Journal of Medicinal Plants Studies 2016; 4(4): 208-214

Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(4): 208-214

Received: 29-05-2016
Accepted: 30-06-2016

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Effect of nitrogen fertilization on antioxidant activity of Mas cotek (*Ficus deltoidea* Jack)

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Abstract
Mas cotek (*Ficus deltoidea* Jack) a medicinal herb has its wider application for treating many disorders e.g. as a cure of disorders of menstrual cycle, gout, diabetes, hypertension and diarrhea. With increasing popularity, area under cultivation of Mas cotek plant is increasing and there is a need to optimize its growing conditions to get maximum produce by limited use of available resources. Present study was conducted to check the response of Mas cotek plant to differential application of nitrogen and its effect on growth, biochemical and antioxidant activity. Five levels of urea (0, 50, 100, 150 and 200 kg N ha^{-1}) were applied, each with four replicates under RCBD in open shelter glasshouse conditions. Data were recorded on growth related parameters, biochemical traits and antioxidant activity. The results showed that nitrogen application improved growth, chlorophyll contents and antioxidant activity of Mas cotek with maxiumum growth @ 100 kg ha^{-1}.

Keywords: antioxidant activity, Mas cotek, nitrogen, fertilizer

1. Introduction

Popularity of herbs and medicinal plants is increasing these days as more and more people are inclined to use herbal remedies in their daily life. It is believed that as much as 80% of the world outside the industrialized countries relies on herbs for health [1]. *Ficus deltoidea* is important medicinal herb. It belongs to *Moraceae* family and locally known as Mas cotek. It is a traditional herb usually used for its medicinal properties. The leaves of *F. deltoidea* are boiled and the decoction is taken by mother after birth. It is believed that it helps to contract the uterine and vaginal muscles, improve blood circulation and regain body strength as well as for treating disorders related to the menstrual cycle. It is also taken as medicinal tea for general health and in treating pneumonia, diabetes, hypertension, diarrhea and gout [2].

For proper growth and optimal yield, nutrients must be available to plants in correct quantity, proportion and in a usable form at the right time. To fulfill these requirements, chemical fertilizers and/or organic manures are needed. Nitrogen (N) is one of the most important nutrients affecting the growth, development, fruit yield and plant quality [3, 4] and the same time is an essential mineral for the biosynthesis of amino acids, proteins and enzymes [5]. Its deficiency causes stunted growth and yield reduction [6]. Plants can use various forms of the nitrogen from soils (e.g. NH^{4+} and nitrate NO^{-3}) and incorporate it in proteins via the amino acids [7]. Nevertheless, urea constitutes another nitrogen source widely used by the plants [8]. Similarly urease is the enzyme responsible for urea hydrolysis and production of ammonium [9].

Antioxidants are the substances which delay or inhibit oxidative damage [10] hence play important and crucial role in disease prevention [11] and growth promotion [12]. However, management practices such as source and rate of fertilizer used can affect plant quality and nutrient composition [13, 14]. In common agricultural practices, inorganic fertilizers are used to maximize the production of foliage for leafy herbs. It has been reported fertilizer affect the antioxidant level [15] and vitamin activity [16, 17]. Antioxidant levels in *Brassica rapa* seemed to decrease as the fertilizer rate increased, especially under conventional fertilization [18]. It was also seen that a plant will contain higher levels of phytochemicals and antioxidant compounds if it has experienced some stress due to the lower rates of fertilization during its development [19, 20]. Increasing nitrogen fertilizer increased the concentration of lutein and carotene in parsley [21] and phenolic compounds and carotenoids in lavender [22], whereas in *Chrysanthemum morifolium* heavy nitrogen fertilization was reported to decrease flavonoids and antioxidant activity of flowers [23]. Quality parameters like dry matter, specific gravity, starch contents, vitamin-C and ash contents were also affected with P and K fertilization [24].

Increased application of fertilizers resulted in an increased vegetative growth and yield of *Aronia melanocarpa* whereas the content of anthocyanins and total acidity decreased [25]. While N is an essential nutrient element for crop growth and quality, little is known about the effect of N supply on the antioxidant activity of Mas cotek.

Success of a plant under given set of conditions is predicted by its vegetative growth as well as the production of valuable reproductive parts (fruits or other economical products). In case of medicinal plants these economical products includes flavonoids, phenolic acids and catechins which are well known for their antioxidant activity and made them attractive for medicinal purpose [26]. Mas cotek is an emerging plant having a wider application in the field of medicine. Keeping in view the increasing future demand of Mas cotek plant, its production technology was needed to be optimized. In the present study, we tried to find the optimum nitrogen level for proper growth and better antioxidant profiling of Mas cotek plant.

