Environment Conservation Journal 17 (3), 1-9,2016
ISSN 0972-3099 (Print) 2278-5124 (Online)
Abstracted and Indexed

Effect of nitrification inhibitors on physio-chemical properties, growth and yield attributes of Mulberry (*Morus*spp.)

Vinod Kumar Yadav✉, P.M.PratheeshKumar and V.Sivaprasad

Received: 25.05.2016 **Revised: 11.08.2016** **Accepted:12.09.2016**

Abstract

Experiments were conducted to assess the effect of urea amended with nitrification inhibitors. Application of urea amended with nitrification inhibitors were influenced physiochemical properties of soil such as pH (7.47) and electrical conductivity (0.25 µmhos/cm) were significantly low with neem oil coating as well as in DCD. Similarly the chemical properties such as available phosphorus (64.88 kg/ha) and potassium (299.88 kg /ha) were also significantly higher and on par due to coating with neeem oil or DCD as nitrification inhibitor. Significantly high soil physical properties such as porosity 45.69 % as well as water holding capacity and low bulk density was found low (1.14 mmhos/cm) were also found in soil treated with both neeem oil and DCD amended urea. The biochemical and physiological attributes *viz*., chlorophyll, sugar, leaf protein, moisture content and moisture retention capacity of mulberry were found higher when neem oil or DCD used as nitrification inhibitor. The yield parameters such as number of shoot per plant (10), plant height (177 cm), length of longest shoot (180 cm), number of leaves/shoot (32.18) and total leaf yield (11353 kg/ha/crop) were observed at par with the use of DCD and neem oil as nitrification inhibitors.

Key Words: biochemical,DCD,Karanj oil, Mulberry, Neem oil, nitrification inhibitors, physiochemical

Introduction

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, sugar as well as physiological parameters such as moisture content and moisture retention (Singhal et al., 2000; Vijaya*et al*.,2009; Rao*et al*., 2011; Babu*et al*., 2013). Among nitrogenous fertilizers, prilled urea is applied as source of N for plants. When applied to soil, urea is hydrolyzed by urease enzyme to form ammonium, which is subsequently converted to nitrite and nitrate by nitrifying bacteria. However, the applied N is lost by several processes such as ammonia volatilization, emission of nitrous oxide by the action of denitrifying organisms and leaching of nitrate form of nitrogen ($NO^-_{3})$ with water. This loss leads to poor recovery of fertilizer nitrogen, which rarely exceeds 50% (Prasad *et al*., 1998). The loss is more pronounced in sandy soils (Katyal*et al*., 1985). Mulberry is the sole food plant of silkworm (*Bombyxmori* L.). The mulberry leaves are repeatedly harvested throughout year for silkworm rearing. Maximization of leaf production per unit area is major concern among mulberry growers. Since fertility of soil have direct impact on qualitative and quantitative mulberry leaf production, and hence, availability of essential nutrient like nitrogen is equally vital. Requirement of nitrogen for mulberry cultivation varies from 250 to 350 kg /ha/year (Purohit*et al*., 1996; Rajanna*et al*.,1993. The nitrogen is highly mobile and unstable mineral nutrient is soil. It is available in soil for very short period after application as it is lost from soil due to nitrification, leaching, volatilization and denitrification (Purakayastha*et al*., 1997). Nitrifying bacteria grow on the surface of soil particles in close proximity to soil are responsible for conversion of ammonical form of nitrogen to nitrate form (Harmsen and Van Schreven, 1955). Nitrifier growth rate is proportional to the nitrification rate in soil and is maximum when the soil moisture is at field capacity (Myers *et al*., 1982). On the other hand, in saturated soils, nitrification nearly stops due to lack of $O_{2;}$ it also stopsin very dry soils (Justice and Smith, 1962; Parker and Larson, 1962). To ensure continuous and optimal supply of N and to increase fertilizer use efficiency, there is a need to retard the

