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EXPLOITING THE NUTRITIONAL REQUIREMENT FOR GROWTH, FLOWER PRODUCTION AND PHYTOCHEMICAL PROFILE OF *Murraya exotica*

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The potential of *Murraya exotica* has never been fully exploited in Pakistan and very little work has been carried out as an important essential oil crop. In present study growth potential and phytochemical profile of *M. exotica* under Faisalabad agro-climatic conditions were explored. The nutrition effects on growth and flowers production *of M. exotica* were investigated. It was noted that values of different growth parameters significantly increased with increase in the fertilizer level whereas, maximum values of plant height (125.07 cm), number of branches (43.6), number of flowers (164.1), size of flowers (1.89 cm), fresh weight of flowers (23.6 g) and dry weight of flowers (7.39 g) were recorded in plants supplied with 20 g NPK/plant/month + 4 kg FYM/plant (T6). In addition, this also significantly enhanced the nutrient contents (N, P and K) and photosynthetic pigments in the flowers. Phytochemical analysis revealed increase in carbohydrates, phenolic compounds, phytosterols, alkaloids, flavonoids, proteins and free amino acids contents.
Keywords: Chlorophyll content, essential oil, fertilizer, FYM, NPK, phytochemicals, Rutaceae

INTRODUCTION

Murraya exotica Synonyms viz. *Murraya paniculata*, *Chalcas exotica*, orange Jasmine, belongs to the family Rutaceae. *M. exotica* is an evergreen shrub, usually grows up to 2 to 3 m in height. Alternate leaves with usually 3-9 leaflets are produced on this plant. The leaflets are 1-5 cm in size, dark green in color, glossy, cuneate or rounded at the base. Small clusters of white fragrant flowers produced at the top of branches. The plant can be pruned to grow topiary and can also be grown as a hedge plant or ornamental plant (Whistler, 2000). *M exotica* are adapted to wide range of conditions. This species grows from nearly sea level to an elevation of 1500 m and native to China, India, Sri Lanka, Northeastern Australia and Taiwan (Parrotta, 2001). Medicinally, it is an important plant as it is possess several chemical compounds like alkaloids, coumarins, carotenoids and flavones etc (Aziz *et al*., 2010; Gautam and Goel, 2012).

The balanced use of fertilizers not only supplied the essential nutrients in sufficient amounts to plants but also enhances the efficiency of the plants to get benefitted from the applied nutrients (Law-Ogbomo and Egharevba, 2009; Younis *et al*., 2010). The main purpose of using the fertilizers is to replace the amount of nutrients that has been depleted in the soil profile because of continuously taken up by the plants needed for their growth (Younis *et al*., 2011).

Nitrogen (N), potassium (K), and phosphorus (P) are the main and most important components in mineral fertilizers and manipulate the growth while applied in various combinations (Younis *et al*., 2013). Nitrogen has more influence on plant growth, quality and yield than any other element does and its excess application enhances vegetative growth (Kashif *et al*., 2014). Potassium is necessary for basic physiological functions of the plants i.e. formation of sugars, starch, synthesis of proteins and cell division. While, transfer of energy, synthesis and breakdown of carbohydrates and photosynthesis are some of the necessary life process that could not be accomplished in the absence of phosphorus (Obreza, 2001). Whereas, organic manures generally improve the soil organic matter content, a basic indicator of soil health and resilience (Riaz *et al*., 2008; Tariq *et al*., 2012; Yasmeen *et al*., 2012). The organic materials enrich the soil fertility and enhance its ability to supply the significant quantities of nutrients required to plants (Krishnamurthy *et al*., 2001).

As compared to other commercial flower crops, very little work has been carried out on *M. exotica*. Therefore, this study was designed to understand the nutritional requirement for enhancing growth and flower yield and to analyze the chemical profile of the plant.

MATERIALS AND METHODS

The present study was conducted during 2011-2013 at Rose project, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The study consisted of two parts: firstly, response of *M. exotica* regarding its vegetative and floral characteristics to organic (FYM) and inorganic (NPK) fertilizers was evaluated. Secondly phytochemical analysis was made under the agro-climatic conditions of Faisalabad, Pakistan.

