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Influence of pyridoxine and spermine on lemongrass (*Cymbopogon citratus*) plants

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Abstract. *Orabi SA, Talaat IM, Balbaa LK, Abdalla AE. 2015. Influence of pyridoxine and spermine on lemongrass (*Cymbopogon citratus*) plants. Nusantara Bioscience 7: 139-143.* Two pot experiments were conducted to investigate the effect of pyridoxine and spermine (each at 50, 100 and 200 mg/L) on plant growth, essential oil yield, and its main constituents. All treatments significantly promoted plant height, fresh and dry mass (g/plant) of *Cymbopogon citratus* L., especially 100 mg/L spermine which mostly recorded the highest increments in total nitrogen, total phosphorous and total potassium mainly in the second cut, followed by 200 mg/L pyridoxine. Total phenols, essential oil % and oil yield recorded the maximum increments with spermine treatments followed by pyridoxine. Treatment of lemongrass plants with 100 mg/L spermine gave the highest relative percentage of citronellol (51.20%). Oxygenated compounds recorded the highest value (89.70%) with 200 mg/L spermine treatment, while the maximum non-oxygenated ones resulted from the untreated plants (13.65%). All treatments under study significantly affected the scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl-hydrate). Application of spermine at the concentration of 100 mg/L recorded the highest increment in phenylalanine ammonia lyase (PAL) enzyme activity (µg/g F.wt.).

Keywords: *Cymbopogon citratus*, antioxidants, essential oil, GLC, medicinal and aromatic plants

INTRODUCTION

In traditional medicine of Brazil, lemongrass (*Cymbopogon citrates*) is considered to be anxiolytic, hypnotic, and anticonvulsant (Rodrigues and Carlini 2006; Blanco et al. 2009). Wang et al. (2014) reported that in the folk medicine of India the leaves of lemongrass plant are used as stimulant, sudorific, antiperiodic, and anticatarrhal. The volatile oil of lemongrass is carminative, depressant, analgesic, antipyretic, antibacterial, and antifungal agent. Wright et al. (2009) made an infusion from *C. citratus* to be used as an inexpensive remedy for the treatment of oral thrush in HIV/AIDS patients. The plant volatile oil can be used to treat patients with an increased risk for Alzheimer’s disease (AD) and in addition to drugs which are able to neutralize reactive oxygen species lemongrass volatile oil may be considered as a very safe and not expensive drug which can be used to prevent inflammatory process that ends in neuronal death (Tschäpe and Hartmann 2006).

Pyridoxine (Vitamin B6) is a strong antioxidant. It can act as a coenzyme for many metabolic enzymes. It is also necessary metabolite in all organisms. It plays an important role in growth and differentiation of some plant species and it is an essential cofactor for many metabolic enzymes, especially amino acids metabolism (Dolatabadian and Sanavy 2008). Samiullah (1997) and Shimasaki and Fukumoto (1998) reported that pyridoxine promotes the growth of root system. Similar results were obtained by Lone et al. (1999) who found that pyridoxine enhances nutrient uptake and lead to higher yield of economic plants. Farrokhi and Paykarestan (2010) reported that pyridoxine improved growth and yield parameters in corn plants.

Polyamines are low-molecular-weight positively charged molecules which regulate nearly all developmental processes such as growth and stress (Flores and Galston 1982; Smith 1985; Altman and Levin 1993; Messiaen et al. 1997). Polyamines are necessary for plant growth and differentiation, related to senescence, and they play an important role in the response of plant to stress (Perez-Adamor et al. 2002). Pegg (1986.) reported that polyamines can affect the transcriptional and translational stages of protein synthesis, it also stabilizes membranes and alters intracellular free calcium levels (Schuber 1989; Khan et al. 1993).

The aim of this work was to study the effect of pyridoxine and spermine on growth, chemical constituents as well as total essential oil and oil fractions of lemongrass plants.

MATERIALS AND METHODS

Two pot experiments were carried out during two successive seasons of 2011 and 2012 in the greenhouse of National Research Centre, Dokki, Giza, Egypt to study the effect of the antioxidants pyridoxine and spermine on growth, yield and essential oil of lemongrass. Transplants were provided from "SEKEM" company and cultivated in nursery on 10th of November, for both seasons.

