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Original paper

Effect of fertilization regime on Murraya exotica plants growth and bioactive compounds

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Abstract Murraya exotica L. (Rutaceae) is an ornamental pot plant also known as a medicinal plant, containing secondary metabolites that have long been used in pharmacology as a treatment for different diseases.

The aim of this work was to evaluate the effect of fertilization regime on Murraya exotica plants growth and on some bioactive compounds contained in its leaves.

Therefore, measurements of morphological parameters (number of the leaves, number of the branches on a plant, plant height) and biochemical analyzes (content in chlorophylls, carotenoids, carbohydrates, phenolics, and the antioxidant capacity) were performed on the leaves of the Murraya plants treated with different fertilizers.

The present study revealed that the application of fertilization treatments exerted beneficial effects, improving the growth and quality of Murraya exotica tested plants.

Keywords Antioxidant activity, decorative potential, fertilizers, *Murraya exotica*

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Introduction

Murraya exotica L. (*Rutaceae*), also known as *Murraya paniculata,* is an evergreen shrub plant, cultivated in Europe mainly as an ornamental pot plant for the fragrance flowers and glossy green leaves. In its native area, which includes China, India, Sri Lanka, north-eastern Australia and Taiwan (PARROTTA [1]), the plant is very appreciated for ambiental decoration and also as a hedge plant because of its vigour and high soil tolerance.

Beside this, for many years *Murraya* is known as a medicinal plant given that its leaves and roots have been traditionally used by the Chinese traditional medicine to treat stomach pains, rheumatalgia, toothache and body pains from injury or trauma (WU & al. [2]) and in India for treatment of diarrhea and dysentery (CHOPRA & al. [3]).There are also studies which showed that the essential oil from *M. exotica* had strong antifungal activity against *Candida albicans* and a modest antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Sarcina lutea* (EI-SAKHAWY & al.[4]).

Murraya plants contain secondary metabolite that has long been used in pharmacology to lower blood cholesterol levels, as anti-obesity (ISWANTINI & al. [5]) and some metabolites are reported to have antioxidant capacity (CASADO & al. [6]).

The phytochemical components with antioxidant activity, such as polyphenols, could be involved in preventing the occurrence of oxidative-stress related diseases, caused by the attack of free radicals on key biocomponents like lipids, proteins or nucleic acids (MAYNE [7]). This oxidative stress is involved in pathogenesis of many human diseases: cancer, cardiovascular diseases, osteoporosis, neurodegenerative processes.

Plant secondary metabolites are unique sources for pharmaceuticals and food additives (KARIMUNA & al. [8]). As a great number of aromatic and medicinal plants contain chemical compounds exhibiting antioxidant properties, there are developed many methods for extracting the bioactive compounds from *Murraya* plant (NASEEM & al. [9]).

The *Murraya* sp. leaves extract was reported to contain a wide range of different compounds consisting of coumarins (SAEED & al. [10]), phenols, flavonoids (SUKARI & al. [11]; ZHANG & al. [12], alkaloids, carotenoids etc. (GAUTAM & al. [13] and different studies demonstrated the high potential of *Murraya* plants as a source of natural antioxidants (ZHANG & al. [12]; GAUTAM & al. [13]). Also the components of leaf essential oils of *Murraya* plants from different parts of the world were evaluated for various biological activities in order to be used as a possible food supplement or in pharmaceutical industry (SAYAR & al. [14]; DOSOKI & al. [15]).

However, some studies reported that many factors, including provenance, weather, soil conditions, different fertilization treatments can change the chemical composition (BOIRA & al. [16]; DOSOKY & al. [15]).

The studies on *M. exotica* nutritional requirements showed that in a good quality of nutritional regime the plant has a very good response, consisting of a good development of growth parameters (number of branches, number of leaves and number of flower) correlated with a high increase of the photosynthetic pigments (NASEEM & al. [17]).