***Effect of organic (FYM) and inorganic (NPK) fertilizers*:** One year old plants of uniform height were planted at 3x3 feet P-P and R-R distance. Garden NPK fertilizer (17:17:17), and well rotten cow manure were used for the first part. The treatments were per plant basis, and NPK was given after each month, while, FYM was added at the start of the experiment. Irrigation and weeding practices were same for all the treatments. Seven treatments with four replications were used in this experiment.

Whereas, the treatment combinations were:

T_0 = Control,
T_1 = 10 g NPK/plant/month + 3 kg FYM/plant,
T_2 = 10 g NPK/plant/month + 4 kg FYM/plant,
T_3 = 15 g NPK/plant/month + 3 kg FYM/plant,
T_4 = 15 g NPK/plant/month + 4 kg FYM/plant,
T_5 = 20 g NPK/plant/month + 3 kg FYM/plant,
T_6 = 20 g NPK/plant/month + 4 kg FYM/plant

***Data collection*:** Data were recorded on plant height (cm) by measuring rod, numbers of branches per plant and numbers of flowers per plant were counted, size (cm) of flowers was done by digital Vernier caliper (Model: HT0403-A1, Cingda Industry Co., Ltd. China) fresh and dry weights (g) of flowers were taken by the digital weighing balance. For leaf mineral (N%, P%, K%) contents, mature and healthy leaves along with their petioles were washed with detergent and then with tap water and finally with well rinsed distilled water for 2-3 times so that the effect of detergent was washed out. Afterwards the leaves were dried under shade for 48 hours and then were packed in perforated paper bags. After labeling, bags were punched and placed them in oven for drying at 60°C for over 48 hours. The dried leaf samples were then taken out and grinded to fine powder in an electric stainless steel grinder (Model (Moulinex, AR1044, Tokyo, Japan). This powder was stored in properly labeled air tight plastic bottles at room temperature for further process. Analysis of N, P and K was done by following Champan and Parker (1961).

Total leaf chlorophyll contents (mg g^{-1}FW), chlorophyll contents were calculated by using the method of Arnon (1949). Fresh leaves of (0.5 g) were chopped into segments of 0.5 cm and extracted with 5 mL acetone (80%) at 10^{o}C overnight. Centrifuge the material at 14000 x g (14,000 times Earth's gravitational force) for 5 min. and measured the absorbance of the supernatant at 645, 652 and 663 nm on spectrophotometer (Model Hitachi U 2001, 121-0032).

***Phytochemical analysis of Murraya exotica*:** Two kilogram fresh flowers of *M. exotica* were collected, washed with distilled water; air dried and powered the material for phytochemical analysis. The powdered plant materials was macerated with petroleum ether to remove fatty substances, the marc (organic residue) was further exhaustively extracted with of 50% ethanol for 3 days. The extract was separated by filtration and concentrated under vacuum using rotary evaporator (EYELA, N-N series, Japan) at 40°C. Ethanolic extract of *M. exotica* was subjected to qualitative tests for the identification of various active constituents (Younis *et al*., 2011; Gautam and Goel, 2012).

***Carbohydrates*:** A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated H_2SO_4 was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrate.

***Fixed oils*:** were checked by taking a few drops of extract and pressed between two filter papers. Appearance of oil stains on the papers indicates the presence of fixed oil.

***Proteins*:** Small quantity of the extract was dissolved in 3 ml of distilled water and equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added in it until pink or purple color appeared as an indicator of proteins and free amino acids presence.

***Saponins*:** The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

***Phenolic Compounds*:** Small volume of the extract was taken in distilled water then added the dilute ferric chloride solution (5% w/v), development of violet color showed the presence of phenolic compounds.

***Phytosterols*:** A small quantity of the extract was taken and dissolved in 5 ml of chloroform. Took 1 ml of chloroform solution, and few drops of concentrated sulphuric acid were added. Brown color produced shows the presence of phytosterols.

***Flavonoids*:** Concentrated sulphuric acid was added in a small portion of extract. Appearance of yellow orange color showed the presence of flavonoids.