Lemongrass transplants were put into the pots (30 cm in diameter), with three replicates for each treatment, each replicate includes six pots. All normal agricultural practices were performed as usual with lemongrass cultivation. Physical and chemical properties of the soil were as follow:

Two cuts were taken during the growing seasons (the first one in July and the second one in October). The following parameters were recorded at each cut: plant height (cm), number of tillers per plant, fresh and dry mass of herb, and essential oil percentage. The percentage of the volatile oil of air-dried herb for each treatment was determined by hydro-distillation according to Guenther (1961). The resulted essential oil was collected, dehydrated over anhydrous sodium sulfate and kept in refrigerator until Gas Liquid Chromatography (GLC) analysis. GLC analysis of volatile oil of each treatment was performed separately with a Hewlett-Packard model 5890. A fused silica capillary column (Carbowax 20M measuring 20m x 0.32 mm internal diameter, film thickness of 0.17μm) was used. The temperature program adopted was maintained at 75°C for 5 min. with an increase of 4°C min-1 until 220°C (10 min). The carrier gas was Helium and the working flow rate was 1.0 mL/min, detector was 9144 HP. The identification of the compounds was achieved by matching their retention times with those of authentic samples injected under the same conditions.

Biochemical constituents determination. Fresh leaves were collected for antioxidant activity estimation of PAL (phenylalanine ammonia lyase) enzyme. At harvest time, the herb samples were collected at time intervals when they were about 10 cm in length, weighed and then the herb yield was determined.

DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) ASSAY. The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method of Shimada et al. (1992). Briefly, 2 mL aliquot of DPPH methanol solution [25µg/mL] was added to 0.5 mL sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. L-Ascorbic acid was used as the standard. Where AC = absorbance of the control and AS = absorbance of reaction mixture [in the presence of sample]. All tests were run in triplicates [n = 3], and the average values were calculated.

Statistical analysis. The data obtained were subjected to standard analysis of variance procedure according to Snedecor and Cochran (1980). The values of L.S.D. were calculated whenever F values were significant at 5% level.

RESULTS AND DISCUSSION

Effect on growth characters

Data presented in Table 1 show that foliar application of pyridoxine at 50, 100 and 200 mg/L significantly increased plant height compared to untreated plants. Maximum plant height was obtained with application of spermine at 100 mg/L followed by pyridoxine at 200 mg/L. Concerning the effect of the polyamine, spermine, on growth of lemongrass plant, data presented in Table 1 indicate that foliar application of spermine to lemongrass plants significantly promoted growth parameters (i.e., plant height (cm), number of tillers/plant, fresh and dry weight (g/plant). The highest recorded values were obtained in plants treated with 100 mg/L spermine. Data also indicate that foliar treatment of lemongrass plants with spermine significantly increased the number of tillers, especially in plants treated with 100 mg/L. The fresh and dry weight of leaves followed the same trend (Table 1).

In this respect, Talaat et al. (2005) reported that exogenous application of putrescine on periwinkle transplants considerably increased plant growth at successive developmental stages. Asghari et al. (2013) studied the effect of treating wheat seeds with pyridoxine on germination speed, the average daily germination, the dry weight of wheat plant, the shoot and root length and the activity of catalase and peroxidase enzymes. They reported that all the above-mentioned criteria were significantly affected, on the level of one percent probability (p>0/01), as a result of pyridoxine treatments and its duration application, except the germination percent and the average daily germination. They also reported that treatment of wheat seeds with pyridoxine can help the farmers in setting and producing suitable wheat plants.

Effect on chemical constituents

Data shown in Table 2 indicate that treatment of lemongrass plants with 100 mg/L spermine significantly increased total nitrogen (%), total potassium and total phosphorous (%). The percentage of essential oil (%) of the herb was significantly increased, especially in plants treated with 100 mg/L spermine, followed by pyridoxine (200 mg/L). Oil yield (mL/plant) followed the same trend. These results hold true for both cuttings.

Supporting these results, Krishnamurthy (1991), Sharma et al. (1997), El-Bassiouny and Bekheta (2001) reported that foliar application of putrescine enhanced the uptake of K, P, Ca, and Mg in rice, , and wheat plants, respectively. These results are in harmony with the findings of Gharib and Hanafy (2005) who found that foliar application of putrescine affected the chemical composition of pea as resulted in a significant increase of N concentration. Talaat et al. (2005) also reported that exogenous application of putrescine on periwinkle transplants at 10^{-3}M putrescine significantly increased photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), total soluble and insoluble sugars, total proteins and total alkaloids in the leaves.

El-Tohamy et al. (2008) also reported that foliar application of putrescine resulted in a significant increment of vegetative growth (including plant height, number of leaves, number of branches and fresh weight of plants) and yield of eggplant compared to control plants as well as a significant increase of N, P and K contents of leaves.

