COMMUN. SOIL SCI. PLANT ANAL., 33(3&4), 451–460 (2002)

NIMIN AND *MENTHA SPICATA* OIL AS NITRIFICATION INHIBITORS FOR OPTIMUM YIELD OF JAPANESE MINT

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ABSTRACT

In order to improve nitrogen utilization efficiency of crops, which seldom exceeds 50%, numerous synthetic chemicals have been examined by several workers, for inhibition of either urea hydrolysis or nitrification in soils. However, for nitrification inhibitors to be popular in developing countries it must be cost effective and biodegradable. Field experiments were conducted for two years (1997–98) to evaluate the performance of two natural products, *Mentha spicata* oil and nimin (tetranortriterpinoids), an alcohol extract of Neem (*Azadirachta indica* Juss) as nitrification inhibitors. Prilled urea was coated with essential oil of *Mentha spicata*, nimin and a synthetic inhibitor dicyandiamide (control) at the rate of 1% on w/w basis and their effects on herb and essential oil yield and nutrient accumulation in Japanese mint (*Mentha arvensis* L.) was studied. The natural products significantly increased the herb and essential oil yield of mint compared to prilled urea applied without any coating material.

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The natural products were also found as effective as DCD. As expected, nitrogen (N) accumulation in the plant also significantly increased with the coating of urea. The average apparent N recovery was estimated to be about 50%, 42%, and 34% at 100, 200, and 300 kg ha^{-1} rate of N application, respectively. Average NO_3–N formation was low in coated urea treated soils. The pattern of inhibition of nitrification and increase in fertilizer N use efficiency by nimin and *M. spicata* oil suggest that these materials may be used for retardation of nitrification in soil.

Key Words: Essential oil; Japanese mint; Nitrification inhibitor; Urea N

INTRODUCTION

Among the nitrogenous fertilizers, prilled urea is the good source of N applied to soils. When applied to soil, urea is hydrolyzed by urease to form ammonium, which is subsequently converted to nitrite and nitrate by nitrifying bacteria. Ammonia volatilization, emission of nitrous oxide by the action of denitrifying organisms and leaching of NO_3 with water are some of the major pathways through which N is lost out of the soil. This results in a poor recovery of fertilizer N, which seldom exceeds 50% (1). This is even more pronounced in paddies and in sandy soils (2). Japanese mint (*Mentha arvensis*), which is primary source of menthol, is being cultivated extensively in Northern Indian plains covering an area of over 120 thousand hectares (3). The oil is used in pharmaceutical products, beverages, medicines, etc. Excessive loss of nitrogen due to leaching is a serious problem in light textured soils (4), which results in a very poor recovery of applied N, which is reported to be less than 30% (5) in mints. To ensure continuous and optimal supply of N, and to increase fertilizer use efficiency, there is a need to retard the rate of either urea hydrolysis or nitrification or both. In this context, several synthetic urease and nitrification inhibitors have been developed (6,7). However, for nitrification inhibitors to be practical in developing countries it is essential that they must be inexpensive, effective and biodegradable.

Some indigenous materials fulfilling these requirements have been tested for their potency in retarding nitrification. Neem (*Azadirachta indica* Juss) cake inhibits nitrification (6,8). Neem bitter (tetranortriterpenoids) present in Neem have been reported to be responsible for nitrification inhibition (9). The major constituents of bitters are nimbin, nimbinin, nimbidin, etc, which are lipid associates and constitutes about 2% (w/w) of oil. A concentrated neem extract nimin containing as much as 5% neem bitters has been developed (10). Nimin has been found to be a useful nitrification inhibitor in mitigating N_2O emission from rice field (11).

Karanjin, a furanoflavonoid from karanj (*Pongamia glabra* Vent) seeds, was compared to nitrapyrin (N-serve) in the laboratory and greenhouse conditions with rice (12) and has been observed to have nitrification inhibitory properties. A number of essential oils find wide application in pharmaceutical preparations as antiseptic agents. Chemical constituents such as menthone iso menthone, carvone, thymol, and pulegone present in mint oils are found to possess antimicrobial properties. Sivropoulou et al. (13) observed that essential oils obtained from *Mentha pulegium* and *Mentha spicata* exhibited antimicrobial properties against gram-positive and gram-negative bacteria. The essential oil at high concentration was extremely bacteriocidal. *Mentha spicata*, oil commonly known as spearmint oil, is the primary source of carvone. Besides carvone the major constituents of the oil are limonene, myrene, 1,8-cineole, salrinin hydrate linalool, etc.

