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Efficiency of Slow Release Urea Fertilizer on Herb Yield and Essential Oil Production of Lemon Balm (*Melissa officinalis* L.) Plant

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Abstract: Recently, the use of slow release fertilizer is a new trend to save fertilizer consumption and to minimize environmental pollution. Thus the aim of the present investigation was carried out to study the efficiency of different urea treatments Viz. prilled urea (Pu), Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU) at the rat of 360 Kg N/ha. on fresh and dry herb yield, nitrogen use efficiency and essential oil production of lemon balm plant. Urea fertilizer significantly increased the dry herb and essential oil yields of the plant compared to control. The dry herb yield increased over prilled urea (Pu) by 25.7, 57.4, 71 and 48.2% by using Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU), respectively. The essential oil yield (l/ha) increased over prilled urea (Pu) by 72.56, 164.32, 253.17 and 102.23 % with adding Pu (2 splits), Pu (4 splits), SCU and NCU, respectively. The application of sulphur-coated urea was more effective in facilitating N uptake by plant and gave the highest increase of apparent N recovery (56.0%), N-agronomy efficiency (17.93 Kg/Kg) and N-physiological efficiency (32.01 Kg/ Kg) compared to other urea treatments. The main compounds of lemon balm oil (citronellal, neral and geranial) were increased by using most of urea treatments and this effect was accompanied with decreasing the relative concentration of geranyl acetate, linalool, citronellol and geraniol as compared with control. The maximum relative concentration of the two monoterpenes, i.e. geranial and neral (54.82%) was recorded with sulphur coated urea (SCU). Citronellal reached the maximum percentages with the application of sulfur coated urea (SCU) and prilled urea Pu (4 splits) which gave 34.21 and 33.86 % followed by Pu (2 splits), neem coated urea (NCU), then prilled urea (Pu) as compared with control which recorded the lowest relative concentration (18.98%).

Key Words: *Melissa officinalis* · Essential oil · Slow release urea fertilizer · Sulphur coated urea (SCU) · Neem coated urea(NCU) · Medicinal · Aromatic plants

INTRODUCTION

Lemon balm, *Melissa officinalis* L. (Lamiaceae) is a perennials herb native to southern climates of Europe and North America. The leave of lemon balm, are widely used in Europe as a herbal tea for their aromatic, digestive and antispasmodic properties in nervous disturbance of sleep and functional gastrointestinal disorders [1].

Some pharmacological properties have been attributed to principal constituents. Rosmarinic acid is antiviral and antioxidant [2], while the essential oil of lemon balm has anti-bacterial, anti-fungal and anti-histaminic activities [3].

The major compounds of the essential oil of the lemon balm leave are citronellal (2-40%) and citral (10-30%), it is a mixture of two monoterpenes, geranial and neral. Due to its intense lemon aroma and flavour, citral is used widely in foods and cosmetics [4], accompanied by ρ-caryophyllene, germacrene D, ocimene and citronellol [5-7].

Nitrogen is the most important mineral element in fertilization programs because plants usually need N in greater amounts than other mineral nutrients. Nitrogen losses are caused by leaching, erosion, volatilization, denitrification and fixation in soil organic matter. Prilled urea is one of the main source of N applied to plant [8] and when applied to soil, it hydrolyzed by urease to the NH_4 form and subsequently converted to nitrate by the action of nitrifying bacteria. The NO_3 is subject to losses either as nitrogen gas or nitrous oxide by the action of

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denitrifying bacteria and through percolation of soil water [9]. Excessive of N due to NO_3 leaching is a serious problem in mint plant cultivation on light textured soils [10]. Appreciable increases in the yields of several crops have been obtained due to the application of nitrogenous fertilizers. However, the costs of inorganic fertilizers are increasing from time to time and becoming unaffordable for subsistent farmers. Nitrogen fertilizer use efficiency of crops is also very low. Maximum of 50% of the applied N is utilized by corn [11]. There are several methods of reducing N loss and improving N-use efficiency of crops. Such methods include proper time and methods of application of fertilizers, use of slow release fertilizers.

