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NITRIFICATION INHIBITORS MEDIATED ENHANCED NITROGEN USE EFFICIENCY IN MULBERRY (*MORUS* SPP.)

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ABSTRACT: Studies were conducted to assess the effect of various nitrification inhibitors on dry biomass, nitrogen content, uptake of nitrogen, apparent nitrogen recovery, population of nitrifying bacteria and yield in mulberry. Neem oil, Karanj oil and dicyandiamide (DCD) were used as nitrification inhibitors for coating urea and applied in the mulberry field in two levels. The results were compared with two levels of urea without coating nitrification inhibitors as well as with recommended dose of urea. Dry biomass of leaf (3374 kg/ha/) and dry biomass of shoot were significantly higher with neem oil coated urea while dry biomass of stem was (1735 kg/ha/) significantly higher due to DCD coating. Likewise, nitrogen content in leaf (3.56 %), stem (1.58%) and shoot (5.14%) was significantly higher with DCD and it was at par when urea coated with neem oil. Nitrogen uptake by mulberry leaf (120 kg/ha), stem (27 kg/ha) and shoot (261 kg/ha) was significantly higher and at par with DCD as well as with neem oil coating. The population of Actinomycetes (2.93 cfu×104 g^{-1} soil), Nitrosomonas (2.90 cfu×103 g^{-1} soil) and Nitrobacter (3.52 cfu×103 g^{-1} soil) was significantly less in DCD as well as neem oil coated urea treated soil. Apparent nitrogen use efficiency was highest with DCD at lower level of nitrogen fertilizer. The study suggests exploration of neem oil as nitrification inhibitor for coating urea to increase nitrogen use efficiency and curtail nitrogen fertilizer dose in mulberry cultivation which is also eco-friendly and cost effective.
Key words: DCD, Karanj oil, Mulberry, Neem oil, nitrification inhibitors

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INTRODUCTION

Nutritive value of mulberry leaf fed to silkworm is the key determinant of the quality of silk produced and hence, fertility of soil where the mulberry is cultivated has direct impact on quality and quantity of mulberry leaf. Among the soil nutrients, nitrogen plays an important role as it is the vital constituent of various bio-molecules such as amino acids, protein, chlorophyll [1,2].Therefore, nitrogenous fertilizers are vital in the growth and yield of mulberry [3,4]. The recommended dose of NPK fertilizer for high yielding mulberry variety V1 is 350:140:140 and for K2 it is 260: 120:120 kg/ha/yr. [5]. Though high dose of nitrogen is applied, the available nitrogen that the plant utilizes for its metabolism is only 30-35%. In plantation crops such as coffee and tea, N recovery is less than 30%, [6] even with the application of 300-900 kg N /ha/yr. This is because major part of applied nitrogen is lost through nitrification and associated processes, [7]. Significant portion of the applied and naturally mineralized fertilizer is leached out of the root zone, [8, 9]. Further excess use of nitrogenous fertilizers results the formation of nitrous oxides which reaches to the stratosphere and destroy the protective ozone layer, [10] that would increase the ultraviolet radiation reaching the earth's surface. Thus Loss of nitrogen and environmental concern warrantes to explore the potential of slow releasing nitrogen fertilizers as alternatives. This method showed promising results in various agricultural crops [11, 12, and 13]. However, though higher dose of N is recommended for mulberry cultivation, the potential of nitrification inhibitors to reduce N dose is not explored.

MATERIALS AND METHODS

Studies were conducted at the experimental garden of Central Sericultural Research and Training Institute, Mysore (12° 18' N latitude and 76° 42' E Longitude), during 2013 and 2014. The mulberry plants (Variety V1) were pruned and the soil samples were collected for analysis before imposing the treatment. The soil of experimental field was red sandy loam in texture. Before initiation of the experiment, the following soil parameters were determined as per standard methods as mentioned. The pH of the soil was 7.82, Electrical conductivity was 0.14 dSm^{-1} [14], organic carbon content was 0.45 % [15], available nitrogen was 295 kg/ha (16), phosphorous was 25 kg/ha [17] and was K 350 kg/ha [18]. The nitrogen content in plant was determined by modified miro-Kjeldahl [19].