The objective of this study was to evaluate the inhibition of urea hydrolysis and nitrification by *Mentha spicata* oil and Nimin (alcohol extract of *Azadirachta indica*, under field conditions.

MATERIALS AND METHODS

Field experiments were conducted for two years, 1997–98 with Japanese mint (*Mentha avensis*) at the research farm of Central Institute of Medicinal and Aromatic Plants (CIMAP) field station, Pantnagar, India situated at 20°N latitude,79.5°E longitude and 24.4 m above mean sea level. The soil of experimental field was a Mollisol, clayey loam in texture having pH 7.20, organic C 12.0 g kg^{-1}, mineralizable N (alkali $KMNO_4$ extractable) 150 mg kg^{-1}, phosphorus (0.5 *M* $NaHCO_3$ extractable) 12.5 mg kg^{-1} potassium (NH_4 OAc extractable) K 98.20 mg kg^{-1} and bulk density 1.36 Mg m^{-3}.

The experiments were arranged in a randomized block design with thirteen treatment combinations comprising three levels of urea N applied at the rate of 100, 200, and 300 kg N ha^{-1}, each with three coating materials or left uncoated. Control check without N and without coating materials was also included. Each treatment was replicated three times. The plot size for each treatment in a replication was $10 \times 12\,m^2$. Mentha (*Mentha arvensis* cv *Kosi*) suckers were planted end to end in the last week of January in both the years, in 5 cm deep furrows, 50 cm apart and covered with loose soil. Immediately after planting the field was lightly irrigated. All plots including the control received P and K at the rate of 60 kg ha^{-1} applied through single super phosphate and muriate of potash, respectively. Urea, both coated and non coated, was applied in three equal splits, i.e., one third at planting and the remaining two-thirds in equal splits at 25 and 75, days after planting. The crop was irrigated periodically as required.

Urea was coated with three coating materials in two steps. First, urea and castor oil (*Ricinus commumis*) in 100:1 proportion (1% w/w basis) were physically mixed and dried for 24 h. The oiled urea granules were mixed separately with *M. spicata* or Nimin at again in 100:1 proportion. The resulting double-coated urea granules were shade dried for another 24 h. The double-coated urea material was used with in a few days of its preparation.

The crop was harvested during the last week of May in both the years. For plant sampling a 1×1 m area in the middle of each plot was harvested one week before the final crop harvest. Extraction of essential oil was done with 200 g fresh samples following hydro-distillation for 3 h. For chemical analysis a representative sample of 100 g was taken from the bulk sample collected for oil extraction. The plant samples were shade dried, oven dried, ground and chemically analyzed for N, P, and K following standard procedures (14).

Soil samples were collected to a depth of 30 cm randomly from three sites in each plot at regular intervals of seven days after application of the fertilizer N treatments till 21 days and at harvest (85 days after transplanting) and were analyzed for ammonical N and nitrate N (15) to evaluate urea N transformation (mineralization, nitrification).

Data on all the observations were subjected to analysis of variance and least significant difference (LSD) were calculated using T-method (16).

RESULTS

Fresh herb yield of mint it significantly increased with N application up to 200 kg ha^{-1}. Average herb yield at 300 kg N ha^{-1} (Table 1) was not different from that at 200 kg N ha^{-1}. With respect to different coating materials, the herb yield at 100 kg N ha^{-1} was, however, not different under different coating materials. An almost similar trend was observed with N application at 200 kg ha^{-1}, as well as at 300 kg ha^{-1}. However, DCD coating resulted in a higher herb yield at 200 kg ha^{-1} and Nimin at 300 kg ha^{-1} N over the rest of coating materials. Considering mean of the four treatments at each level of N, herb yield increased to an extent of 61%, 117%, and 120% over the control with the application of 100, 200, and 300 kg N ha^{-1}, respectively. The oil yield also followed the similar trend. There was a significant increase in oil yield over the control with the application of up to 300 kg N ha^{-1}. On average the oil yield of mint increased over the control by 37%, 81%, and 105% with N application at 100, 200, and 300 kg ha^{-1}, respectively.

Table 1. Influence of Different Treatment Combinations on Herb and Essential Oil Yield of Japanese Mint

Treatments			
Levels of N ($kg\,ha^{-1}$)	Coating Materials	Herb Yield ($Mg\,ha^{-1}$)	Oil Yield ($l\,ha^{-1}$)
Control (0)	—	20.9a	146a
100	None	30.5b	186b
	MS oil[a]	34.5c	193b
	Nimin	34.6c	200b
	DCD	35.0c	220c
200	None	36.8c	200c
	MS oil	42.0d	277d
	Nimin	45.0d	292d
	DCD	57.5e	286d
300	None	43.4d	272d
	MS oil	45.5d	297d
	Nimin	51.8c	326e
	DCD	43.0d	300d

[a] MS oil: *Mentha spicata* oil.
Figure within column followed by same letters are not significantly different at 5% level of significance.