Recently, the use of slow release fertilizers is a new trend to save fertilizer consumption and to minimize environmental pollution [12,13]. Slow release fertilizers are made to release their nutrient contents gradually and to coincide with the nutrient requirement of a plant. These fertilizers can be physically prepared by coating granules of conventional fertilizers with various materials that reduce their dissolution rate. The release and dissolution rates of water-soluble fertilizers depend on the coating materials. Coating materials degrade ability is an important focus of the research in this field because of the renewed attention towards environmental protection issues [14]. Furthermore, the use of coating materials may result in high production costs and even soil contamination after their release into soil. To solve these problems, by mixing common urea with industrial organic wastes and controlled release inorganic materials [15] and those by mixing inorganic compound fertilizer with N-rich and high-quality organic fertilizer [16]. The amount of N fertilizer recovery by the plant in relation to the total amount of N fertilizer added is directly related to the level of applied N fertilizer and inversely related to split N fertilizer applications [17].

The aim of this investigation was conducted to study the efficiency of slow release urea fertilizers Viz. prilled urea (Pu), Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU) on yield, nitrogen use efficiency and essential oil production of lemon balm plant.

MATERIALS AND METHODS

The present investigation was carried out to study the efficiency of different urea fertilizers Viz. prilled urea (Pu), Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU) at 360 Kg N/ha.on the herb yield, nitrogen use efficiency and essential oil production of lemon balm plant.

Two field trials were conducted in randomized complet block design with 3 replications during the seasons 2005 and 2006 at the Experimental Farm of Faculty of Agriculture, Giza, Egypt. The soil of the experimental site was clay loam textured i.e. 23 % sand, 46 % silt and 31 % clay, with pH 8.1, EC 1.0 dS/m and 1.50 % organic matter.The available N, P and K were 168, 31 and 25 ppm, respectively. The physical and chemical properties of the investigated soil were determined according to Cottenie *et al.* [18].

Six- weeks old seedlings of lemon balm were transplanted on 1-February 2005 and 2006 at 50 cm x 60 cm hill spacing using one seedling / hill. Organic manure ($30m^3$/ha), calcium superphosphate (400 kg/ha) and potassium sulphate (100 kg/ha) were applied 15 days before transplanting. Full dose (360 Kg N/ha) of prilled urea (Pu, 46%N) was applied at transplanting. In the case of Pu (2 splits), 50 % was applied at transplanting and the 50 % one month later. In the case of Pu (4 splits), 25% of N was applied at transplanting then repeated every month from transplanting. Sulphur coated urea (SCU,32 % N) and neem coated urea (NCU, 34%N) were incorporated into the soil before transplanting. Urea fertilizers coated with different materials were synthesized using coating technique [19]. The plants were harvested on first June and October during the growing seasons and the following data were recorded.

Fresh and dry weight of herb yield) g/plant and ton/ha): Total nitrogen in dry weight of herb was determined by kjeldahl method as described by Bremner and Mulvaney [20], N-efficiency parameters [21] were calculated as follows:

$$\text{Apparent N recovery (\%)} = \frac{\text{100 (Uptake of N from the added fertilizer (Kg/ha)-Uptakeof N from control without fertilizer (Kg/ha)}}{\text{Amount of nitrogen applied (Kg/ha).}}$$

$$\text{Agronomic efficiency (Kg/Kg)} = \frac{\text{Dry herb yield with N fertilizer (Kg/ha)- Dry herb yield without fertilizer (Kg/ha)}}{\text{Amount of nitrogen applied (Kg /ha).}}$$

$$\text{N physiological efficiency (Kg/Kg)} = \frac{\text{Dry herb yield with N fertilizer (Kg/ha) - Dry herb yield without fertilizer (Kg/ha)}}{\text{Uptake of N from the added fertilizer (Kg/ha)-Uptakeof N from control without N fertilizer (Kg/ha)}}$$

The essential oils of the fresh herb were obtained by hydrodistillation in a Clevenger- type apparatus for 3 h, according to the Egyptian Pharmacopoeia [22]. The essential oil percentage and yield (ml/plant and l/ha) were calculated. Subsequently, the obtained essential oil from each treatment was dehydrated over anhydrous sodium sulfate and then subjected to GLC analysis with Varian VISTA 6000 FID model. The separation was carried out with 2-mx 1/8 stainless steel 3 % OV-101 column. The flow rate of the carrier gas (nitrogen) was maintained at 50 ml/min. The column temperature was programmed from 50° to 200°C at the rate of 2.5°C/min. The injection port temperature was maintained at 180°C and detector at 240°C. The relative percentages of the different compounds were determined by Varian 4270 integrator. The identification of these compounds was achieved by matching their retention times with those of authentic standers injected with the same conditions.

The obtained data were subjected to statistical analyses according to Snedecor and Cochran [23] using L.S.D at the level of 5%.