The experiment comprised of six treatment combinations formed with two levels (300 kg and 250 kg/ha/yr) of nitrogen coated with three different nitrification inhibitors. Also, three controls comprising two levels of nitrogen without coating nitrification (T4 and T8) inhibitors and a recommended dose of nitrogen (T9) were kept for comparison as detailed below.

T_1: 300 N coated with Neem oil (0.5% v/w): 140 P: 140 K kg/ha
T_2: 300 N coated with Karanj oil (0.5% v/w): 140 P: 140 K kg/ha
T_3: 300 N coated with DCD (1.0% w/w):140:140 N P K kg/ha
T_4: 300 N: 140 P: 140 K kg/ha (control for nitrogen level 300 kg/ha)
T_5: 250 N coated with Neem oil (0.5%v/w): 140 P: 140 K kg/ha
T_6: 250 N coated with Karanj oil (0.5% v/w): 140 P: 140 K kg/ha
T_7: 250 N coated with DCD (1.0% w/w): 140 P: 140 K kg/ha.
T_8: 250 N: 140 P:140 K kg/ha ((control for nitrogen level 250 kg/ha)),
T_9: 350 N: 140 P: 140 K kg/ha (Recommended dose)

Area of each plot was 8.64 m^2 with 12 plants per plot planted in a paired row (150+90) cm x 60 cm system. The experiment was laid out in RBD with four replications per each treatment and controls. Castor oil is used as sticker for coating the nitrification inhibitor on the nitrogen fertilizer. Prilled urea was coated with castor oil in 100:1 (1 % w/v) proportion and shade dried for 24 h. Thereafter, it was coated either with Neem oil 0.5% (v/w), Karanj oil (0.5% v/w) or DCD 0.5% (w/w) separately and kept for 24 hours in shade for mineralization. This nitrification inhibitors coated urea was used in the experiment as mentioned above in the treatment details. The urea without coating nitrification inhibitors (T_4 & T_8) and recommended dose (T_9) served as controls. The soil samples were collected from experimental plots after the shoots attaining 65 days old just before harvesting. Similarly, leaf and stem samples were collected for analysis of nitrogen content.
The uptake of nitrogen and apparent nitrogen recovery were calculated by following formula.

Nutrient uptake (kg/ha) = Dry biomass × Nutrient content in plant/100
Apparent N recovery **(%)** = Uptake of N in treated plots - Uptake of
N in control plots ×100) / quantity of nitrogen applied

The soil samples were collected from all the plots just before harvesting and analysed for population of nitrifying bacteria *viz*., *Nitrosomonas*, *Nitrobacter* and actinomycetes by serial dilution method (20).

The data were subjected for analysis of variance (ANOVA) and means were compared to assess their significance.

RESULTS AND DISCUSSION

There was significant ($P<0.05$) difference in dry biomass of leaf due to different treatments. Significantly highest dry biomass of leaf was observed in T1 (3374 kg/ha) followed by in T3 (3354 kg/ha) when compared to recommended dose of nitrogen (3274 kg/ha). There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4) in dry biomass of leaf where all the treatments coated with nitrification inhibitor given significantly higher dry biomass. Similarly, the difference in dry biomass of leaf varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher dry biomass compared with non- coated urea. However, compared with recommended dose (T9) two treatments (T1 and T3) only showed significantly higher dry biomass.

Nitrogen content in mulberry leaf due to coating urea with different nitrification inhibitors increased significantly over non-coated at both levels of nitrogen (Table1) compared to recommended dose of nitrogen (3.30%). There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4) nitrogen content in leaf where all the treatments coated with nitrification inhibitor given significantly higher nitrogen content. Similarly the difference in nitrogen content varied between lower level (250 kg/ha) of coated and non-coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher nitrogen content compared with non-coated urea. However compared with recommended dose (T9), the difference was not significant at lower level of urea with nitrification inhibitors.