Author's Address
Department of Agronomy, Central Sericultural Research and Training Institute, Srirampura, Mysore India
E-mail:vinodkumarydv@gmail.com

rate of urea hydrolysis or nitrification or both. To improve availability and use efficiency nitrogen in soil several synthetic chemicals such as N- serve, DCD, CS2, sodium chlorate and BHC, have been examined for inhibition of urea hydrolysis and nitrification or both in soils. However, the use of many of these chemicals has been restricted academic studies because, of high cost, lack of availability and hazardous effects. Plant materials such as Karanj (*Pongamiaglabra*), Neem (*Azadirachta indica*) and Tea (*Camellia sinensis*) wastes were used effectively to inhibit nitrification in many agricultural crops (Prasad *et al*., 1971; Sahrawat*et al*., 1975; Prasad *et al*., 1995 &Patra*et al*., 2001, 2002 and 2006) and are also locally available, cost effective and eco-friendly effective alternatives. Since mulberry is cultivated in about 2.10 lakhs ha in the country, a huge quantity of nitrogen is recommended for mulberry cultivation. In order to reduce the nitrogen fertilizer application, reduce the production cost as well as to reduce the hazards due to environment pollution, the possibility of application of slow releasing nitrogenous fertilizers in mulberry leaf production is warranted.

Materials and Method

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 (Jackson, 1973), organic carbon content was 0.45 % (Walkely & Black, 1934), available nitrogen was 295 kg/ha (Subbiah and Asija, 1956), phosphorous was 25 kg/ha (Murphy and Riley, 1962) and was K 350 kg/ha (Jackson, 1973). The nitrogen content in plant was determined by modified miro-Kjeldahl (Jackson, 1973).

The biochemical parameters such as chlorophyll (Arnon, 1949), Sugar (Dubious at al., 1956) and protein (Lowry et al., 1951) were estimated as per standard procedure. The moisture percentage was determined from difference in dry and fresh weight and moisture retention was calculated by following the formula.

Moisture% = (Weight of fresh leaves–Weight of dry leaves) ×100/ Weight of fresh leaves.

Moisture Retention Capacity = (Weight of leaves after 6 hrs- dry weight of leaves) ×100/ weight of leaves after 6 hrs.

The experiment comprised of six treatments 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.

T1: 300 N coated with Neem oil (0.5% v/w): 140 P: 140 K kg/ha

T2: 300 N coated with Karanj oil (0.5% v/w): 140 P: 140 K kg/ha

T3: 300 N coated with DCD (1.0% w/w):140:140 N P K kg/ha

T4: 300 N: 140 P: 140 K kg/ha (control for nitrogen level 300 kg/ha)

T5: 250 N coated with Neem oil (0.5%v/w): 140 P: 140 K kg/ha

T6: 250 N coated with Karanj oil (0.5% v/w): 140 P: 140 K kg/ha

T7: 250 N coated with DCD (1.0% w/w): 140 P: 140 K kg/ha.

T8: 250 N: 140 P:140 K kg/ha ((control for nitrogen level 250 kg/ha)),

T9: 350 N: 140 P: 140 K kg/ha (Recommended dose)

Area of each plot was 31.68 m2 with 44 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 (T4 & T8) and