RESULTS AND DISCUSSION

Urea fertilizer significantly increased the fresh and dry herb yield (gm/plant and ton/h.) of plants compared to the control treatment (Tables 1&2). In the first season the dry herb yield (ton/ha.) increased over control by 31.3, 65.1, 106.6, 124.5 and 94.5 % by using prilled urea (Pu), Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU), respectively. In the first season, the dry herb yield increased over prilled urea (Pu) by 25.7, 57.4,71 and 48.2%, with adding Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU), respectively. The corresponding increases in dry herb yield at the second season were 11.7, 26, 39.8 and 23.2%, respectively. The data indicated that sulfur coated urea (SCU) was more effective than the other urea treatments. The increase of herb yield might be due to the higher utilization of nitrogen by the plant as a result of decreasing loss of fertilizer nitrogen. Similar resulted had been reported by Ram *et al.* [24]on Japanese mint, Singh and Singh [25]on citronella java, also Singh *et al.* [26]on *Coriandrum sativum* and Singh [27] on *Pelargonium graveolens* as well as Aziz and El-Ashry [28]showed that, coating urea with various coating materials, i.e. rock phosphate, pentonite and elemental sulphur significantly increased fresh and dry weight of lemongrass herbage as compared with both uncoated fertilizers and control.

Nitrogen uptake (Table 3 and 4) increased significantly with all urea fertilizer as compared with control during the two seasons. In the first season total N uptake (Kg/h) increased over prilled urea (Pu) treatment by 35.2, 76.4, 96 and 64.5% by application of Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU), respectively and over 14.7, 31.2, 50 and 29%, respectively during the second season. The application of sulphur coated urea (SCU) was more effective in facilitating N uptake by plant owing to the higher herbage yield and N concentration. The application of sulphur coated urea (SCU) gave the highest increased of apparent N recovery (56.0%), N-agronomy efficiency (17.93 Kg/ Kg) and N-physiological efficiency (32.01 Kg/ Kg). Similar trends were obtained in the second season.Thus sulphur coated urea being proved significantly superior to other N treatments including split applied prilled urea. This effect might be due to higher utilization of nitrogen by the plant as a result of retardation of losses of fertilizer by the regulation of urea hydrolysis and nitrification [9] and subsequently higher N use efficiency due to regulation of urea-N transformation [29]. Moreover, Aziz and El-Ashry[28] showed that coating ammonium nitrate and urea fertilizer with various coating materials, i.e. rock phosphate, pentonite and elemental sulphur reduced its solubility, releasing into the soil and consequently higher availability of N was maintained to lemongrass the plant.

Table 1: Influence of slow release urea fertilizers on fresh and dry herb yields of lemon balm during 2005 season

Treatment	Fresh herb yield (g/ plant) 1cut	Fresh herb yield (g/ plant) 2 cut	Dry herb yield (g /plant) 1cut	Dry herb yield (g /plant) 2 cut	Total fresh herb yield g/plant	Total dry herb yield g/plant	Fresh herb yield ton /ha	Dry herb yield ton /ha
Control	430.22	436.92	116.26	123.56	867.14	239.82	18.73	5.18
Prilled urea (Pu)	552.59	567.88	155.58	159.03	1120.47	314.61	24.20	6.80
Pu (2 splits)	681.91	726.93	190.95	204.72	1408.85	395.67	30.43	8.55
Pu (4 splits)	859.24	894.56	245.22	250.21	1753.80	495.43	37.88	10.70
SCU	928.02	990.88	261.92	276.69	1918.90	538.61	41.45	11.63
NCU	807.64	877.54	228.16	238.47	1685.18	466.63	36.40	10.08
L.S.D at 0.05	14.99	9.18	1.59	3.10	14.65	3.57	0.32	0.08

Prilled urea (Pu), Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem coated urea (NCU)

Table 2: Influence of slow release urea fertilizers on fresh and dry herb yields of lemon balm during 2006 season

Treatment	Fresh herb yield (g/ plant)		Dry herb yield (g /plant)		Total fresh herb yield g/plant	Total dry herb yield g/plant	Fresh herb yield ton /ha	Dry herb yield ton /ha
	1st cut	2nd cut	1st cut	2nd cut				
Control	364.43	413.51	104.29	117.95	777.94	222.23	16.80	4.80
Prilled urea (Pu)	524.52	590.38	145.44	168.64	1114.90	314.08	24.08	6.78
Pu (2 splits)	617.23	632.36	170.00	180.42	1249.59	350.43	26.99	7.57
Pu (4 splits)	691.35	706.99	194.90	200.52	1398.34	395.42	30.20	8.54
SCU	788.69	800.16	213.55	225.34	1588.84	438.90	34.32	9.48
NCU	663.49	683.46	190.22	196.26	1346.95	386.48	29.09	8.35
L.S.D at 0.05	7.43	7.03	4.95	2.97	10.14	6.73	0.22	0.15