2. Materials and Methods

2.1 Soil and plant selection

Present study was conducted using Bungor soil series (Kaolinitic, Iso-hyperthermic, Typic Paleudult, pH = 4.8, extractable P = concentration 4.92 mg/kg, and Se = concentration, 0.78 mg kg^{-1}). Five levels of urea (0, 50, 100, 150 and 200 kg ha^{-1}) were applied. To meet the other nutritional requirements of plants based on the recommendation of Malaysia Agriculture Research Development Institute (MARDI) for the cultivation of *Ficus deltoidea*, phosphorous (43 kg P ha^{-1}) and potassium (82 kg K ha^{-1}) fertilizer were also added. The experiment was conducted in the glasshouse condition using completely randomized design. Each treatment was repeated four times. *F. deltoidea* plant selected for study were tow month old. Plants were transplanted in polybags containing 10 kg soil. Plants were watered daily with deionized water during the whole growth period (4 months). Data was recorded on plant height (cm), root length (cm), plant weight (g), chlorophyll contents (mg/g), number of branches, leaves and fruits. Samples from different parts of the plants were subjected to wet digestion method using nitric acid (HNO_3) using Havlin and Soltanpour [27] protocols. Total nitrogen in the plant sample was determined by Kjeldahl method [28]).

2.2 Antioxidant activity of mas cotek *(F. deltoidea)*

The influence of different urea rates on the antioxidant activity was determined by measuring the free radical scavenging effect with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in the extracts from the dried leaf sample. Also, the extraction of antioxidant compounds was conducted employing standard method [29]. A total of 0.5 g dried leaves was placed in 150 ml conical flask and the flask was covered with aluminum foil. The conical flasks containing the samples were placed on an orbital shaker at room temperature for 1 h in the dark. After 1 h, the samples were filtered using a Whatman No. 1 filter paper and the extracts were stored in the freezer at -80°C. DPPH free radical scavenging assay was measured using DPPH free radical test. The initial absorbance of DPPH in methanol was measured using a Spectramax+ 384 spectrophotometer at the wavelength of 515 nm until the absorbance remained constant. A total of 40 µl of extracts was added to 3 ml of 0.1 mM methalonic DPPH solution. The mixture was incubated at room temperature for 30 min before the change in absorbance at 515 nm was measured. The percent of inhibition was calculated using the following formula.

Inhibition (%) = [(A515 of control – A515 of sample)/A515 of control] × 100

A stands for absorbance at wavelength of 515 nm.

2.3 Statistical analysis

Average data were subjected to analysis of variance (ANOVA) and means were separated with Tukeye' s Honestly Significant Difference (HSD) test at 5% significance level using Statistics 8.1 software.

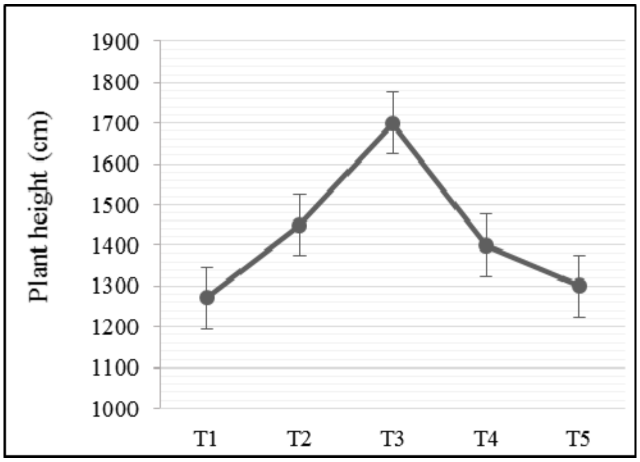
3. Results

The present experiment was conducted to check the effect of nitrogen fertilization on growth, biochemical and antioxidant activity of Mas cotek. Data were collected and analysis of variance (ANOVA) was performed which showed that all the treatments differed significantly from each other for all studied traits (Table 1). Further, means were separated using HSD at 5% level of confidence (Table 2 and Table 3) for morphological traits and nutrient composition respectively.