Table 1. Effect of nitrification inhibitors on nitrogen use efficiency and dry biomass

Treatments	Dry biomass leaf (kg/ha)	Nitrogen content leaf (%)	Nitrogen uptake leaf (kg/ha)	Dry biomass stem (kg/ha)	Nitrogen content stem (%)	Nitrogen uptake stem(kg/ha)
T1	3374	3.54	119	1732	1.51	26
T2	3110	3.27	102	1575	1.40	22
T3	3354	3.56	120	1735	1.58	27
T4	3082	2.73	84	1549	1.00	15
T5	2944	3.25	96	1488	1.22	18
T6	2865	3.11	89	1443	1.17	17
T7	3026	3.26	99	1531	1.25	19
T8	2635	2.17	57	1306	1.03	13
T9	3274	3.30	108	1665	1.35	23
LSD(P-0.05)	68.7	0.09	3.63	32.24	0.14	1.99

Data presented are average of eight crops

In case of nitrogen uptake by leaf (Table-1), at higher level (300 kg/ha), the nitrogen uptake was significantly more in T3 (120 kg/ha) followed by in T1 (119 kg/ha) compared with recommended dose of nitrogen (108 kg/ha). However, nitrogen uptake of leaf in lower level (250 kg/ha) was found less (57-96 kg/ha) compared with recommended dose (T9).

The treatments significantly influenced the dry biomass of stem compared to non-coated urea at both nitrogen levels (300 kg/ha & 250 kg/ha). Significantly highest biomass of stem was observed in T3 (1735 kg/ha) followed by in T1 (1732 kg/ha) compared with recommended dose of nitrogen (1665 kg/ha). There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4). All the treatments coated with nitrification inhibitor given significantly higher dry biomass stem. Similarly the difference in dry biomass stem varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher dry biomass stem compared with non coated urea. However, compared with recommended dose (T9) two treatments (T3 and T1) only showed significantly higher dry biomass stem.

Regarding Nitrogen content in stem, it increased significantly due to coating over non-coated at both levels of nitrogen. Significantly highest nitrogen content was obtained in T3 (1.58 %) followed by in T1 (1.51 %) compared to recommended dose of nitrogen (1.35 %). There was significant difference in higher level (300 kg/ ha) of urea coated with nitrification inhibitors (T1-T4), all the treatments coated with nitrification inhibitor given significantly higher value. Similarly the difference in nitrogen content varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher nitrogen content compared with non-coated urea. The highest nitrogen content in stem was recorded in T7 (1.25 %) followed by in T5 (1.22%). However, compared with recommended dose (T9) it was not significant at lower levels.

Significant ($P<0.05$) difference in nitrogen uptake by stem was observed due to different treatments. The highest nitrogen uptake was found in T3 (27 kg/ha) followed by in T1 (26 kg/ha) compared to recommended dose T9 (23 kg/ha). In case of higher level of nitrogen (T1-T4) the nitrogen uptake in all the treatments coated with nitrification inhibitor given significantly higher nitrogen uptake. Correspondingly, the difference in nitrogen uptake varied between lower level (250 kg/ha) also (T5-T8) was significant compared with non coated urea. However, compared with recommended dose (T9) treatments T3 and T1 only showed significantly higher nitrogen uptake.

Dry Shoot biomass due to different treatments was significantly varied due to treatments. Highest shoot dry biomass was observed in T1 (5108 kg/ha) followed by in T3 (5086 kg/ha) compared to recommended dose of nitrogen (4939 kg/ha). There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4) in dry shoot biomass where all the treatments coated with nitrification inhibitor given significantly higher dry shoot biomass. Similarly the same varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher dry shoot biomass compared with non coated urea. The significantly higher value (4548 kg/ha) was recorded in T7.

Table 2: Effect of nitrification inhibitors on mulberry dry shoot biomass and nitrogen use efficiency

Treatments	Dry shoot biomass (kg/ha)	Nitrogen plant (%)	Nitrogen uptake shoot (kg/ha)	Apparent nitrogen recovery (%)
T1	5108	5.05	258	28.3
T2	4686	4.67	219	15.3
T3	5086	5.14	261	29.3
T4	4631	3.73	173	0.00
T5	4432	4.46	198	28.8
T6	4307	4.28	184	23.2
T7	4558	4.51	205	31.6
T8	3941	3.20	126	0.00
T9	4939	4.65	230	19.0
LSD(P-0.05)	99.71	0.13	6.93	

Data presented are average of eight crops

Nitrogen content in shoot increased significantly over non-coated at both levels of nitrogen application (Table 2). Significantly highest nitrogen content was obtained with T3 (5.14 %) followed by T1 (5.05 %) compared to recommended dose of nitrogen (4.65 %). Significantly higher value of nitrogen content in shoot was recorded in T7 (4.51%) followed by in T5 (4.46) against non-coated urea at lower level (250 kg/ha).