Table 3: Influence of slow release urea fertilizers on N content, N uptake, N efficiency of lemon balm during 2005 season

Treatment	Plant N %	N uptake Kg/ha	Apparent nitrogen recovery %	N agronomic efficiency Kg/ Kg	N physiological efficiency Kg/ Kg
Control	1.58	81.67	-	-	-
Prilled urea (Pu)	2.13	144.52	17.46	4.49	25.74
Pu (2 splits)	2.29	195.43	31.60	9.35	29.59
Pu (4 splits)	2.38	254.87	48.11	15.34	31.88
SCU	2.44	283.29	56.00	17.93	32.01
NCU	2.36	237.70	43.34	13.61	31.40
L.S.D at 0.05	0.03	2.80	0.78	0.21	0.95

Table 4: Influence of slow release urea fertilizers on N content, N uptake, N efficiency of lemon balm during 2006 season

Treatment	Plant N %	N uptake Kg/ha	Apparent nitrogen recovery %	N agronomic efficiency Kg/ Kg	N physiological efficiency Kg/ Kg
Control	1.61	77.45	0.00	-	0.00
Prilled urea (Pu)	2.24	151.86	20.67	5.51	26.65
Pu (2 splits)	2.30	174.22	26.88	7.69	28.61
Pu (4 splits)	2.33	199.30	33.85	10.39	30.70
SCU	2.40	227.84	41.78	13.00	31.12
NCU	2.35	195.76	32.86	9.85	29.98
L.S.D at 0.05	0.01	3.45	0.96	0.40	0.63

The essential oil yield (ml/plant and l/ha) was significantly increased by addition of urea fertilizers treatments compared with the control (Table 5&6). In the first season, the essential oil yield (l/ha) increased over prilled urea (Pu) by 72.56, 164.32, 253.17 and 102.23 % by using Pu (2 splits), Pu (4 splits), SCU and NCU, respectively. The corresponding increases the essential oil yield (l/ha) at the second season were 39.64, 105.12, 170.27 and 72.41%, respectively. Sulphur coated urea (SCU) had a significant beneficial influence on the essential oil yield than other urea treatments. This effect may be attributed to decreasing N-leaching losses from coated fertilizers and consequently increasing N- availability in the soil which was maintained to the plant. Similar findings were obtained in experiments indicate some beneficial influence of the coating materials in terms of increasing N recovery, herb and oil yield [9] as well as Aziz and El-Ashry [28] showed that applying different nitrogenous fertilizers caused a primitive effect on increasing the essential oil percentage of lemongrass plants compared with the control, moreover the increases were much greater with urea and ammonium nitrate coated with different material than uncoated fertilizer.

The identified components of lemon balm essential oil (Table 7) were 1-octen-3-ol, *trans*-β- ocimene, citronella, linalool, neral, geranial, geranyl acetate, citronellol, geraniol and caryophyllene oxide. The oil was charachtrized with high relative concentration of monoterpenes. The major compounds were citronellal (18.98-34.21%), neral (13.23-31.78%), geranial (13.35-28.27%) and geranyl acetate (6.39-13.51%) which represented about (63.47-95.42%) of the oil at all treatments. These results agreed with that reported by Enjalbert *et al.* [4] Sarer and Kiikdil, [5]; Adzet *et al.* [6] and Kreis and Mosandl [7].

Table 5: Influence of slow release urea fertilizers on essential oil yield of lemon balm during 2005 season

	1st cut		2nd cut			
Treatment	Oil %	ml / plant	Oil %	ml / plant	Total oil yield ml/plant	Oil yield l/ha.
Control	0.05	0.20	0.03	0.13	0.33	7.18
Prilled urea (Pu)	0.06	0.33	0.04	0.21	0.54	11.66
Pu (2 splits)	0.08	0.57	0.05	0.36	0.93	20.12
Pu (4 splits)	0.10	0.83	0.07	0.60	1.43	30.82
SCU	0.12	1.11	0.08	0.79	1.91	41.18
NCU	0.07	0.57	0.06	0.53	1.09	23.58
L.S.D at 0.05	0.01	0.05	0.01	0.05	0.05	1.17