recommended dose (T9) served as controls. The soil samples were collected from experimental plots after the shoots attaining 65 days old just before harvesting. Similarly, the growth attribute parameters were recorded.
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 no of shoots per plant due to different treatments. Significantly highest No. of shoots/ plant (10) was observed in T3 followed by in T1 (9) which was less (8) due to recommended dose of nitrogen (Table1). There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4) in number. of shoots/ plant where all the treatments coated with nitrification inhibitor given significantly higher number of shoots/ plant. Similarly the difference in No. of shoots/ plant 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 No. of shoots/ plant compared with non-coated urea application. However, compared with recommended dose (T9) two treatments (T1 and T3) only showed significantly higher No. of shoots/ plant. Nitrification inhibitors coated urea had significant impact on height of plant with significantly in T1 (177cm), which was at par with T3 (177cm). .Plant height significantly increased due to coating urea with nitrification inhibitors. There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4) plant height where all the treatments coated with nitrification inhibitor showed significantly higher plant height. The difference in plant height 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 plant height compared with non-coated urea. The higher value was recorded in T7 (156 cm) followed by in T6 (152 cm) compared with non-coating at lower nitrogen level. However compared with recommended dose (T9), the difference was non significant at lower level of urea coated with nitrification inhibitors.
In case of length of longest shoot, at higher level (300 kg/ha), higher plant height was found in T1 (180 cm) which was at par with T3 (180 cm) and less due to recommended dose of nitrogen (171 cm). At lower nitrogen level plant height was maximum with T5 (156 cm) at par with T3 (156 cm) and least at lower level (250 kg/ha) was found less (146 cm-156 cm).
Regarding number of leaves/shoot, it increased significantly due to coating over non-coated at both level of nitrogen. Significantly number of leaves/shoot was obtained in T3 (32.18) followed by in T1 (31.75) compared to recommended dose of nitrogen (28.63). 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 number of leaves/shoot 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 Number of leaves/shoot compared with non-coated urea. The highest number of leaves/shoot was recorded in T5 (24.02) followed by in T7 (23.68). However, compared with recommended dose (T9) it was not significant at lower level.Significant ($P<0.05$) difference in leaf yield was observed due to different treatments. The highest leaf yield was found in T1 (11353 kg/ha/crop) followed by in T3 (11311 kg/ha/crop) compared to recommended dose T9 (9718 kg/ha/crop).In case of higher level of nitrogen (T1-T4) the in leaf yield all the treatments coated with nitrification inhibitor given significantly higher leaf yield. Correspondingly the difference in Leaf yield varied between lower level (250 kg/ha) also (T5-T8) was significant compared with non coated urea. However, compared with recommended dose (T9) treatments T1 and T3 only showed significantly higher leaf yield. The application of urea with nitrification inhibitors were increased growth attribute as well as mulberry leaf yield compared without nitrification inhibitors in both level of nitrogenthis is probably due to the inhibitory action of nitrification inhibitors in coated compared to non coated urea. The neem oil were found as effective nitrification inhibitor in term of growth of mulberry

Treatments	Shoots/ plant	Plant height (cm)	Length of longest shoot(cm)	Leaves/ shoot	Leaf yield (kg/ha/crop)
T1	9.0	177	180	31.75	11353
T2	8.0	172	171	28.44	9334
T3	10	177	180	32.18	11311
T4	7.0	158	158	23.89	8167
T5	7.0	151	156	24.02	8450
T6	7.0	152	152	23.02	7915
T7	7.0	156	156	23.68	8668
T8	6.0	146	146	19.21	6241
T9	8.0	171	171	28.63	9718
C.D at 5 %	0.21	6.42	3.77	0.59	256

Table1. Effect of nitrification inhibitors on growth and yield attributes of mulberry

Treatments	pH	EC (mmhos /cm)	BD (g/cm^3)	Porosity (%)	WHC (%)	Available P kg /ha	Available K kg/ha
T1	7.47	0.25	1.14	45.69	36.23	64.88	299.88
T2	7.46	0.26	1.16	44.91	35.99	58.74	293.74
T3	7.51	0.27	1.17	45.02	35.67	55.99	290.99
T4	7.60	0.29	1.24	44.45	34.94	46.83	281.83
T5	7.51	0.24	1.18	44.80	35.75	59.57	294.57
T6	7.48	0.25	1.17	44.61	35.97	61.38	292.81
T7	7.56	0.26	1.18	44.68	35.09	54.91	289.91
T8	7.63	0.32	1.25	44.00	34.61	44.89	279.89
T9	7.75	0.30	1.26	44.55	34.93	54.60	289.60
SEm±	0.05	0.01	0.02	0.20	0.37	3.37	3.37
CD at 5%	0.15	0.03	0.08	0.58	1.08	9.84	9.84

Table 2. Effect of nitrification inhibitors on physiochemical properties of soil

attribute compared to non-.coated urea. Similar observations were also made by PrakasaRao and bhat (1984) and PrakasaRao and Puttanna (1987),The Significantly lower soil pH was observed in T2 (7.47) followed by in T1 (9) when compared to recommended dose of nitrogen (7.75). There was no significant difference were found between higher and lower level (300 kg/ ha & 250 kg/ ha) of non-coated and coated urea (T1-T4 and T5-T8). In general higher value of pH was recorded in T9