The nutrients for plants can be derived from the soil and from the applied fertilizer treatments. Nitrogen, potassium, and phosphorus are the most important components in mineral fertilizers applied in various combinations (YOUNIS & al. [18]). Nitrogen exerts the greatest influence on plant growth, quality, and yield, its excess application enhancing vegetative growth (KASHIF & al. [19]). Potassium is necessary for basic physiological functions of the plants like starch and protein biosynthesis and cell division, while phosphorus has essential roles in the transfer of energy, carbohydrates metabolism and photosynthesis (HAVLIN & al. [20]). Besides these, Fe is important for plant growth, including the formation of chlorophyll, oxidation-reduction in respiration and biosynthesis of enzymes and proteins and Zn is important for formulation of growth hormone, catalyst formation and maturation of seed proteins (CONWAY & PRETTY [21]).

Generally, all applied treatments increased the growth parameters like plant height, the number of branches, fresh and dry weights, photosynthetic pigments (chlorophyll a, b and carotenoids), minerals (N, P, K) and total sugars (MOHAMED & al. [22]). However, recently some efforts are made to reduce the amounts of chemical fertilizers applied to medicinal and aromatic plants in order to reduce the environmental pollution without decrease of yield (SIMAEI & al. [23]; MOHAMED, & al. [24]).

The aim of this work was to evaluate the benefits of *Murraya* plants treatments with some kind of fertilizer on growth and on some bioactive compounds contained in the leaves.

Therefore, measurements of morphological parameters (number of the leaves, number of the branches on a plant and plant height) were periodically made during the growth period of the plants in some experimental variants of fertilizer treatments. At the end of experiment period biochemical analyses were performed on matured plants for determination in leaves of the chlorophylls content, carotenoids, carbohydrates and phenolics and also for establish the antioxidant capacity of the *Murraya* plants treated with different fertilizers. Thus, the study was designed to identify a proper fertilization regime to obtain available plant not only for commercial use, as a starting material for future high decorative plants but also as natural sources of antioxidants.

Materials and methods

Experimental variants

The experiment was conducted in greenhouses at the Hortinvest Center of the U.S.A.M.V Bucharest (44º 26' N and 26º 06' E *latitude* and *longitude,* respectively). Experimental variants were established: 75% of the plant was subjected to a bi-monthly fertilization regime with different commercial substances, and 25% of the plants formed the control variant, without any fertilization (Table 1).

Besides the usual chemical fertilizer *NPK complex (1:1:1)*, other fortified variants of fertilizers were applied, promoting fast root growth, vegetative growth, flowering, and fruiting:

- *Vitaflora*, an organo-mineral fertilizer on the basis of salts of humic acids with a complex of macro- and micro minerals (N, P, K, Fe, Mn, Mo, Zn, Mg, Cu, B);
- *Cropmax*, a foliar fertilizer, which is a powerful stimulator of plant metabolism which contains in ultraconcentrated form the entire spectrum of required growth regulators: growth promoters (auxins, cytokinins, gibberellins), plant vitamins, polysaccharides, enzymes, aminoacids and macro and microelements (N, P, K, Fe, Zn, Mg, Cu, Mn, B, Ca, Mo, Co, Ni).

The new plants, obtained by sowing were placed in plastic pots with 10-12 cm diameter, filled with a usual substrate for ornamental plants, consisted of peat (80%) and perlite (20%). Throughout the period of growth there were applied specific works complex care for directing micro-climate factors, pest and disease etc.

Table 1. Experimental variants

Description	Fertilizer concentration (%)	Number of plants
Unfertilized	-	15
NPK complex	0.1	15
Vitaflora	0.1	15
Cropmax	0.1	15

Morphological measurements

There were made periodically observations and measurements on the main vegetative parameters such as the number of the leaves, the number of the branches on a plant and the plant height.