Nitrogen uptake by the shoot significantly ($P<0.05$) deferred due to treatment. The highest nitrogen uptake was found in T3 (261 kg/ha) followed by in T1 (258 kg/ha) compared to recommended dose of nitrogen T9 (230 kg/ha). There was a significant difference between higher level (300 kg/ ha) of non--coated and coated urea (T1-T4) with all the treatments coated with nitrification inhibitor showed significantly higher nitrogen uptake. The difference in nitrogen uptake varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) also where all the treatments coated with nitrification inhibitor recorded significantly higher nitrogen uptake compared with non coated urea. The maximum nitrogen uptake was recorded in T7 (205 kg/ha). DCD and neem oil has long been known as a most effective retardant of nitrification inhibitor [21]. Also, high Nitrogen content with use of several natural products have been reported in rice [22, 23]

Apparent nitrogen recovery in nitrification inhibitors coated urea increased over non-coated at both levels of nitrogen (Table 2) with highest apparent nitrogen recovery in T7 (31.6 %) followed by T3 (29.30 %) while in case of recommended dose of nitrogen it was less (19%). The dry biomass yield, nitrogen content and uptake of nitrogen by mulberry leaf, stem shoot (Table 1 & 2) were increased with application of coated urea. Corresponding result was recorded in rice crop [24]. Increase in leaf, stem and shoot dry biomass, nitrogen content, uptake of nitrogen and apparent nitrogen recovery in coated urea treatment might due to high utilization of N from soil by mulberry plant as a result of retardant loss of N fertilizer by nitrification inhibitors. Neem oil, karanj oil and DCD have nitrification inhibiting properties as in several naturally occurring plant materials, because of presence of constituent such as nimin in neem oil and karanjin in karanj oil [25, 26, 27, 28, 29, 30, 31, and 32]. Since these bacteria are vital for transformation of ammonical form of nitrogen (NH_4^+) to nitrate (NO_3) form which is absorbed by the plants. The reduction in bacterial population which reduces the conversion rate and hence the applied nitrogen will be retained in the soil for a longer period. This longer period of nitrogen availability in the soil might have increased the nitrogen use efficiency of the plants. Similar observation on increase in nitrogen use efficiency and biomass was reported in Mentha-spent and pyrethrum flowers [33] and paddy (Bruce *et al*., 2013) due to application of nitrification inhibitors.

There was significant ($P<0.05$) difference in protein of due to different treatments. The highest protein content was observed in T1 (22.51 %) followed by in T3 (22.43%) compared with that observed in recommended dose of nitrogen (20.44%). In case of higher level (300 kg/ ha), all the treatments coated with nitrification inhibitor given significantly higher protein content. Similarly, the difference in protein varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher protein content compared with non- coated urea. However, compared with recommended dose (T9), three treatments (T1, T2 and T3) only showed significantly higher protein. Sugar content in mulberry leaf due to coating urea with different nitrification inhibitors increased significantly over non-coated at both levels of nitrogen (Table-3) compared with recommended dose of nitrogen (12.49%). There was significant difference between higher levels (300 kg/ ha) of non-coated and coated urea (T1-T4) sugar content in leaf where all the treatments coated with nitrification inhibitor given significantly higher sugar content. The highest sugar content was recorded in mulberry leaf with T1 (13.27 %) followed by in T3 (13.14 %).

