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Research Paper

Phytochemistry quality of marigold (*Calendula officinalis*): The effect of harvest time and organic fertilization

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ABSTRACT

Calendula officinalis (marigold) is cultivated in Brazil and widely used for medicinal purposes such as anti-inflammatory and antiseptic. Brazilian's market does not provide medicinal plants with biomarkers, therefore it is necessary develop adequate technologies to produce medicinal plants in quantity and phytochemistry quality. This study aimed to determine the effect of harvest time and organic fertilization on flavonoids and total phenolic compounds yield in marigold flowers. The data obtained by (ANOVA) and the averages were compared using Tukey 5%. Flavonoids yield was higher at afternoon harvest in orange flowers. Higher concentrations of total phenolic compounds (931.86 mg/g) were found in orange flowers with fertilization of 30 t/ha and lower (368.53 mg/g) in yellow flowers fertilized with the highest dose of cattle manure (120 t/ha). The result of the research proves that the phytochemical quality of the marigold flowers was influenced by the organic fertilization and harvest time so that we can benefit nutritionally a better quantity and quality of the chemical composition, according to the required concentration of fertilizers and time of harvest.

Key words: Medicinal plant, phenolic, flavonoids, antioxidant, farming system.

INTRODUCTION

Calendula officinalis L. (Asteraceae) is a medicinal species cultivated in several regions of Brazil for therapeutic, cosmetic and ornamental purposes besides being used also in cooking. Its flowers are very showy, with coloration varieties between yellow and orange. It is one of the most used medicinal plants in the country, being popularly known as flower-of-all-the-male or marigold (Lorenzi and Matos 2002; Vieira et al., 2006).

Several compounds were isolated from its flowers and leaves: triterpenes, flavonoids, phenolic compounds (chlorogenic acid, tannins, rutin, narcissin and quercetin), carotenoids and hydroxycoumarins and essential oil extracted (Fernandes et al., 2013; Gazim et al., 2007). Some of these compounds, especially flavonoids, are used as markers to evaluate the quality of the medicinal plants.

Among its pharmacological uses were related topical anti-inflammatory actions, healing, emollient and antimicrobial. In the cosmetic industry, it is used in making creams, shampoos, lotions, deodorants and toothpastes. It also works as repellent for some insects and acts as inhibitor of the growth of pathogens (Mazaro et al., 2013; Rigane et al., 2013).

In Brazil, because of the implementation of "Live Pharmacies or Green Pharmacies", the production of herbal medicine became of great importance, especially

for the implementation of Phytotherapy in the Unified Health System (SUS). Among the medicinal species selected by the Ministry of Health, it is worth mentioning the marigold, which also appears in the National Relation of Medicinal Plants of Interest to SUS (Brasil, 2009).

In addition, the production of medicinal species in an organic farming system can increase the income of the small farmers. When it comes to medicinal plants, the production of biomarkers or bioactive compounds is tied to the conditions of domestication and cultivation.

Physiological responses to biotic and abiotic factors condition result in more or less production of these compounds. Thus, factors related to organic cultivation, such as fertilization, are necessary to obtain higher productivity in biomass and better phytochemical quality of the plant material.

Marigold is a medicinal specie that develop best on wells with good drainage and rich in organic matter. Studies on fertilization and nutrition of marigold have reported an increase in the production of biomass (Araújo et al., 2009; Leite et al., 2005). The organic fertilization usually recommended for the crop varies from 20 to 60 t/ha of cattle manure (Borella et al., 2011; Leite et al., 2005).

The nutritional status of the plants is influenced by several factors, including fertilization, which reflects on its productivity. In this context, there are direct relationship between doses of fertilizer and production (Faquin, 2002).

The standardization of inflorescences is also very important, since the flowering phase lasts several days, resulting in heterogeneous flowers. This lack of uniformity may compromise the quality of raw material supplied for the manufacture of herbal products (Bertoni et al., 2006). The aim of this study was to evaluate the influence of harvest and organic fertilization on the yield of calendula flowers as well as on the phenolic compound contents and antioxidant capacity.

MATERIALS AND METHODS

This study was conducted under field conditions at Oratórios, Brazil (coordinates 20°25′49′′S and 42°48′20′′W, at 422 m of altitude). Average maximum annual temperature was 21.8°C and average minimum was 19.5°C. Average annual precipitation was 1.250 mm.

The soil used was classified as red-yellow Argisoil and presented, in the 0-20 cm depth layer, the following characteristics: pH (water 1:2.5)= 5.1, P (Mehlich) = 9.9 mg dm^{-3}, P (Remainder)= 30.3 mg L^{-1}, K^{+} = 71 mg.dm^{-3}, Ca^{++} = 2.00 cmolc dm^{-3}, Mg^{++} = 0.9 cmolc dm^{-3}, Al^{+++}= 0.1 cmolc dm^{-3}, H+Al= 3.47 cmolc dm^{-3}, Sb= 3.08 cmol dm^{-3}, t= 3.18 cmolc dm^{-3}, T= 6.55 cmol dm^{-3}, V= 47%, organic matter= 3.3 dag/kg.

The soil preparation consisted of harrowing and raising beds, and marigolds (“Dobrada Sortida”) were cultivated by organic farming system using five doses of cattle manure (0; 30; 60; 90; 120 t/ha). The dry cattle manure had these characteristics (%): N = 1.02; P = 0.3; K = 0.64; Ca = 0.65; Mg = 0.27; S = 0.25 and Organic Carbon = 14.8.

Seedlings were transplanted in the experimental area and with growth spacing of 0.4 × 0.4 m. For each treatment, orange and yellow flowers were harvested in the morning and afternoon and dried in air circulating oven (40°C) until reaching 10% moisture. After drying, marigold flowers were macerated in ethanol. The extract was evaporated (40°C) to obtain the crude extracts.