***Alkaloids*:** Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the Mayer's reagent, appearance of cream precipitate showed the presence of alkaloids.

RESULTS

Effect of different combinations of NPK and FYM on growth parameters of *M. exotica* was examined and highly significant effect ($P < 0.05$) was noted. It was observed that the plants maintained maximum plant height (125.07 cm) by the application of 20 g NPK/plant/month + 4 kg FYM/plant (T_6) closely followed (117.64 cm) by 20 g NPK/plant/month + 3 kg FYM/plant (T_5). A significant increase in plant height (108.38 cm) was also noted in plants applied with 15 g NPK/plant/month + 3 kg FYM/plant (T_4). No fertilizer treatment (T_0) resulted in the minimum value (69.14 cm) for this variable (Fig. 1). The data regarding number of branches per plant also found significant ($P < 0.05$) among various fertilizer treatments where maximum value (43.62) was recorded. NPK and FYM applied @ 20 g NPK/plant/month and 4 kg FYM/plant statistically related to T_5 (41.38) treatment (20 g NPK/plant/month + 3 kg FYM/plant). The minimum number of branches (19.62) was observed in control (T_0) plants grown without any fertilizer treatment (Fig. 2).

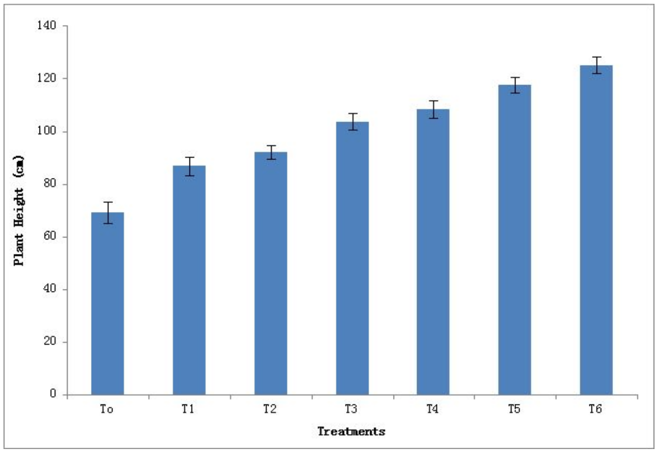

Figure 1. Effect of fertilizer treatments on plant height (cm).

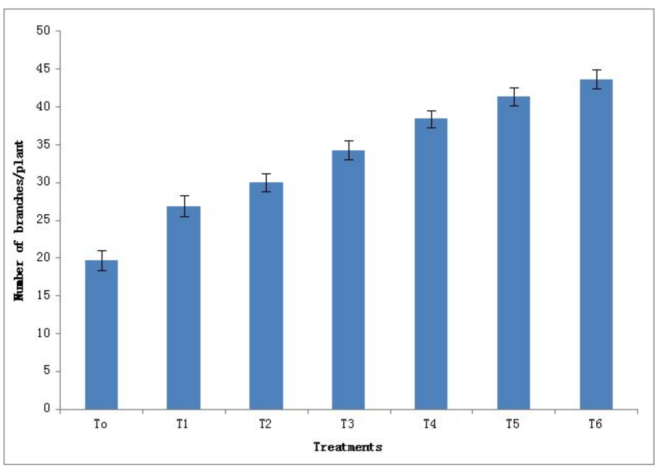

Figure 2. Effect of fertilizer treatments on number of branches/plant.

Application of various NPK treatments in combination with FYM significantly affected the number of flowers produced by each plant. The plants applied with 20 g NPK/plant/month + 4 kg FYM/plant (T_6) produced the maximum number of flowers (164.1) and were followed by those fertilized with 20 g NPK/plant/month + 3 kg FYM/plant (T_5) which produced 154.65 flowers per plant. A significant increase in number of flowers was also observed by the application of 15 g NPK/plant/month + 4 kg FYM/plant (T_4) and 15 g NPK/plant/month + 3 kg FYM/plant (T_3). Plants with no fertilizer application resulted in the minimum number of flowers (87.15) per plant (Fig. 3).