(7.75) and lower value was recorded with T2 (7.46). In general wherever, urea was coated with nitrification inhibitors soil pH slightly decreased (Table 2). In case of soil electrical conductivity had no significant difference was recorded and lower Ec value was recorded in T5 (0.24 mmhos/cm) and maximum value was found in T8 (0.32 mmhos/cm).The data revealed that bulk density of soil was decreased with application nitrification inhibitors. Significantly minimum value was recorded in T1 (1.14 g/cm3) followed by T2 (1.16 g/cm3) as compared to the recommended dose nitrogen T9 (1.26 g/cm3).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 registered significantly minimum value .Similarly the difference in the bulk density varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor were yielded significantly lower bulk density compared with non coated urea. However, compared with recommended dose (T9) treatments (T1, T2, T3 and T6) showed significantly lower soil bulk density.Concerning porosity of soil, it increased significantly due to coating over non-coated at higher level of nitrogen application. All the treatment coated wit nitrification inhibitors registered higher value as against to non-coated urea. Significantly maximum value had noticed with T1 (45.02 %), T2 (45.02%) followed by T3 (44.91 %) compared to recommended dose of nitrogen (44.55 %). 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. Likewise the variation in soil porosity varied between lower level (250 kg/ha) of coated and non coated urea (T5-T8) where all the treatments coated with nitrification inhibitor showed s higher value of porosity against non-coated urea. In case of lower level of nitrogen highest value was recorded in T5 (44.80 %) followed by T7 (44.68 %). In case of water holding capacity at higher level (300 kg/ha), higher water holding capacity value was found with T1 (36.23 %). It was at par with T2 (35.99 %) and T3 (35.67 against recommended dose of nitrogen (34.93 %). All the treatment coated with nitrification inhibitors was found higher value in both level of nitrogen against non-coated urea nitrogen. Though, water holding capacity at lower level (250 kg/ha) was found less (34.61 % - 35.75 %) compared with recommended dose (T9).Regarding available phosphorous in soil it was increased due to coating compared with non-coated at both level of nitrogen. The highest value of available phosphorus noticed with T1 (64.88 kg /ha), it was significantly higher against recommended dose of nitrogen, other treatments were not significant. In case of lower level of nitrogen, highest available phosphorus was recorded with T6 (61.38 kg/ha) followed by in T5 (59.57 kg /ha). Available potassium in soil was increased due to coating at both level of nitrogen. The highest available potassium was noticed with T1 (299.88 kg /ha) other treatment did not showed significant increase. In lower level of nitrogen, significantly higher available potassium was recorded with T5 (294.57 kg/ha) followed by in T6 (292.91 kg /ha)over non- coated urea. Soil physical and chemical properties plays major role on available nutrient to the plant (Strong et al., 1999). In the soil environment, porosity and water holding are critical factors that influence nitrification activity (Focht and Verstraete, 1977; Yuan *et al.,* 2005). Oxygen concentration in the soil is reduced at higher soil moisture as most of the soil pore spaces are filled with water (Focht and Verstraete, 1977. Optimum pH and EC for the growth of nitrifying bacteria is in the range of 6.7 to 8.5 and < 1 mmhos/cm, respectively (Kyveryga et al., 2004).

Significant difference in organic carbon in soil was observed due to different treatments. The highest organic carbon was found in T1 (0.64 %) followed by in T3 (0.62 %) compared to recommended dose T9 (0.43 %). In case of higher level of nitrogen (T1-T4), all the treatments coated with nitrification inhibitor showed significantly higher soil organic carbon. Correspondingly the difference in organic carbon varied between lower level (250 kg/ha) also (T5-T8) was significant compared with non coated urea. However, compared with recommended dose (T9), treatments T1, T2, T3 and T7 showed significantly higher organic carbon in soil (Fig. 1).

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 recommended dose of nitrogen (20.44%). In case of higher level (300kg/ha), all the treatments coated with nitrification inhibitor given significantly higher protein content. Similarly, the difference in protein varied betweenlower 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.

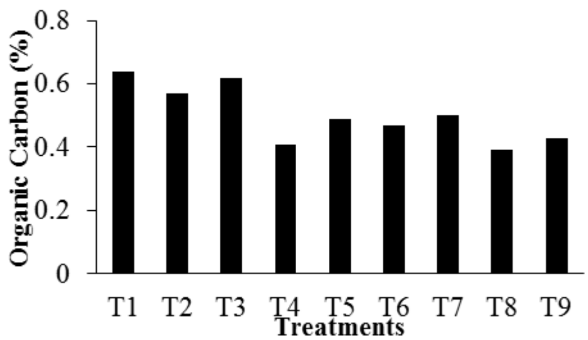

Fig1. Effect of nitrification inhibitors on organic carbon content in soil

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 yotal 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.Significant (P<0.05) difference in leaf moisturewas observed due to different treatments.