Biochemical analysis methods

At the end of the experiments, in order to estimate the impact of the fertilizers treatment occurred on cell level, characteristic parameters were analyzed, such as assimilatory pigments content, total carbohydrates content, total phenolic content and the antioxidant capacity using appropriate analysis methods. The determinations were made in triplicate, using fresh leaves. The extractions were conducted according to the protocol used for each determination.

- ***Determinations of the assimilatory pigments content*** in the active leaves**:** c*hlorophyll* and *carotenoid pigments* were extracted in 80% acetone and determined spectrophotometrically (wavelengths 663 nm, 647 nm and 480 nm) using the extinction coefficients and equations described by SCHOPFER [25]. The results were expressed in mg/g fresh weight.
- ***Total phenolic content*** was performed according to the modified Folin-Ciocalteu assay (SINGLETON & al.[26]). The method consists in chemical reduction of Folin-Ciocalteu reagent (which is a mixture of tungsten and molybdenum oxides) and measuring the intensity of the obtained blue colour at 750 nm. The measurements were achieved with a UV/Visible ThermoSpectronic Helios spectrophotometer. Total phenolic values were expressed in terms of gallic acid equivalents (mg GAE/g fresh weight), which is a common reference compound.
- ***Determination of total soluble carbohydrates*** was performed according to the Somogyi-Nelson method (IORDACHESCU and DUMITRU [27]; SOMOGYI [28]). For determination of the total soluble carbohydrates non-reducing carbohydrates were first transformed in reducing carbohydrates by hydrolysis with hydrochloric acid. The reducing carbohydrates when heated with alkaline copper tartrate reduce the copper from the cupric to cuprous state and thus cuprous oxide is formed. When cuprous oxide is treated with arsenomolybdic acid, the reduction of molybdic acid to molybdenum blue takes place. The measurements of absorbance were achieved at 620 nm with a UV/Visible ThermoSpectronic Helios spectrophotometer. The results were expressed in mg/g fresh weight.
- ***Total antioxidant capacity*** (radical scavenging activity) was determined using the stable free radical diphenylpicrylhydrazyl (DPPH) method according to BLOIS [29] procedure adaptated by BRAND-WILLIAMS & al. [30], for complex matrices. Briefly, a 100 µM solution of DPPH in methanol was prepared and 2 ml of this solution was mixed with 1 ml of different concentrations of *Murraya* leaves extract in 80% aqueous methanol. After 30 min incubation in dark at room temperature, absorbance (A) was measured at 515 nm. The percentage of the radical scavenging activity (RSA) was calculated as follows:

$$\% \text{ RSA} = (1-[A_{sample}/A_{control\ t=0}])/100$$

DPPH solution in 80% methanol was used as a control. The EC_{50} parameter for each sample, defined as the concentration of sample which is required to scavenge 50% of DPPH free radicals, was calculated from the linear regression curve of the sample extracts (mg/ml) against the percentage of the radical scavenging activity.

- ***Statistical analysis*** was performed using the one-way Analysis of Variance (ANOVA). Also Pearson's correlation coefficient (*r*) was used to calculate the relationship between the EC_{50} values and the antioxidant compounds (chlorophylls, carotenoids, phenolics) contents in all the experimental variants. Moreover, the relationship between the chlorophylls and carbohydrates content was investigated.

Results and discussions

Supplying plants with sufficient amounts of macro- and micronutrients is necessary for their growth and development as these elements are involved in the biosynthesis of proteins, enzymes, hormones. Fertilizers treatments are used in order to increase the soil availability to provide the nutrients amount required by plants.

1. Influence of fertilization regime on the morphological parameters

Effect of different fertilizers on ***plant height*** of *M. exotica* was examined. Significant difference (P<0.05) was noted at the end of experiment between control plants (untreated variant) and the plants which received fertilization treatments. Between the variants treated with NPK and Vitaflora no significant difference (P>0.05) was found (Figure 1).