Similarly the difference in sugar content varied between lower levels (250 kg/ha) of coated and non-coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher sugar content compared with non-coated urea. The maximum sugar was found in T5 (12.37 %) followed by in T7 (12.32 %). However compared with recommended dose (T9), the difference was not significant at lower level of urea with nitrification inhibitors. Compared to non-coated urea at both nitrogen levels (300 kg/ha & 250 kg/ha). Total chlorophyll (mg/g) varied significantly. Highest chlorophyll was observed in T1 (3.48 mg/g) followed by in T3 (3.29 mg/g) compared with recommended dose of nitrogen (3.19 mg/g). There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4). All the treatments coated with nitrification inhibitor given significantly higher Total chlorophyll. Similarly the difference in Total chlorophyll varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed significantly higher chlorophyll compared with non coated urea. However, compared with recommended dose (T9) two treatments (T1 and T3) only showed significantly higher chlorophyll content.

Population of nitrifying bacteria and actinomycetes significantly varied with application of coated urea (Table 3). Significant ($P<0.05$) reduction was observed in case of all the treatments irrespective of levels of nitrogen. Significantly ($P<0.05$) low actinomycetes population was observed in case of T3 (2.93 cfu$\times10^4$ g^{-1} soil) followed by T2 (3.36 cfu$\times10^4$ g^{-1} soil) against highest (5.24 cfu$\times10^4$ g^{-1} soil) observed in (T8).

Table 3. Effect of nitrification inhibitors on population of *Actinomycetes* and nitrifying bacteria

Treatments	Total *Actinomycetes* (cfu$\times10^4$)g^{-1}soil	*Nitrosomonas* (cfu$\times10^3$) g^{-1} soil	*Nitrobacter* (cfu$\times10^3$) g-^{1} soil
T1	3.36	2.98	4.02
T2	3.77	3.05	4.69
T3	2.93	2.90	3.52
T4	5.03	4.72	12.26
T5	3.63	3.02	4.55
T6	3.80	3.20	5.74
T7	3.15	2.78	3.77
T8	5.24	4.79	11.98
T9	5.19	4.69	12.02
SEm+	0.12	0.11	0.24
CD ($P<0.05$)	0.35	0.32	0.71

Similarly, population of *nitrosomonas* significantly ($P<0.05$) reduced due to the treatments. Least number of *nitrosomonas* population was recorded in T7 (2.78 cfu$\times10^3$ g^{-1} soil) followed by T3 (2.90 cfu$\times10^3$ g^{-1} soil) when compared to recommended dose of nitrogen (4.69 cfu$\times10^3$ g^{-1} soil). The highest number of *nitrosomonas* population was recorded in T8 (4.79 cfu$\times10^3$ g^{-1} soil) followed by inT9 (4.69 cfu$\times10^3$ g^{-1} soil). However, compared with recommended dose (T9) and non-coated urea, all nitrification inhibitors coated urea significantly reduced total of *nitrosomonas* population in the soil. Likewise, the treatments influenced the population of *Nitrobacter*. Population was least in T3 (3.52 cfu$\times10^3$ g^{-1} soil) followed by T7 (3.77 cfu$\times10^3$) g^{-1} soil) compared with recommended dose of nitrogen (12.02 cfu$\times10^3$ g^{-1} soil). The highest number of *Nitrobacter* population was recorded in T4 (12.26 cfu$\times10^3$ g^{-1} soil) followed by in T9 (12.02 cfu$\times10^3$ g^{-1} soil). However, compared with recommended dose (T9) all nitrification inhibitors coated urea significantly reduced *Nitrobacter* population in soil (Table 3). Identical observations were made [34, 35, and 36]. The result on the population of nitrifying bacteria was in corroboration with the reports of Goring [37], where retardation of nitrification by N-serve is reported due to the toxicity to ammonium oxidizing autotrophic *Nitrosomonas,* and *Nitrobacter.* Similar observations on reduction of nitrifying bacteria due to nitrification inhibitors were reported in various agriculture crops also [38, 39]

CONCLUSION

The study shows significantly higher leaf, stem shoot and nitrogen use efficiency by the use of either DCD or Neem oil as nitrification inhibitors compared with recommended dose of nitrogen. The DCD is a proven nitrification inhibitor which is not economical and also is reported to be phototoxic. The results shows effectiveness of neem oil at par with that of DCD and it is easily available, inexpensive and biodegradable. Therefore, neem oil could be successfully used as nitrification inhibitor to reduce the nitrogen fertilizer use in mulberry cultivation and also to reduce fertilizer related environmental hazards.

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