Treatments	Proteins (%)	Sugar (%)	Total chlorophyll (mg/g f. wt)	Leaf moisture (%)	Shoot moisture (%)
T1	22.51	13.27	3.48	75.06	67.75
T2	21.37	12.89	3.23	72.78	65.53
T3	22.43	13.14	3.29	74.98	67.70
T4	19.92	11.50	3.10	71.73	64.48
T5	19.41	12.37	3.11	72.48	65.23
T6	19.19	12.21	3.03	72.00	64.75
T7	20.24	12.32	3.10	72.65	65.40
T8	17.91	11.13	2.92	68.72	61.47
T9	20.44	12.49	3.19	73.29	66.04
SEm±	0.37	0.15	0.05	0.20	0.20
CD at 5%	1.08	0.45	0.14	0.58	0.59

Table 3. Effect of nitrification inhibitors on quality of mulberry leaf

The highest leaf moisture was found in T1 (75.04 %) followed by in T3 (74.98 %) compared to recommended dose T9 (73.29 %). In case of higher level of nitrogen (T1-T4) the leaf moisture in all the treatments coated with nitrification inhibitor given significantly higher leaf moisture. Correspondingly, the difference in leaf moisture varied between lower level (250 kg/ha) also (T5-T8) was significant compared with non coated urea. However, compared with recommended dose (T9) treatments T1 and T3 only showed significantly higher leaf moisture. Shoot moisture due to different treatments was significantly varied with high moisture observed in T1 (67.75 %) followed by in T3 (67.70 %) compared with recommended dose of nitrogen (66.04 %). There was a significant difference between higher level (300 kg/ ha) of non-coated and coated urea (T1-T4) in shoot moisture where all the treatments coated with nitrification inhibitor given significantly higher shoot moisture. 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 shoot moisture compared with non coated urea. The significantly higher value (65.23%) was recorded in T5. Nitrogen is an important constituent of protein and chlorophyll and helps water absorption which in turns increases moisture retention. Similar observations were reported in various agriculture crops and mulberry also (Matsuma *et al*., 1955; Tangamani R and Vivekanandan 1984; Paul *et al*., 1992; Bongale *et al*., 1994 and Chaluvachari, 1995).

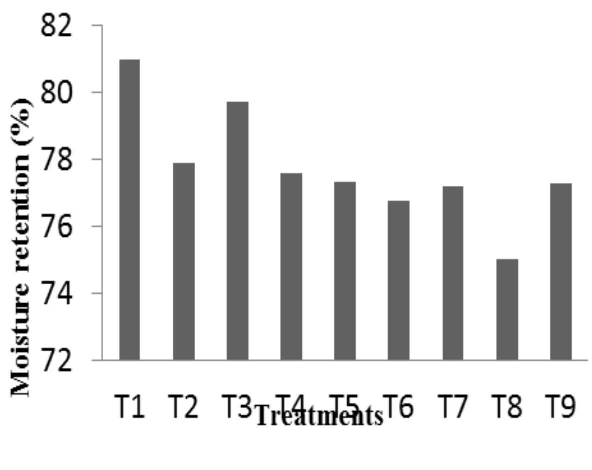

Fig. 2: Effect of nitrogen inhibitors on moisture retention of mulberry

Regarding moisture retention (%) in leaf, it increased significantly due to coating over non-coated at both levels of nitrogen (Fig. 2). Significantly highest moisture retention was obtained in T1 (80.98 %) followed by in T3 (79.74 %) compared to recommended dose of nitrogen (77.30 %). 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 moisture retention 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 moisture retention compared with non-coated urea. The highest moisture retention in leaf was recorded in T5 (77.32%) followed by in T7 (77.21%). However, compared with recommended dose (T9) it was not significant at lower levels.