3.1 Nitrogen fertilization and plant growth

Nitrogen is the most essential mineral nutrient that promotes plant growth and consequently yield. The improvement in growth of plants as compared to control was observed in all treatments as depicted by increase in plant height, root length, plant fresh weight and dry weight. It was observed that although all the growth related parameters showed significant increase in all treatments as compared to control, ideal growth was shown by plants under T_3 (where the application of fertilizer was 100 kg urea ha^{-1}). Plant height was significantly increased owing to application of nitrogen fertilizer. Fertilizer application up to 100 kg ha^{-1} caused the highest increase in plant height (25%) as compared with control treatment (Figure 1a). Nitrogen application had a positive effect on root growth of Mas cotek plant (Fig. 1b). Maximum increase in root growth (21.66%) was observed when nitrogen was applied @ 100 kg ha^{-1}.

Fresh and dry weight of the plants was also positively affected by the application of nitrogenous fertilizer. A progressive increase in plant weight was observed with increasing nitrogen rate. Maximum increase in fresh and dry weight per plant (12.6% and 38.9%) was recorded when nitrogen was applied at the rate of 100 kg ha^{-1}. It was also observed that higher rates of fertilizer seemed unnecessary in improving the fresh and dry weight of Mas cotek plants (Fig. 1 c & d).



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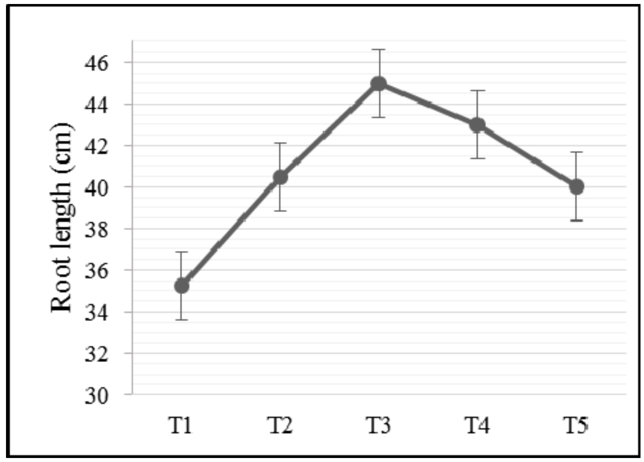

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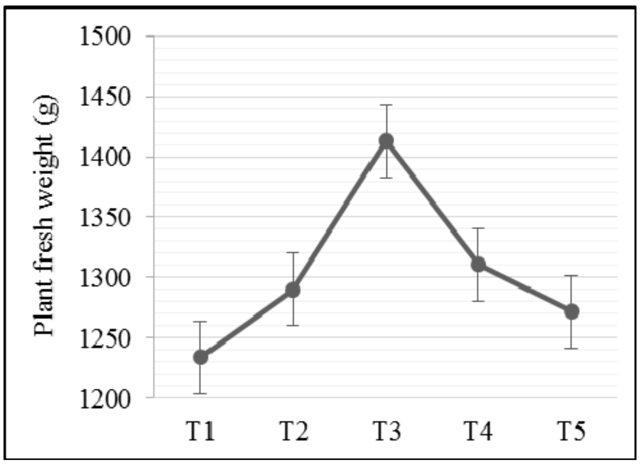

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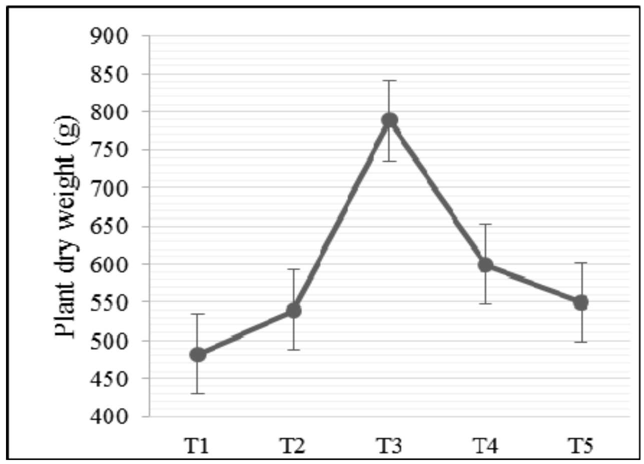
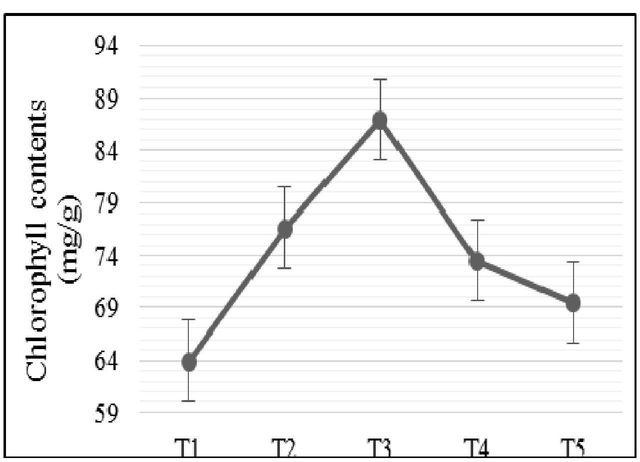


(d)

Fig 1: Performance of mas cotek plants under different rates of nitrogen application.