Table 6: Influence of slow release urea fertilizers on essential oil yield of lemon balm during 2006 season

	1st cut		2nd cut			
Treatment	Oil %	ml / plant	Oil %	ml / plant	Total oil yield ml/plant	Oil yield l/ha
Control	0.06	0.21	0.04	0.17	0.37	8.04
Prilled urea (Pu)	0.08	0.42	0.05	0.30	0.71	15.44
Pu (2 splits)	0.09	0.56	0.07	0.44	1.00	21.56
Pu (4 splits)	0.12	0.81	0.09	0.66	1.47	31.67
SCU	0.13	1.03	0.11	0.91	1.93	41.73
NCU	0.10	0.69	0.08	0.55	1.23	26.62
L.S.D at 0.05	0.01	0.05	0.01	0.04	0.07	1.45

Table 7: Chemical composition of lemon balm essential oil as affected by slow release urea fertilizers

Essential oil composition	control	prilled urea (Pu)	Pu (2 splits)	Pu (4 splits)	SCU	NCU
1-Octen-3-ol	1.88	0.72	2.37	0.27	0.01	0.48
Trans-β- ocimene	7.01	2.00	1.44	0.19	1.05	1.01
Citronellal	18.98	29.81	32.83	33.86	34.21	30.71
Linalool	8.08	3.22	2.01	1.29	0.54	2.65
Neral	13.23	31.78	31.23	24.58	26.55	27.69
Geranial	17.75	14.06	13.35	25.29	28.27	21.01
Geranyl acetate	13.51	8.18	9.88	10.03	6.39	10.16
Citronellol	1.16	0.07	0.51	0.61	0.15	0.32
Geraniol	2.29	0.56	0.14	0.96	0.21	0.04
Caryophyllene oxide	1.48	5.86	4.47	0.41	1.11	5.43
Total identified	85.37	96.26	98.23	97.49	98.49	99.50

prilled urea (Pu), Pu (2 splits), Pu (4 splits), sulfur coated urea (SCU) and neem cake coated urea (NCU)

The main compounds of lemon balm oil (citronellal, neral and geranial) were increased by using most of urea treatments and this effect was accompanied with decreasing the relative concentration of geranyl acetate, linalool, citronellol and geraniol as compared with control. The maximum relative concentration of the two monoterpenes,i.e. geranial and neral (54.82%)was recorded with sulphur coated urea (SCU). Moreover citronellal reached the maximum percentages with sulfur coated urea (SCU) and Pu (4 splits) which gave 34.21 and 33.86 % followed by Pu (2 splits), neem coated urea (NCU), then prilled urea (Pu) as compared with control which recorded the lowest relative concentration (18.98%). The application of sulphur coated urea (SCU) was more effective in affecting productivity and yield of secondary metabolites. Thus sulphur improves nitrogenase activity, N fixation and consistently increased the protein and oil contents of plants [30], as well as it is required for the synthesis of S-containing amino acids, protein, chlorophyll and oil [31].

The quality of those metabolites is affected either indirectly by affecting availability of photosynthates provided by primary metabolism [32] or directly, through some factors responsible for efficient utilization of precursors coming from primary synthesis [33]. The essential oil biosynthesis takes place in epidermal oil glands, which are carbon heterotrophic [34] and hence depend on the adjoining photosynthesizing cells for continuous supply of carbon precursors. Such as CO_2, sucrose, glucose, acetate and mevalonate, however, CO_2 and sucrose are the best precursors of isoprenoid biosynthesis [35]. Moreover, the essential oil biosynthesis in plant is the integration of several metabolic pathways, which requires linking of several steps such as continuous production of precursors, their transport and translocation to the active site of synthesis and finally depends upon normal functioning of associated metabolic pathways such as carbon fixation, respiration and isoprenoid pathway. Any disruption in normal metabolic pathway affects the sequence of steps of the oil biosynthesis. Slow release fertilizers play an important role in improving fertilizer use efficiency by plants by reducing the frequency of fertilization, thereby mitigating environmental pollution and leading to the development of sustainable agriculture. [36].

In conclusions the obtained data revealed that the application of slow release urea fertilizer as sulphur coated urea had the beneficial influence in terms of increasing N-efficiency, herb and oil yield as well as had favorable effect for increasing the main constituents of lemon balm essential oil.

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