Dry Matter Yield, N Accumulation, and Apparent N Recovery

As with the fresh herb yield, the dry matter yield significantly increased over control by N fertilizer application (Table 2). Similarly, coating urea with different materials, increased the dry matter yield. At 100 kg N application the increase over the non-coated urea, with *Mentha spicata* oil, nimin, and DCD was 6%, 16%, and 31%, respectively. The corresponding increase at 200 and 300 kg $N\,ha^{-1}$ application were 20%, 29%, and 64%, and 13%, 19%, and 18%, respectively. The mean dry matter yield at 100, 200, and 300 kg N application was 8.53, 12.82, and 11.70 $Mg\,ha^{-1}$, respectively. Plant accumulation of N also followed an identical pattern. There has been a significant increase in N uptake due to N application up to 300 $kg\,ha^{-1}$. The influence of coating of urea on N accumulation over non-coated urea was more pronounced at 200 and 300 $kg\,ha^{-1}$ than at 100 $kg\,ha^{-1}$. The average N uptake was 57%, 103%, and 116% higher over the control with 100, 200, and 300 kg N application. The apparent N recovery, however, decreased with increase in the dose of N application (Table 2). Coating of urea with natural and synthetic materials augmented the N recovery over the non-coated urea at

Table 2. Influence of Different Treatment Combinations on Dry Matter Yield, N Accumulation, and Apparent N Recovery in Japanese Mint

Treatments				
Level of N ($kg\,ha^{-1}$)	Coating Materials	Dry Matter Yield ($Mg\,ha^{-1}$)	N Uptake ($Kg\,ha^{-1}$)	Apparent N Recovery (%)
Control (0)	—	5.48a	87.64a	—
100	None	7.53b	120.40b	32.76
	MS oil[a]	8.01c	128.20b	40.56
	Nimin	8.73c	140.70e	53.06
	DCD	9.88c	161.10d	73.46
200	None	9.90d	135.07c	23.72
	MS oil	11.86e	166.04d	39.21
	Nimin	12.82e	192.30e	52.33
	DCD	16.24f	227.40f	69.88
300	None	10.58d	148.58c	20.31
	MS oil	12.09e	185.26e	32.54
	Nimin	12.63e	209.50f	40.62
	DCD	12.49e	212.90f	41.75

[a] MS oil: *Mentha spicata* oil.

all levels of applications. The highest N recovery was observed with DCD followed by nimin and *Mentha spicata* oil.

Mineral N Status of Soil

All three coating materials inhibited nitrification at all levels of N application (Table 3). At 100 $kg\,ha^{-1}$ N application, the estimate of mineral N on day 7 indicate that the NO_3 formation from the coated urea decreased by 48%, 45%, and 52% over the non coated urea with *M. spicata* oil, Nimin, and DCD, respectively. The most significant observation was the highest accumulation of NH_4 in the DCD treated soil. An almost similar trend was observed at day 14 and 21. At harvest, the NO_3-N in *M. spicata*, Nimin, and DCD treated soils decreased by 33%, 62%, and 58%, respectively, over urea alone. Taking an average of four determinations estimates (i.e., day 7, 14, and 21 and at harvest), the NO_3 formation was estimated to be lowered by 40%, 47%, and 54%, respectively, as compared to urea alone applied at 100 kg $N\,ha^{-1}$. Similarly, the average NH_4 accumulation was higher in the DCD treated soils. At 200 kg N application the reduction in NO_3 formation with the *Mentha spicata* oil, Nimin, and DCD treated soil were 44%, 45%, and 58%, respectively, over prilled urea alone.

Estimates of NH_4 and NO_3-N in soil at harvest also indicates a lower NO_3 formation in all the treatments receiving coated urea as compared to non-coated urea. Taking the average of the four estimates at different intervals the data indicate that there was a mean reduction of nitrification by 46, 44, and 52% over control with *Mentha spicata* oil, nimin and DCD, respectively. The trend in the status of mineral N at 300 kg ha^{-1} application also was similar to that under 100 and 200 kg N ha^{-1}. Comparing the data on day 7, the NO_3 formation under mint oil, Nimin, and DCD were 38%, 38%, and 112%, respectively. The average retardation of NO_3 formation in the different treatments was 43%, 35%, and 50%, respectively, with mint oil, Nimin, and DCD. Data on the mineral N ($NH_4 + NO_3$) determined at regular interval, as well as their mean value, indicate that NO_3 formation was significantly retarded due to the coated materials. Both the natural products were as effective as the synthetic inhibitor DCD.