Data presented in Table 3 indicate that total phenols increased gradually as a result of pyridoxine treatments. The highest value was found in plants treated with 200 mg/L pyridoxine. Application of spermine at 50 and 200 mg/L was less effective and the best results were obtained in plants treated with 100 mg/L spermine. Treatment of lemongrass plants with pyridoxine significantly increased

Table 1. Effect of pyridoxine and spermine on growth of lemongrass plants

Treatment	Plant height (cm)		No. of tillers/plant		Fresh weight of leaves (g/plant)		Dry weight of leaves (g/plant)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	84.33	89.33	11.00	11.50	113.31	166.67	24.97	42.70
Pyridoxine 50	86.00	93.33	12.00	15.33	148.20	260.00	33.36	60.05
Pyridoxine 100	87.67	97.67	12.67	17.00	176.02	265.00	35.11	64.72
Pyridoxine 200	90.00	99.00	16.00	19.50	178.12	278.33	38.88	68.09
Spermine 50	88.00	87.67	12.50	13.00	153.21	233.33	29.67	62.47
Spermine 100	92.50	105.67	18.00	22.00	195.71	265.00	35.39	68.61
Spermine 200	89.00	87.33	13.50	15.33	132.40	197.67	28.52	48.15
LSD (5%)	2.97	7.45	2.99	2.31	5.87	7.61	1.06	1.23

Table 2. Effect of pyridoxine and spermine on chemical constituents of lemongrass plants

Treatment	Nitrogen %		Phosphorous %		Potassium %		Oil %		Oil yield (mL/plant)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	3.62	3.00	0.11	0.18	2.15	2.30	0.40	0.25	0.46	0.42
Pyridoxine 50	4.44	3.35	0.21	0.19	2.20	2.30	0.42	0.27	0.63	0.69
Pyridoxine 100	4.62	3.39	0.23	0.20	2.25	2.60	0.44	0.31	0.77	0.82
Pyridoxine 200	5.06	4.23	0.34	0.34	2.40	2.75	0.51	0.34	0.91	0.95
Spermine 50	4.52	3.56	0.16	0.29	2.30	2.75	0.47	0.31	0.72	0.72
Spermine 100	6.03	5.75	0.36	0.39	2.50	2.90	0.58	0.40	1.14	1.06
Spermine 200	4.72	4.31	0.24	0.21	2.35	2.45	0.42	0.27	0.56	0.54
LSD (5%)	0.04	0.03	0.03	0.03	0.01	0.01	0.08	0.09	0.05	0.05

Table 3. Effect of pyridoxine and spermine on total phenols, PAL enzyme activity, DPPH scavenging activity and total glutathione of lemongrass plants

Treatment	Total phenols (mg/g F.wt.)		PAL enzyme (μg/g F.wt.)		DPPH radical scavenging activity % (100μg/mL)		Glutathione (GSH) (μmol/g F.wt.)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	0.93	1.25	11.20	14.19	62.09	65.90	2.06	2.57
Pyridoxine 50	1.51	1.83	14.93	17.77	66.29	69.33	2.30	2.79
Pyridoxine 100	1.89	2.12	18.29	22.09	69.52	70.48	2.70	3.62
Pyridoxine 200	2.03	2.40	19.08	22.25	71.43	74.48	3.24	4.01
Spermine 50	1.43	1.73	14.08	16.26	71.62	75.24	2.81	3.67
Spermine 100	1.53	1.86	15.24	18.21	74.47	77.33	3.90	4.53
Spermine 200	1.16	1.55	12.24	15.58	72.19	75.81	3.19	3.86
LSD (5%)	0.16	0.13	0.87	1.48	2.14	1.87	0.23	0.21

Table 4. Effect of pyridoxine and spermine on essential oil composition of lemongrass plants