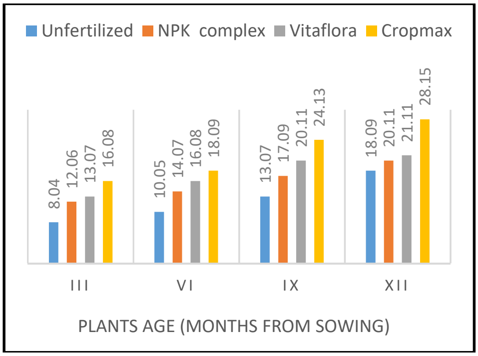

Figure 1. Effect of fertilization regime on the plant height (cm) at *M. exotica*

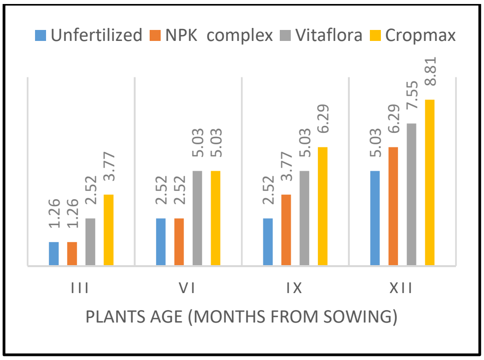

Figure 2. Effect of fertilization regime on the number of branches/plant at *M. exotica*

Similar results regarding the positive effect of some fertilizers on the plant height were reported for different plants like *Zinnia* (KASHIF [19], *Dahlia* (KASHIF & al. [31]), *Ocimum basilicum* (MOHAMED & al. [24]) indicating a beneficial influence on the plant growth.

Researches carried out on *Murraya exotica* by NASEEM & al. [9] showed that values of different growth parameters were significantly higher with increase in the fertilizer level reporting maximum values of plant height (125.07 cm) and number of branches (43.6) in plants supplied with 20 g NPK/plant/month + 4 kg organic fertilizer/plant.

Also with respect to the ***number of branches/plant*** the analysis of variance revealed a significant difference (P<0.05) not only between the control and the fertilized variants, but also among various fertilizer treatments at the end of the experiment (Figure 2).

Literature reported that increasing amount of chemical or organic fertilizers determined increase in the number of branches per plant at *Murraya koenigii* (SIDDAPPA and HEGDE [32]), *Gerbera* (KHOSA & al. [33]), *Murraya exotica* (NASEEM & al. [9]), which suggests that supplementation of the nutrients intake could determine an improvement of the photoassimilates production in plants.

The ***number of leaves/plant*** at the end of the experiment was favourably affected by different fertilization treatments.

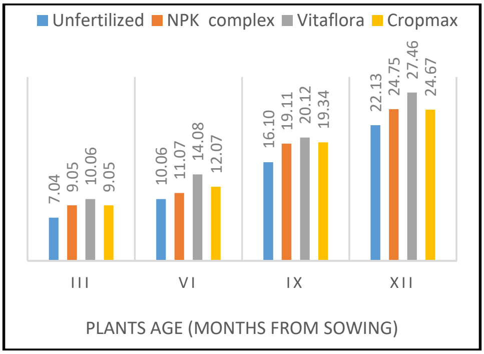

Figure 3. Effect of fertilization regime on the number of leaves/plant at *M. exotica*

The data regarding this growth parameter found significant differences (P<0.05) between untreated plants and various fertilizer treatments. Among these, high values were recorded in Vitaflora (27.46) and Cropmax variant (24.67) (Figure 3). On the contrary, no significant difference (P>0.05) was noted between variants treated with NPK and Cropmax. Similar results obtained ŞELARU & al. [34]), confirming the positive influence of a sustained fertilization regime on the evolution of biometric parameters of plants, which in fact define their decorative potential.

Some authors observed that high fertilizer doses increase the availability of mineral nutrients involved in metabolic processes which determined the plant growth (AMARJEET & al. [35]) but, although number of leaves/plant increased with higher fertilization level, fertilizer amount must never exceed certain levels because can negatively influenced the development of plants (KHOSA & al. [32]).