3.2 Nutrient fertilization and biochemical activity

Chlorophyll contents were also affected positively by application of nitrogenous fertilizer (Fig. 2). Maximum increase in chlorophyll contents (22.03%) was observed at 100 kg ha^{-1} nitrogen application. It was also observed that chlorophyll contents were statistically similar above 100 kg ha^{-1} fertilizer rate (Figure 2a). The quality of fresh leafy herbs can be evaluated by the nutrient compositions in aerial plant tissues. Mineral content in leaf tissues was influenced by fertilizer rates. Nitrogen application had a positive effect on the mineral contents especially on nitrogen concentration in different parts of the plants viz. root, shoot and leaves (Figure 2 b, c & d). Nutritional analysis showed that nitrogen contents of plants increased with increasing fertilizer rates. In the case of leaves the highest concentration of N was observed under the treatment containing N @ 200 kg ha^{-1}. Results were also analysed for N concentration in shoot and leaves. It was seen that the treatment containing N @200 kg ha^{-1} improved the N concentration in the plant parts as compared to control and showed 14, 32 and 26% increase for leaves, shoot and roots, respectively.



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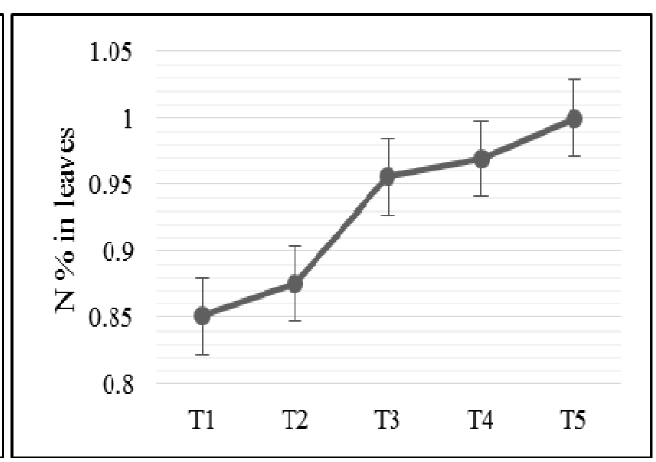

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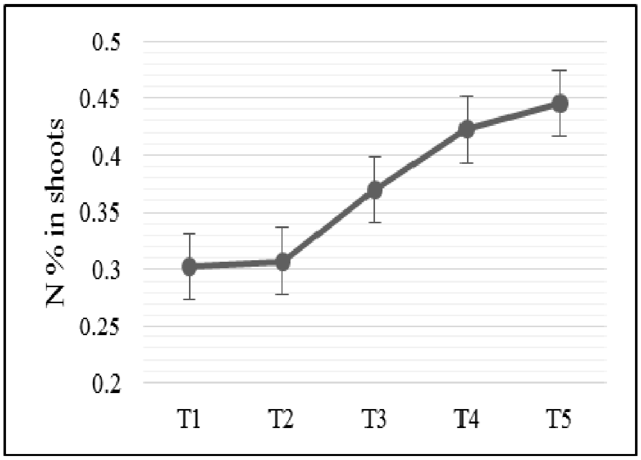

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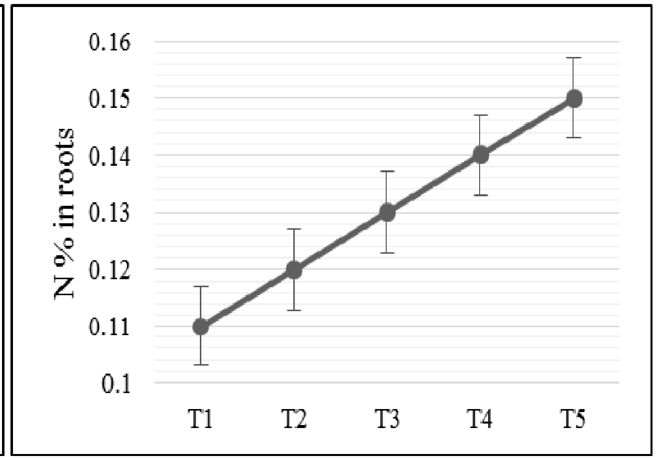

(d)

Fig 2: Effect of N application on biochemical activity of Mas cotek plant.