DISCUSSION

In general the NH_4-N content was comparatively high in the DCD treated soil. This is probably due to the inhibitory of action of DCD treated soil on nitrifiers as compared to the other materials. The natural products, nimin and *Mentha spicata* oil, were found as effective as nitrification inhibitors in terms of yield obtained as compared to the control and subsequently with respect to the status of mineral N in soil. The role of DCD as a chemical inhibitor is well established, but it has little effect on urea hydrolysis (6). Natural products like Nimin and especially *Mentha spicata* influenced the urea hydrolysis also which is indicated by a lower concentration of NH_4 in natural product coated urea treated soil. Although mint responds to as N level as high as 400 kg N (4,17), in the present investigation no significant response was observed beyond 200 kg, which could be due to higher recovery of N as a consequence of coating urea with natural and synthetic materials.

Dry matter yield, N accumulation and apparent N recovery increased due to the use of coating materials. The apparent recovery, however, was low at the higher dose of fertilizer N application. This could be attributed to a possibly higher loss of fertilizer at higher doses. As such, DCD had a slight edge over the two natural product with regard to N recovery. Similar results were reported by Patra et al (18). *Mentha spicata* oil is known for its antimicrobial properties (13). The terpenoids, tannins, and polyphenol content in essential oil are supposed to inhibit nitrification in soil (19). Bremner and McCarty (20), however, did not support such a hypothesis. They found that when such materials are added to the soil, soil microorganisms immobilized the NH_4-N. The activity of the Neem

Table 3. Influence of Different Treatment Combinations on Mineral N in Soil (0–30) at Different Stages of Growth of Japanese Mint

Treatments		Mineral N ($mg\,kg^{-1}$), Days After N Application									
		7		14		21		At Harvest		Average	
Levels of N ($kg\,ha^{-1}$)	Coating Materials	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
Control (0)	—	1.45	92.3	5.8	86.7	10.2	74.5	2.12	46.2	5.14	74.9
100	—	3.26	193.2	19.16	107.4	3.8	110.8	3.5	93.2	8.68	126.2
	MS Oil[a]	3.18	100.0	10.20	52.5	16.8	86.5	0.5	62.5	7.67	75.4
	Nimin	5.85	106.2	9.3	67.5	24.8	55.0	4.5	35.0	11.11	65.9
	DCD	17.81	92.5	43.7	52.5	15.0	48.0	12.2	38.8	22.43	57.9
200	—	7.80	348.0	21.9	232.4	13.4	209.4	2.29	72.39	11.34	215.6
	MS Oil	10.28	194.0	40.1	85.34	21.67	124.0	4.18	59.73	19.06	115.8
	Nimin	7.05	190.5	17.43	94.9	14.85	136.6	6.20	58.7	11.63	120.2
	DCD	16.38	148.0	41.0	78.9	23.67	122.2	14.8	60.32	23.96	102.3
300	—	14.87	520.0	42.0	384.2	18.5	342.0	4.2	120.0	17.44	341.5
	MS Oil	17.50	322.0	35.5	138.2	35.8	224.0	6.93	98.5	23.90	195.7
	Nimin	12.0	321.0	30.5	221.5	26.7	241.0	10.5	96.7	19.92	220.0
	DCD	26.50	245.0	49.5	130.4	40.2	201.0	23.9	99.9	35.02	168.8
	LSD($P = 0.05$)	3.21	12.7	2.5	56.7	1.8	10.2	2.10	4.56	—	—

[a]MS oil: *Mentha spicata* oil.

extract and nimin (10) as nitrification inhibitor have already been reported (21). Nimin which is primarily an alcohol extract of Neem oil and chemically tetranortriterpenoides, is reported to be responsible for retardation of nitrification (10). The mode of action of these materials is not well understood but both natural products have some regulatory influence on urea hydrolysis and subsequent nitrification. Unlike DCD these materials probably possess urease inhibitory properties. A detail study on a active ingredients responsible for such regulation as well as a microbial assay of urease activity and nitrifiers is needed.

ACKNOWLEDGMENTS

The authors are thankful to the Director, CIMAP, Lucknow for providing the necessary facilities, and the Scientist-in-Charge, CIMAP field station, Pantnagar for his help in conducting the experiments.

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