunoides*) on the yield of coconut palm

Treatment	Nuts $palm^{-1}$ yr^{-1} Pre-treatment	Nuts $palm^{-1}$ yr^{-1} Post-treatment[1]	Cumulative yield[2] (nuts $palm^{-1}$)	Copra yield[3] (kg $palm^{-1}$ yr^{-1})
500 g N (Control)	24.50	65.48	246.31	12.03
M. invisa + 250 g N	26.87	76.86	278.18	15.83
M. invisa + 375 g N	25.50	73.45	287.00	12.10
C. mucunoides + 250 g N	25.63	73.62	260.50	14.47
C. mucunoides + 375 g N	25.19	73.28	268.18	13.89
Mean integrated treatment		72.84	273.47	14.10
SE $plot^{-1}$		20.09	78.83	4.44
CV (%)		27.58	29.41	32.46
$LSD_{0.05}$		ns	ns	ns

[1]Adjusted mean yield of 3rd and 4th year using covariance analysis taking pre-treatment yield as ancillary variable
[2]During the four years experimental period
[3]Means of 3rd and 4th year
SE, Standard Error; CV, Coefficient of Variation; LSD, Least Significant Difference
ns, Not significant

is judged not only by the number of nuts harvested but also on the basis of nut characters, particularly the copra output. The weight of copra, the dried kernel of the nut used for oil extraction, showed an increasing trend in integrated treatments when compared to the control.

Palms under both *C. mucunoides* and *M. invisa* treatments with 50% and 75% of recommended inorganic N produced an average increase of 8–10 nuts $palm^{-1}$ yr^{-1} in the post-treatment period than those palms which received the full dose of recommended fertilizer-N. This indicated that legumes effectively substituted up to 50% of the inorganic N requirement of coconut palm. The importance of N_2 fixation may be judged from the amount of N fertilizer required to attain comparable yield in plantation crops when a legume is not used. Cadigal *et al.* (1983) reported that ipil-ipil (*Leucaena leucocephala*) leaves substituted for ammonium sulphate in coconut seedlings in the Philippines. In the present study, the higher coconut yield recorded under the integrated treatment, compared with inorganic fertilizer alone, can be attributed to supply of N_2 and other nutrients and the improvement in soil fertility parameters as a result of cultivation and incorporation of the legumes.

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