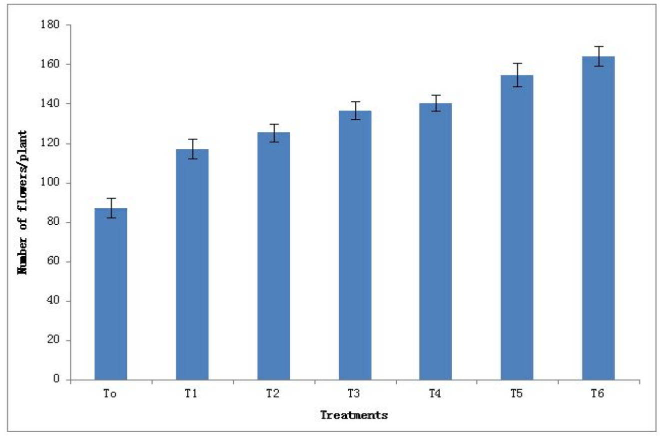

Figure 3. Effect of fertilizer treatments on number of flowers/plant.

A significant flower size difference ($P < 0.05$) was observed among various fertilizer treatments. The flower size was maximum in plants (1.89 cm) treated with 20 g NPK/plant/month + 4 kg FYM/plant (T_6). Non-significant differences were observed between T_6 and T_5 treatment (20 g NPK/plant/month + 3 kg FYM/plant) for this variable. The size of the flower was significantly decreased with reduction of fertilizer level. While, control plants applied with no fertilizer produced the smallest (1.21 cm) flowers (Fig. 4). In the same manner highly significant effect ($P < 0.05$) of different fertilizer treatments was also noted on fresh weight and dry weight of flowers. It was found that the supply of 20 g NPK/month + 4 kg FYM/plant (T_6) resulted in the production of flowers with the highest fresh weight (23.6 g) and dry weight (7.39 g).

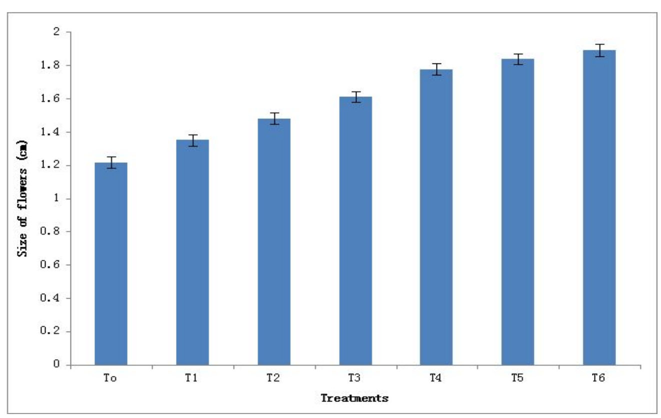

Figure 4. Effect of fertilizer treatments on size of flowers (cm).

***Leaf minerals and chlorophyll contents*:** Analysis of variance for the data regarding leaf nitrogen (N) percentage indicated highly significant ($P < 0.05$) effect of fertilizers application on this variable. The leaf N content increased by increasing fertilizer doses. The highest value (2.53%) was recorded in plants supplied with 20 g NPK per plant per month in combination with 4 kg FYM per plant (T_6).

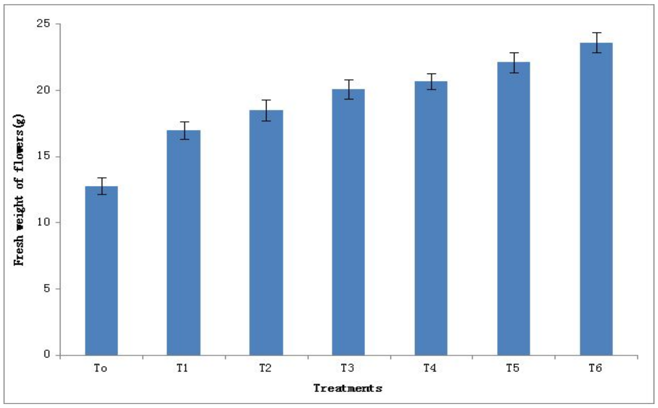

Figure 5. Effect of fertilizer treatments on fresh weight of flowers (g).