Treatment	Control	Pyridoxine 50	Pyridoxine 100	Pyridoxine 200	Spermine 50	Spermine 100	Spermine 200
Myrcene	7.93	0.57	6.37	9.99	8.28	8.53	10.47
limonene	0.38	11.33	0.26	0.30	0.21	0.26	0.18
p-cymene	0.43	0.78	0.65	0.64	0.16	0.74	0.80
b-caryophyllene	0.35	0.97	0.24	0.31	0.84	0.41	0.13
Citronellal	1.10	0.98	0.63	0.85	0.79	0.73	0.52
Linalool	3.37	1.36	1.05	1.28	1.59	1.11	0.89
Neral	0.48	0.63	0.48	0.39	0.36	0.29	2.05
Geranial	27.40	31.30	32.51	30.43	32.07	32.88	32.07
Citronellol	40.72	44.32	51.27	51.00	49.56	51.20	49.84
Geranyl formate	1.23	1.77	0.81	0.30	1.97	1.25	0.37
Neryl acetate	0.71	1.06	0.24	0.50	0.23	0.40	0.23
Caryophyllene oxide	0.82	0.00	2.71	1.40	0.41	1.02	0.48
Non-oxygenated	9.09	13.65	7.53	11.23	9.49	9.94	11.58
Oxygenated	75.82	81.41	89.70	86.13	86.99	88.87	86.46
Total identified	84.91	95.06	97.23	97.37	96.48	98.81	98.04

lemongrass plants with pyridoxine significantly increased the activity of PAL enzyme, especially in plants treated with 200 mg/L followed by plants treated with 100 mg/L pyridoxine and spermine 100 mg/L. These results hold true for both cuttings.

Data are shown in Table 3 also indicate that glutathione content significantly increased as a result of spermine treatments, especially in plants treated with 100 mg/L, followed by plants treated with pyridoxine 200 mg/L. The scavenging effect of methanolic extracts of lemongrass plant on DPPH radicals rapidly increased from 50-100 mg/L (Table 3). The scavenging effects of plants treated with 100 mg/L spermine and 200 mg/L pyridoxine were 74.47% and 72.19 %, respectively.

Ha et al. (1998) reported that the polyamine spermine, which is normally found in millimolar concentrations in the nucleus, is shown to function directly as a free radical scavenger. These data suggest that spermine is a major natural intracellular compound capable of protecting DNA from free radical attack. Huang et al. (1999) also reported that at 5.0 mg/mL, the methanolic extracts from fresh and air-dried fruit bodies scavenged DPPH radicals by 99.1% and 96.3% at 2.5 mg/mL, respectively. In addition, the scavenging effect was 97.1% at 5.0 mg/mL for white mycelia (Huang et al. 1999).

Several studies on the effect of pyridoxine on cell culture indicate that pyridoxine (vitamin B6) functions as a protectant against reactive oxygen species produced in the human body (Merrill and Henderson 1987; Di Salvo et al. 2012; Fitzpatrick et al. 2012).

The oil of lemongrass plant from different treatments as well as untreated control was subjected to fractionation using gas-liquid chromatography (GLC) and the data are represented in Table 4. The identified compounds ranged from 84.91% to 98.81%. Twelve hydrocarbon and oxygenated terpenes were markedly identified which are grouped into three classes, i.e., major constituents (more than 10 %), minor constituents (less than 10 %) and traces (less than 1 %). Accordingly, it is clear from the obtained data that citronellol and geranial represent the major compounds which ranged from 40.72 to 51.27 and 27.40 to 32.88%, respectively.

Hydrocarbon terpenes ranged from 7.53% -13.65 %, while the oxygenated compounds ranged from 75.82 to 89.70%. It is also clear from data presented in Table 4 that plants with 50 mg/L pyridoxine recorded the highest level of total hydrocarbons (13.65 %). Meanwhile, control plants recorded the lowest content of total oxygenated compounds (75.82 %).

These results are in agreement with those obtained by (Schaneberg and Khan 2002) who reported that Lemongrass contains mainly citronellol and geranial which define the oil quality. Pengelly (2004) reported that geranial and neral of lemongrass are two isomeric aldehydes. Other unusual active components are limonene and citronellal (Schaneberg and Khan 2002).

Abd El-Wahed and Gamal El Din (2004) also reported that the application of spermidine at 100 mg/1 improved chamomile growth, oil quantity, and quality by increasing the level of farnesene, bisabolol oxide B, α-bisabolol, chamazulene, and bisabolol oxide A. Concerning the increase in citronellol and geranial contents as a result of pyridoxine and spermine treatments, Prins et al. (2010) reported that the biosynthesis of essential oil depends on genetic factors and the developmental stage of plant growth in addition to other factors which could alter biochemical and physiological changes in plants and modifies the quantity and quality of the essential oil.

In conclusion, by comparing the effect of foliar treatments of lemongrass plants with pyridoxine and spermine, it is apparent from the previous results that foliar treatment with spermine (200 mg/L) or pyridoxine (100 mg/L) had beneficial influence for increasing plant growth, antioxidant enzyme activities and essential oil content. It is also worth to mention, that spermine (200 mg/L) was more effective in influencing lemongrass growth than pyridoxine (100 mg/L).

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