Conclusion

The mulberry leaves are harvested every two months throughout the year for silkworm rearing. Fertility of soil has direct impact on quality and quantity of mulberry leaf and maximization of leaf production per unit area is major concern among mulberry growers. The recommended dose of nitrogen in mulberry cultivation is very high which is lost in a short period due to various nitrification associated process and hence meagre part of nitrogen applied is available for mulberry. The present study shows the effect of neem oil at par with that of DCD for nitrification inhibition and in turn positive influence on physio-chemical and growth attributes of mulberry since the applied nitrogen is available for the plant for a long time due to nitrification inhibition. This study recommends use of neem oil as nitrification inhibitor in mulberry which is ecofriendly and cost effective.

References

Arnon, O. L., 1949. Copper enzymes in isolated chloroplasts polyphenoxidase in *Beta vulgaris*. *Pl. Physiol*, 24:1-15.

Babu, C. M., Dandin, S. B., Thippeswamy, T. and Renukeswarappa, J. P., 2013. Nutritional status of mulberry leaf produced through organic farming and its impact on cocoon production. *Indian J. Seric*, 52:14-18.

Dubious, M. K., Gilles, A. Hamilton, J. K., Rebers., P. A. and Smith, F., 1956. Calorimetric method for determination of sugars and related substances. *Anal. Chem*, 28: 350- 356..

Focht, D.D., and Verstraete, W., 1977. Biochemical ecology of nitrification and denitrification. *Advances in Microbial Ecology,* 1:135-214.

Geethalakshmi, V., Christopher, L. A., Maragatham, N., 1998. Nitrification retardation property of some plant products and their effect on N uptake and nitrogen use efficiency in cotton. *Indian Journal of Agricultural Research,* 32: 271-277.

Harmsen, G. W., and Van Schreven, D. A., 1955. Mineralization of organic nitrogen in soil. *Adv. Agron*, 7: 299-398.

Jackson, M. L., 1973. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd. New Delhi

Justice, J. K., and Smith, R. L., 1962. Nitrification of ammonium sulfate in a calcareous soil as influenced by combinations of moisture, temperature, and levels of added nitrogen. *Soil Sci. Soc. Am. Proc*., 26: 246-250.

Katyal, J. C., , B, Vlek, P. L. G.. and Craswell, E. 1985. Fate and efficiency of nitrogen fertilizers applied to wetland rice: II.Punjab, India. Nutr Cycl Agroecosyst. 6: 279–290.

Kelling, K . A, Wolkowski, R. P., Raurk, M. P., 2011. Potato response to nitrogen form and nitrification inhibitors. *Am. J. Potato Res*, 88: 459-469.

Kiran, U. and Patra, D.D., 2002. Augmenting yield and Urea-N utilization efficiency in wheat (*Triticum aesitivum*) through use of natural essential oils and dicyandiamide coated urea in light textured soils of central Uttar Pradesh, India. *Communication in Soil Science and Plant Analysis*, 33: 1375-1388.

Kyveryga, P. M., Blackmer, A. M., Ellsworth, J. W. and Isla, R., 2004. Soil pH effects on nitrification of fall-applied anhydrous ammonia. *Soil Sci. Soc. Am. J*., 68: 545-551.

Lowry, O. H., Rosenbrough., N. J., Farr, A., Randall, R. J. J., 1951. Protein measurement with the folin phenol reagent. *Biol. Chem*., 193: 265-75

Matsuma, S., Tanaka, S. and Kosaka, T., 1955. Studies on the nutrition in popular varieties of the silkworm, Sanshi shikenjo Hokoku. *Tech. Bull*., 69: 1-47.

Murphy, J. and Riley, J. P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta*., 27:31-36.

Myers, R. J. K., Campbell, C. A. and Weier, K. L., 1982. Quantitative relationship between net nitrogen mineralization and moisture content of soils. *Can. J. Soil Sci.,* 62: 111-124.

Parker, D. T. and Larson, W. E., 1962. Nitrification as affected by temperature and moisture content of mulched soils. *Soil Sci. Soc. Am. Proc*., 26: 238-242.

Patra, D.D., Anwar, M., Chand, S., 2002. Nimin and *Mentha spicata* oil as nitrification inhibitors for optimum yield of Japanese mint (*Mentha arvensis*). *Communication in Soil Science and Plant Analysis*, 33: 451-460.

Patra, D.D., Anwar, M., Chand, S., 2001. Use of mint essential oil as an agrichemical: Control of N-loss in crop fields by using mint essential oil- coated urea as fertilizer. *Current Science*, 81: 1526-1528.