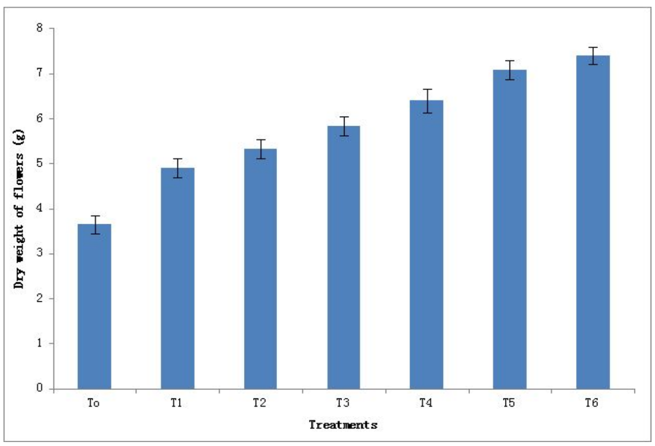

Figure 6. Effect of fertilizer treatments on dry weight of flowers (g).

The application of 20 g NPK + 3 kg FYM/plant (T_5) also significantly increased the N contents in leaf and gave the second highest value (2.33%) statistically at par with the value (2.20%) obtained by supplying 15 g NPK + 4 kg FYM/plant (T_4) to the plants. The lowest leaf N (1.2%) was noted in plants applied with no fertilizer. The application of various fertilizer treatments significantly ($P < 0.05$) influenced the accumulation of phosphorous (P) in the leaves of *M. exotica*. The plants applied with 20 g NPK/month + 4kg FYM/plant (T_6) maintained the maximum concentration of P (0.31%) in leaves. A significant increase in leaf P contents was also observed in plants supplemented with 20 g NPK + 3 kg FYM/plant (T_5) and 15 g NPK + 4 kg FYM/plant (T_4) which gave the second and third highest values i.e. 0.28% and 0.25%, respectively. No fertilizers application (T_0) resulted in the lowest value (0.12%) for this variable. Data regarding leaf potassium (K) contents (%) revealed the highly significant effect of different fertilizer treatments on this variable. It was observed that the plants accumulated maximum K (1.65%) in leaves by the application of 20 g NPK per month + 4 kg FYM per plant (T_6). The leaf K contents were also significantly increased by other fertilizer treatments that gave higher values i.e. 1.57% (T_5), 1.46% (T_4), 1.36 (T_3), 1.27 (T_2), 1.16 (T_1) than no fertilizer supply (T_0) to the plants which resulted in the minimum value (0.93%) for this variable (Table 1).

A significant effect of various fertilizer treatments was noted on the total chlorophyll contents of *M. exotica*. The plants supplemented with 20 g NPK per month + 4 kg FYM per plant exhibited the highest (2.49 mg g^{-1} FW) chlorophyll contents in the leaves. The other fertilizer treatments viz. T_5 (20 g NPK/month + 3 kg FYM/plant), T_4 (15 g NPK/month + 4kg FYM/plant), T_3 (15 g NPK/month + 3 kg FYM/plant), T_2 (10 g NPK/month + 4 kg FYM/plant), T_1 (10 g NPK/month + 3 kg FYM/plant) also significantly increased the leaf chlorophyll contents i.e. 2.38, 2.19, 1.92, 1.70, 1.62 mg g^{-1} FW, respectively with respect to no fertilizer supply (1.47 mg g^{-1} FW) to the plants (Table 1).

***Phytochemical profile of M. exotica*:** Phytochemical analysis of *M. exotica* was done to identify the carbohydrates, alkaloids, flavonoids, saponins and phenolic compounds. It was found that carbohydrates, phenolic compounds, phytosterols, alkaloids, flavonoids and proteins were present, whereas, fixed oils and saponins were absent in *M. exotica*. The results are presented in Table 2.

Table 1. Effect of fertilizer treatments on N, P, K percentage and total chlorophyll contents of *M. exotica*.