3.3 Nitrogen fertilization and antioxidant activity

The N fertilizer rates influenced the antioxidant activity of the Mas cotek. Highest antioxidant activity was observed in leaves for all treatments as compared to stem and roots (Fig. 3). Moreover, it was observed that T_3 treatment exhibited the highest antioxidant activity in all plant organs. The trend of increase in antioxidant activity was parallel with the nitrogen fertilization up to T_3 (100 kg ha^{-1}), but above this level increasing fertilizer rate decreased the antioxidant activity in all plant organs. It showed that normal growth of mas cotek was at T_3 (100 kg ha^{-1}), hence the antioxidant activity was also maximum at that rate.

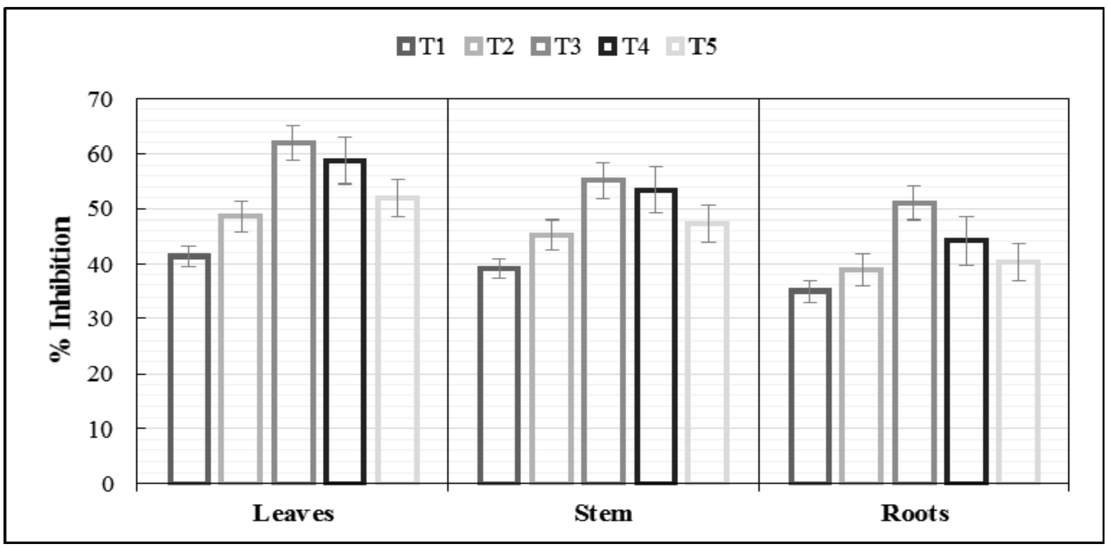

Fig 3: Comparative antioxidant activity of different plant organs under various nitrogen treatments.

4. Discussion

Significant variation was observed among the treatments for all the studied traits in accordance with the previous studies on other medicinal plants [30, 31, 32]. It showed that plants performed differently under all treatments.

It was seen that optimum growth of plants (plant height, root length, fresh and dry plant weight) was observed under T_3 (100 kg ha^{-1}) and these results are in line with the previous results [33, 34]. Increased root length provided better uptake of nutrition and water from soil, in return growth of plants was increased. It also increased the uptake of N, P and K in plants and hence their concentration in different plant parts. Enhanced nutrition then up regulated the photosynthetic machinery as was seen by increased chlorophyll contents and also the rate of photosynthesis was increased which resulted in production of more biomass. Nitrogen is the building block for amino acids and protein synthesis as well as chlorophyll composition. Improvement of N concentration resulted from higher fertilizer application had led to significant improvement in chlorophyll contents. Our results are in line with [35]. who reported that nitrogen application increased the chlorophyll content and leaf surface area. The formation of photosynthetic pigments and the enzymes taking part in the carbon reduction and membrane system of chloroplasts were also affected by the application of nitrogen nutrition. Production and deposition of more photosynthates has produced more number of leaves, branches and fruits which ultimately increased plant weight. Increase in growth and yield owing to the application of N-fertilizers may be attributed to the fact that nitrogen being important constituent of nucleotides, proteins, chlorophyll and enzymes, involves in various metabolic processes which have direct impact on vegetative and reproductive phases of plants [36, 37].