3.2. Influence of fertilization regime on the bioactive compounds

Generally, increased amounts of N, P, K in leaf is positively correlated not only with leaf dry weight, but also with leaf bioactive contents (KARIMUNA & al. [8]).

Chlorophylls play an important role in the photochemical reactions of photosynthesis (TAIZ & ZEIGER. [36]), therefore changes in the chlorophyll content will influence photosynthetic efficiency.

Analysis of variance for the data regarding leaf chlorophylls content indicated significant ($P<0.05$) effect of fertilizers application on this parameter. The untreated plants (control) recorded 1.03 mg/g FW total chlorophyll in the leaves, while the treated plants exhibited higher values, between 1.21 mg/g FW in the variant supplemented with NPK complex and 1.48 mg/g FW when Vitaflora was the applied fertiliser (Table 2). Between Cropmax and Vitaflora variants no significant difference ($P>0.05$) was observed.

Other researches performed on *M. exotica* in different fertilization variants showed that the level of NPK fertilization influences the accumulation of chlorophyll in the leaves: plants supplemented with 20 g NPK per month + 4 kg organic fertilizer per plant registered the highest (2.49 mg/g FW) chlorophyll content in the leaves (NASEEM & al. [9]). Also YOUNIS & al. [18] reported a positive influence of nitrogen fertilization on chlorophyll contents in some rose cultivars, while BALAN & al. [37] noted that Cropmax treatment induced the highest amount of assimilatory pigments in the leaves of pepper seedlings. As a result of researches on *Heliconia* SUSHMA & al. [38] suggested that the increase in chlorophyll contents registered after fertilizers application could be due to greater availability and uptake of nutrients by plants.

Table 2. Assimilatory pigments and total phenols in *M.exotica* leaves depending on fertilization regime

Experimental variants	Assimilatory pigments (mg/g FW)		Total phenolics (mg GAE/g FW)
	Total chlorophylls	Total carotenoids	
Unfertilized	1.03 ± 5.71	0.043 ± 0.07	6.40 ± 7.46
NPK complex	1.21 ± 4.85	0.045 ± 0.08	6.49 ± 5.15
Vitaflora	1.48 ± 6.81	0.058 ± 0.12	6.67 ± 6.12
Cropmax	1.33 ± 7.72	0.055 ± 0.11	6.91 ± 6.08

Note: values are mean ± SD of 3 experiments in each group.

Carotenoids are an important class of antioxidants involved in the protection of plants against photo-oxidative processes (STAHL & al. [39]; GRAMZA-MICHALOWSKA & al. [40]).

Changes in the carotenoids content of *Murraya* leaves were noted in fertilized variants (between 0.045 mg/g FW under NPK treatment and 0.058 mg/g FW in the variant treated with Vitaflora) compare to control variant, which registered 0.043 mg/g FW carotenoids (Table 2). Data processing by analysis of variance revealed significant differences ($P<0.05$) between the untreated and the fertilized variants, except the variant with NPK applied. Similar results were recorded in the variants treated with Vitaflora or Cropmax ($P>0.05$).

Phenolics are bioactive compounds with potent antioxidant activity widespread in many herbs and spices. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species involved in producing of oxidative stress, leading to cellular damage.

In general, the fertilization regime determined a high accumulation of total phenolic in the leaves of analyzed *Murraya* plants. Analysis of the variance showed significant differences ($P<0.05$) between control variant and the variants treated with Vitaflora or Cropmax, but no significant ($P>0.05$) between control and NPK variant. So, the obtained data indicated the highest level of total phenolics in the leaves of *Murraya* plants in variant treated with Cropmax (6.91 mg GAE/g FW), while the control variant registered 6.40 mg GAE/g FW (Table 2).