Patra, D. D., Kiran, U., Pande, P., 2006. Urease and nitrification retardation properties in natural essential oils and their by- products. *Communication in Soil Science and Plant Analysis,* 37: 1663-1673.

Paul, D. C., Subba Rao., G. and Reb, D. C., 1992. Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* and concomitant larval duration. *J. Insect Physiol*, 38: 229-235.

Prakasa Rao, E. V. S. and Bhat, P. R., 1984. Ammonia volatilization losses from prilled urea, supergranuls and sulpher coated urea, when surface applied and deep placed. *Fertil. News*, 29 : 31

Prakasa Rao, E. V. S. and Puttanna, K., 1986. Nitrification and Ammonia volatilization losses from urea and dicyandiamide treated urea in a sandy loam soil. *Plant soil*, . 97: 201-206

Prasad, R., Power, J. F., 1995. Nitrification inhibitors for the agriculture, health and environment. *Advances in Agronomy*, 54: 233-281.

Prasad, R., Rajale, G. B., Lakhdive, B. A., 1971. Nitrification retarders and slow release nitrogenous fertilizers. *Advances in Agronomy*, 23:337-405

Prasad, R., 1998. Fertilizer Urea, Food Security, Health and the Environment. *Curr. Sci*., 75 : 677– 683.

Purakayastha, T. J., Katyal, J. C, Goswami, N. N., 1997. Evaluation of some modified urea fertilizers applied to rice. *Fertilizer News*, 42: 53-56.

Purohit, M. K. and Kumar, Pavan., 1996. Influence of various agronomical practices in India on the leaf quality in mulberry. *A review. Sericologa,* 36: 27-39.

Rajanna, L. and Dandin, S. B., 1993. Effect of sources and level of nitrogen on mulberry an economic evaluation. *Sericologia*, 33: 541-544.

Ram, M., Yadav, R.L., Chatterjee, B.N., Singh, D.V., 1989. Relative Efficacy of Nitrogen at Different Rate and Time of Application on Growth and Yield of Japanese Mint (*Mentha arvensis*). *J. Agric. Sci*., 59 : 236 –241

Rao, D. M. R, Chikkanna., Vindhya, G. S., Reddy, M. M., Dasappa and Qadri, S. M. H., 2011. Effect of green manure and seri-compost on soil health, leaf quality and quantity traits of mulberry under tropical conditions. *Green Farming,* 2: 290-293.

Sahrawat, K. L., Parmar, B. S., 1975. Alcohol extract of 'neem' as nitrification inhibitors. *Journal of IndianSociety of Soil Science,* 23: 131-134.

Singhal, B. K., Chakraborti, S. Mala, V. R., Sarkar, A. and Datta, R. K., 2000. Photosynthesis for crop improvement in mulberry (*Morus spp*.) - A review. *Sericologia*, 40: 27-55.

Thomas, J. and Prasad, R., 1987. Relative efficiency of prilled urea, urea supergranuls, sulfer coated urea and nitrification inhibitor N-serve blended urea for direct seeded rice. *J. Agric. Sci*., 106:185-190.

Strong, D. T., Sale, P. W. G. and Helyar, K. R., 1999. The influence of soil matrix on nitrogen mineralisation and nitrification. IV. Texture. *Aust J. Soil Res*., 37: 329-344.

Tangamani, R. and Vivekanandan, M., 1984. Physiological studies and leaf nutrient analysis in the evaluation of best varieties. *Sericologia*. 24: 317-324.

Vijaya, D., Yeledhalli, N. A., Ravi, M. V., Nagangoud, A. and Nagalikar, V. P., 2009. Effect of fertilizer levels and foliar nutrients on M-5 mulberry leaf nutrient content, quality and cocoon production. *Karnataka J. Agric. Sci.* 22: 1006-1012.

Villar, J. M. and Guilaumes, E., 2010. Use of nitrification inhibitors in irrigated wheat on a calcareous soil. *Spanish J. Agric, Res*.. 8: 1218-1230

Yuan, F., Ran, W., Shen, Q. and Wang, D., 2005. Characterization of nitrifying bacteria communities of soils from different ecological regions of China by molecular and conventional methods. *Biol. Fertil. Soils,* 41: 22-27.