Treatments	N (%)	P (%)	K (%)	Total chlorophyll content (mg g^{-1} FW)
T_o	1.2 f	0.12 g	0.93 g	1.47 g
T_1	1.5 e	0.16 f	1.16 f	1.62 f
T_2	1.75 d	0.18 e	1.27 e	1.7 e
T_3	1.93 c	0.23 d	1.36 d	1.92 d
T_4	2.2 b	0.25 c	1.46 c	2.19 c
T_5	2.33 b	0.28 b	1.57 b	2.38 b
T_6	2.53 a	0.31 a	1.65 a	2.49 a

Table 2. Phytochemical analysis of *M. exotica.*

Sr #	Phytochemicals	Result
1	Carbohydrates	Present
2	Fixed oils	Absent
3	Saponins	Absent
4	Phenolic compounds	Present
5	Phytosterols	Present
6	Alkaloids	Present
7	Flavonoids	Present
8	Proteins	Present

DISCUSSION

The increase in fertilizer dose caused a parallel increase in plant height. Literature indicated a positive correlation between plant height and fertilizer application rate in different crops, like *Dahlia* (Bhattacharjee and Mukherjee, 1983), *Viola* (John *et al*., 1984), *Chrysanthemum* (Barman and Pal, 1999) and *Zinnia* (Kashif, 2001). The application of 20 g NPK/plant/month + 4 kg, FYM/plant resulted in the highest plant height, which suggests that high fertilizer doses increase the availability of mineral nutrients to plants by enhancing soil structure and improving water holding capacity, aeration and cation exchange capacity of soil. The increase in plant height might be due to the significant influence of nutrients on metabolic processes required for plant growth. An adequate supply of nitrogen (N) and phosphorous (P) is associated with vigorous vegetative growth and improved flower quality. Similarly, potassium (K) plays a key role in a vast array of physiological processes vital for plant growth, from protein synthesis to plant water balance. Whereas, organic manures are added to the soil that not only provides the nutrients but also increase its fertility by providing carbon and other constituents that effect the humus content of soil, biological activity and by improving the soil structure which ultimately result in better uptake of nutrients. Reports regarding increase in the number of branches per plant by increasing the amount of NPK fertilizer are also consistent (Baboo and Sharma, 1997; Gurav *et al*., 2002; John *et al.,* 1984; Katsoulas *et al*., 2006; Patil *et al*., 1999; Palai *et al.,* 2002; Singh and Gupta, 1996; Obreza *et al*., 2008). Our results showed similar trend that *M. exotica* plants produced the highest number of branches with the application of 20 g NPK/plant/month + 4 kg FYM/plant. Similarly, the same treatment resulted in a significant increase in the size of the flowers. These results indicated that the optimum doses of NPK fertilizers along with FYM are necessary for obtaining healthy plants of *M. exotica* but these fertilizers may become toxic at higher levels so care must be taken in their application (Amitabha *et al.,* 1989; Baboo and Sharma, 1997; Mishra, 1998). The maximum increase in number of flowers per plant by the application of 20 g NPK/plant/month + 4 kg FYM/plant is in line with findings of Singh (2003) and Singh and Gupta (1996) who observed that NPK fertilization increased flower production in chrysanthemum and dahlia, respectively. The positive effect of NPK on flower production is well reported in literature (Anamika and Lavania, 1990; Bankar and Mukhopadhyay, 1990).

It was observed that the plants applied with high doses of NPK and FYM maintained maximum growth and yield. Rathore *et al*. (1985) and Siddappa and Hegde (2011) reported the same results in African marigold and curry leaf (*M. koenigii),* respectively. Whereas, Belorkar *et al*. (1992) and Chadaha *et al*. (1999) suggested that high fertilizer treatments help plants to make more photosynthates that result in more and early yields. An increase in fresh and dry weight of flowers of *M. exotica* may be related to improved flower size due to supply of NPK and FYM. There exists a positive correlation between flower size and fresh and dry weight of flowers and an adequate supply of NPK is characterized by improved flower quality, greater flower size, increased number of branches, and high fresh and dry weights (Uma and Gowda, 1987; Chadaha *et al*., 1999; Ghaffoor *et al*., 2000; Zekri and Obreza, 2003; Khan *et al*., 2007; Qasim *et al*., 2014).