Also LUO & al. [41] noted that fertilization increased generally the concentration and accumulation of phenolics, as well as DPPH scavenging ratio at *Artemisia annua*. However, the concentrations of the fertilizers applied could exert different effects. Thus, NGUYEN and NIEMEYER [42] reported that nitrogen fertilization was found to have a significant effect on total phenolic level in *Ocimum basilicum*, which was high only at the lowest applied nitrogen treatment, while OTALORA & al. [43] observed that foliar nitrogen fertilization in elevated concentration determined low levels of total phenolic in *Cichorium endivia.*

The rich content in phenolics and others compounds with antioxidant potential, such as chlorophylls and carotenoids of the leaves may cause the antioxidant properties of *Murraya* plants.

Antioxidant capacity (free radical-scavenging activities) of extracts from all experimental variants was evaluated and the EC_{50} values for every tested plant were calculated for further comparison.

For this purpose the extracts of *Murraya* leaves in all fertilized variants were screened for their possible radical scavenging activity (RSA). Extracts in different concentrations of selected variants exhibited antioxidant activity, expressed as percentage of DPPH reduction (Figure 4).

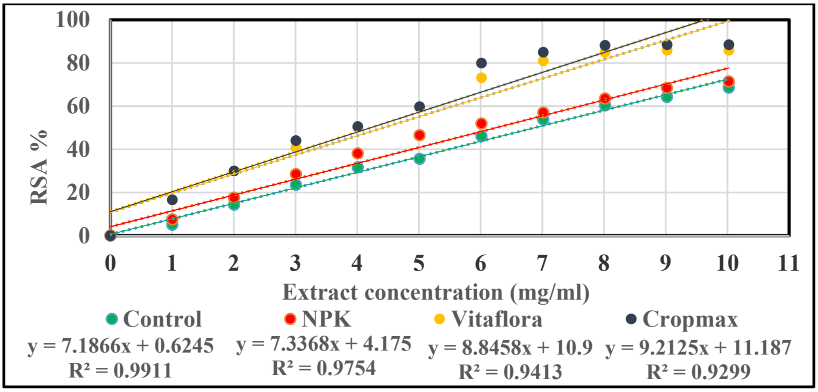

Figure 4. Radical scavenging activity (%) of the tested samples

The measurements indicated the highest antioxidant activity for the plants fertilized with Cropmax (4.21 mg/ml expressed as EC_{50} value), confirming the expectations due to their rich content in total phenolics (Table 3). The lowest scavenging capacity was noted in the leaves of unfertilized variant, which required a higher concentration (6.87 mg/ml) to scavenge 50% of DPPH free radicals.

Table 3. EC_{50} values of DPPH scavenging activities of the tested variants

Experimental variants	EC_{50} (mg/ml)
Unfertilized	6.87
NPK complex	6.25
Vitaflora	4.42
Cropmax	4.21

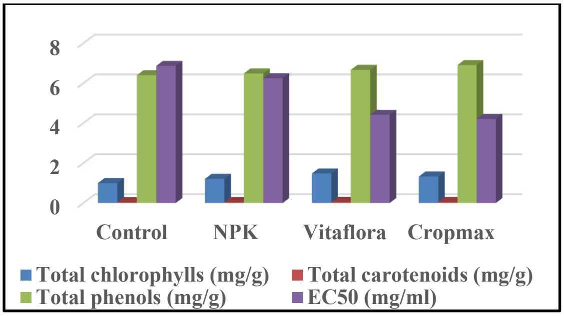

Figure 5. Antioxidants content and radical scavenging activity in *M. exotica* leaves

A good antioxidant power, similar to that of vitamin C, was revealed also by CASADO & al. [6] through qualitative DPPH analysis on *Murraya paniculata*.

However, there are poor data in literature regarding the effect of fertilization on antioxidant activity of *Murraya exotica*, so the results from the present study are supported by researches performed on *Ficus deltoidea* (SHEIKH and ISHAK [44]) showing that nitrogen application improves antioxidant activity. Also the antioxidant activity of lavender flowers was higher in treatment fertilized with nitrogen in dose of 50 kg N/ha, but decreased at higher dose (BIESIADA & al. [45]).