However, treatments containing more than 100 kg ha^{-1} N application have resulted in mineral toxicity in the root zone. Although they show improved growth as compared to control but due to mineral toxicity the roots were unable to grow as long as was observed under 100 kg ha^{-1} N application at which plants showed ideal growth. Mineral toxicity also reduced the number of root hairs present on the root surface which has ultimately limited the supply of nutrients to the aerial parts of the plants. This lead to reduced growth of aerial parts of plants producing plants with reduced height, less weight and low fresh as well as dry weight as was previously reported in rice [38].

Increasing concentration of nitrogen in the root zone burned the root hairs causing mineral toxicity and reduced uptake of phosphorus and potassium in the plants as root hairs play a crucial role in the uptake of phosphorus and potassium [39, 40]. Due to reduced uptake of phosphorus and potassium, chlorophyll contents were also decreased in those plants, as both phosphorus and potassium play important role in the building up of chlorophyll molecules [41].

Comparative study of antioxidant activity of different plant organs showed that maximum antioxidant activity was observed in leaves of Mas cotek followed by stem and roots respectively. Our results are in line with [42]. It was observed that inhibition due to antioxidants was best observed @ 100 kg ha^{-1} and treatments with higher or lower rates of fertilization have reduced inhibition. Increasing the nitrogen rate above the optimum level stimulated the production of reactive oxygen species (ROS) and although production of antioxidants was also increased but the inhibition was reduced as the production of ROS outnumbered the production of antioxidants. Decreased inhibition of antioxidant molecules has caused negative impacts on growth and hence growth was also reduced. Our results are in line with the previous study [43] reported that nutritional stress increased ROS production as well as antioxidant activity in plants. Antioxidant activity and nitrogen concentration in leaves, root and stems were also parallel to each other as was previously reported by Yañez-Mansilla [44].

5. Conclusion

Nitrogen is an important macronutrient and its application to plants influences the growth and quality of plants. It is concluded from the results that nitrogen application had a positive effect on the growth and chlorophyll contents of Mas cotek. Urea application at 100 kg ha^{-1} gave optimum growth of Mas cotek and higher rates of fertilizer were unnecessary for getting increased growth and also were fatal for growth. Also nitrogen application at higher rates negatively affected the antioxidant activity of Mas cotek.

6. Acknowledgements

The authors would like to thank the Department of Land Management, Universiti Putra Malaysia, for providing the necessary support for the study.

Table 1: Statistics results.

Traits	Mean	Mean Squares	CV%
Plant height (cm)	1424	96.32*	5.61
Root length (cm)	40.75	12.23**	4.32
Dry weight (g)	666.68	4742.0*	2.92
Fresh weight (g)	1304	400.4**	4.5
Chlorophyll contents (mg/g)	74.08	17.92**	2.36
N in leaves (%)	1.63	0.93*	1.97
N in shoot (%)	0.25	0.005*	1.24
N in roots (%)	0.88	0.30**	1.35

* Significant at alpha 5% ** Significant at alpha 1%.

Table 2: Dosage effect for some investigated agronomic traits.

Doses	PH	RL	FPW	DPW
N 0 kg ha^{-1}	1270 d	35.25 c	1234 e	482 e
N 50 kg ha^{-1}	1450 c	40.5 b	1290 c	562 d
N 100 kg ha^{-1}	1700 a	45 a	1413 a	789 a
N 150 kg ha^{-1}	1400 b	43 ab	1311 b	778 b
N 200 kg ha^{-1}	1300 d	40 b	1272 d	723 c
HSD Value	30.1	4.1	10.5	6.8

*PH = Plant height, RL = Root length, FPW = Fresh plant weight, DPW = Dry plant weight

Table 3: Dosage effect for chlorophyll content and nutrient concentration in the plant.

Traits	Chlorophyll content	N in leaves	N in shoot	N in roots
N 0 kg ha^{-1}	63.9 c	0.7 c	0.3025 d	0.11 e
N 50 kg ha^{-1}	76.62 b	1.6 b	0.3075 d	0.12 d
N 100 kg ha^{-1}	86.9 a	1.85 a	0.37 c	0.13 c
N 150 kg ha^{-1}	73.5 b	1.9 a	0.4225 b	0.14 b
N 200 kg ha^{-1}	69.5 b	2.1 a	0.445 a	0.15 a
HSD Value	10.1	0.5	0.03	0.005

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