It is imperative to supply optimum doses of N, P and K for obtaining vigorous vegetative growth, improved flower and fruit quality, and to enhanced resistance against biotic and abiotic environmental stresses. The deficiency of these nutrients at any stage of crop may reduce plant growth, delay maturity, and eventually low yield and quality. It was noted that application of NPK fertilizers significantly increased leaf tissue content of these nutrients. In addition, organic fertilizers applied as FYM also contributed in this regard. Low doses of both organic and inorganic fertilizers applied in combination did not significantly affect the accumulation of N, P and K in leaves. Optimal N, P and K availability is compulsory to sustain the growth under normal and stressed conditions because these are the main constituent of the hormones, enzymes and proteins (Poole and Conover, 1990; Hanafy, 1994; Amarjeet *et al*., 2000; Abou-Dahab, 1992). The N and K significantly enhance enzymatic activity and play critical role in the transportation and restoration of protein synthesis (Abd EL-Latif *et al.,* 2011) that results in improved flower yield and quality which is very true for present study.

Chlorophyll, a pigment responsible for photosynthesis in leaves, is an indicator of photosynthetic capacity of plant tissues (Wright and Nageswara, 1994; Nageswara *et al*., 2001). Maintenance of chlorophyll contents is necessary for maximum photosynthetic capacity of plants. The level of NPK fertilization affects the accumulation of chlorophyll in the leaves (Skwarylo-Bednarz and Krzepilko, 2013). It was observed that the plants supplied with 20 g NPK/plant/month + 4 kg FYM/plant maintained the maximum chlorophyll contents. Our results are consistent with the findings of many researchers such as Sabo *et al.* (2002), Bojovic and Stojanovic (2005), Fritshi and Ray (2007) and Houles *et al.* (2007). Also, Younis *et al.* (2013) demonstrated a positive relationship between leaf nitrogen fertilization, rate and chlorophyll contents in rose cultivars. This increase in photosynthetic capacity of plant leaves with NPK fertilization is due to the fact that proteins of Calvin cycle and thylakoids represent the majority of leaf nitrogen (Gibson, 2005). The fertilization of plants with N increases photosynthetic pigments and also increases the stability of the chlorophyll, protein lipid complex and photosynthetic activity. Similarly, K reduces electron leakage and increases chlorophyll contents. Significant dependence between nitrogen fertilization and chlorophyll a and a + b content was also noted in two varieties of amaranth by Skwarylo-Bednarz and Krzepilko (2013). Girish (2006) and Sushma *et al.* (2012) suggested that the increase in chlorophyll contents due to fertilizers application might be due to greater availability and uptake of nutrients by plants.

***Phytochemical analysis of Murraya exotica*:** Phytochemicals are the naturally occurring, non-nutrient, bioactive compounds of plants. They are very important compounds and found to have certain application in medical sciences (Okarter *et al*., 2009). Alkaloids are compounds that possess antibacterial, antimalarial, analgesic and antiseptic characteristics (Evans, 2002). Whereas, phenolic compounds have wide applications for regulating the immune system (Anamika *et al*., 2010), and Saponins are natural antibiotics that are produced by the plants to counter the bacterial and fungal attacks (Okwu and Emenike, 2006).

Phytochemical analysis was carried out for *M. exotica* and results revealed the presence of carbohydrates, phenolic compounds, proteins and amino acids, alkaloids, phytosterols and flavonoids, whereas saponins and fixed oils were found to be absent. These results are in line with the observations of Gautam and Goel (2012); Gupta and Chandra (1974), and contrary to the results of Venkata *et al*. (2010) where alkaloids and terpenoids were present, while flavonoid and steroid were found to be absent.

***Conclusion*:** It can be concluded from the present study that the application of inorganic (NPK) and organic (FYM) fertilizers are necessary for improving the growth and quality of *Murraya exotica*. The supply of fertilizers at the rate of 20 g NPK/plant/month + 4 kg FYM/plant was found as the optimum rate for obtaining high growth and quality. Moreover, *M. exotica* is an important medicinal plant as it possess certain phytochemicals that have wide application in many industries like pharmaceutical, food, and cosmetics.

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