In the present paper a correlation study was performed between the radical scavenging activity (expressed as EC_{50}) and the content in total phenols, total chlorophylls and total carotenoids in order to reveal the contribution of these biochemical compounds to the total antioxidant capacity of the *Murraya* leaves (Figure 5). Good linear correlation was obtained for all the investigated parameters of which the highest value of Pearson's correlation coefficient was registered for the total phenolic content (-0.9300). Also the total content of chlorophylls in the leaves was well correlated to EC_{50} value (-0.8981), while for carotenoids the linear relationship had a lower value, respective -0.8102.

Good correlation between total phenolic content and antioxidant capacity was found also in other plants, such as *Primula veris* (LUPITU & al. [46]), *Taraxacum officinale* (PETKOVA & al. [47]), which could be used as medicinal herb or in foods with the potential to prevent from oxidative stress-related diseases.

Carbohydrates are considered important metabolites, being not only the first organic complex compounds formed in the leaves as a result of photosynthesis, but also a major respiratory substrate. Also carbohydrates are involved in plant protection against wound and infections, as well as in cell detoxification (KAUR & al. [48]).

A significant effect ($P<0.05$) of various fertilizer treatments was noted on the total soluble carbohydrates contents of *M. exotica*. Biochemical analyzes performed revealed the lowest content of total soluble carbohydrates in the leaves of untreated variant of *Murraya* plants (1.11 mg/g FW), while the highest value was noted as effect of Cropmax application (2.64 mg/g FW) (Table 5).

Treatment with Cropmax determined also a high content in total chlorophyll (Table 2), therefore exerted a positive influence on plant capacity of photosynthesis and carbohydrates biosynthesis. In this regard, a high value (-0.9885) of Pearson's correlation coefficient was calculated in order to assess the relationship between chlorophylls and carbohydrates, which indicates a good linear correlation.

Table 5. The total soluble carbohydrates content in *M. exotica* leaves depending on fertilization regime

Experimental variants	Total soluble carbohydrates (mg/g FW)
Unfertilized	1.11 ± 6.19
Complex NPK	1.83 ± 8.09
Vitaflora	3.06 ± 6.41
Cropmax	2.64 ± 7.42

Note: values are mean ± SD of 3 experiments in each group.

Regarding the influence of fertilization on the carbohydrates amounts similar results were reported on *Ocimum basilicum* by NURZYŃSKA-WIERDAK & al. [49] and MOHAMED & al. [24], on *Viola odorata* by MOHAMED and GHATAS [50] and on *Stevia rebaudiana* by PATIL [51], which showed that the treatment with different fertilizers determined high accumulation of carbohydrates in plant leaves.

Conclusions

Each type of fertilizer has its advantages and disadvantages over crop growth and soil fertility. The use of combined fertilizers as a safe and rich source of macro- and microminerals, phytohormones, vitamins, aminoacids, enzymes in order to improve plant growth and productivity has acquired a great attention nowadays. Thus, a good management of fertilization ensures an improved quality and safety of the environment.

It can be concluded from the present study that the application of NPK, Cropmax, Vitaflora fertilizers exerted beneficial effects, improving the growth and quality of *Murraya exotica* tested plants.

The best results were noted in the experimental variant treated with foliar fertilizer Cropmax, which activated the plants metabolism, increasing the assimilatory pigments biosynthesis and the accumulation of primary metabolites (carbohydrates) as well as secondary metabolites (polyphenols) in the leaves.

The improvement effect exerted by Cropmax can be attributed to its improving the availability and the uptake of all nutrients required by the plant, allowing the crops to realize their full potential.

A good antioxidant activity of *Murraya exotica* leaves was determined, especially in the variant treated with Cropmax, which supports a possible use of the plant as a medicinal remedy, food supplement or in pharmaceutical industry, as possible therapeutic alternative.

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