The spectrophotometry method adapted by Nabavi et al. (2008) was used to determine the total flavonoid content of the marigold’s flowers. The absorbance was measured in spectrophotometer (760 nm). Gallic acid and quercetin were used in the standard curve of total phenolic compounds and flavonoids respectively and the results were expressed in mg/g.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and the averages were compared using Tukey 5% probability, by using SAEG software.

RESULTS

The production of fresh and dry mass of marigold flowers cultivated using cattle manure doses is shown in Figure 1(a and b), respectively. There was a linear increase in the production of fresh and dry mass of flowers with the doses of cattle manure applied.

The mean contents of total phenolic compounds were 679.20 ± 173.65 mg/g (CV=9.67%). Higher concentrations of total phenolic compounds (931.86 mg/g) were found in orange flowers with fertilization of 30 t/ha and lower (368.53 mg/g) in yellow flowers fertilized with the highest dose of cattle manure (120 t/ha). Treatments with fertilization of 30 t/ha showed higher levels of flavonoids in orange flower extracts too, probably in response to the adaptive need of the cultivation conditions. The dose-response curve was inversely proportional to cattle manure doses (30; 60, 90 and 120 t/ha) in all treatments (Figure 2).

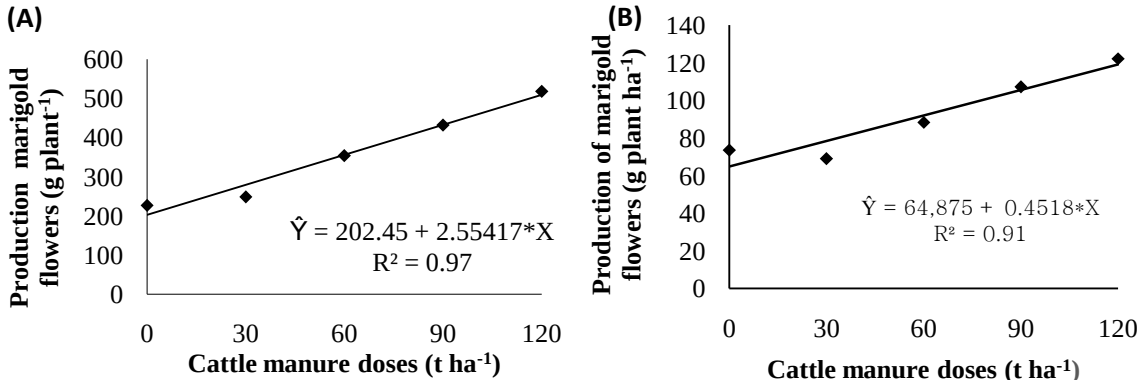

Figure 1: Production of fresh (A) and dry (B) mass of marigold flowers (g/plant) in function of cattle manure doses used in organic fertilization.

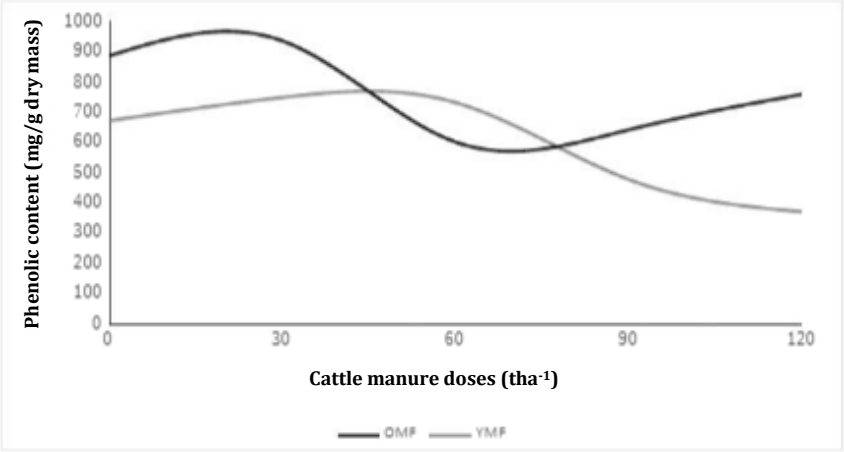

Figure 2: Relationship between cattle manure doses (0, 30, 60, 90 and 120 t/ha) and phenolic compounds contents in the dry matter mass of orange (OMF) and yellow (YMF) marigold flowers.

The results show that the best harvest time of marigold flowers for higher flavonoid yield is in the afternoon (14 h) and that orange flowers contain a higher content of flavonoids than yellow flowers (Figure 3(a-d)).

The antioxidant capacity in the BHA control (4.0 mg/mL) is 10 times lower than those used for extracts (40 mg/mL) which was 52.40% at time zero and 79.80% at time thirty minutes (Table 1).

DISCUSSION

The addition of organic fertilizer (cattle manure) linearly increased the mass production of fresh and dry matter of the marigold flowers, this probably occurs due to the increase of nitrogen (N) supply in the plants. Mota et al. (2008) also found a positive correlation between N and dry matter production in medicinal species. The highest yield of fresh and dry mass of marigold flowers were obtained at the highest dose (120 t/ha) of cattle manure. Araújo et al. (2009) also obtained an increase in biomass production with the increase of organic compound doses applied in the marigold crop. Gassi et al. (2009) showed that higher doses of chicken litter resulted in higher dry mass production of medicinal species.

Treatments with fertilization of 30 t/ha showed higher levels of flavonoids in orange flower extracts as well, probably in response to the adaptive need of the cultivation conditions. The dose-response curve was inversely proportional to cattle manure fertilization doses (Figure 2). Studies report the variation in the levels of bioactive compounds, especially carotenoids

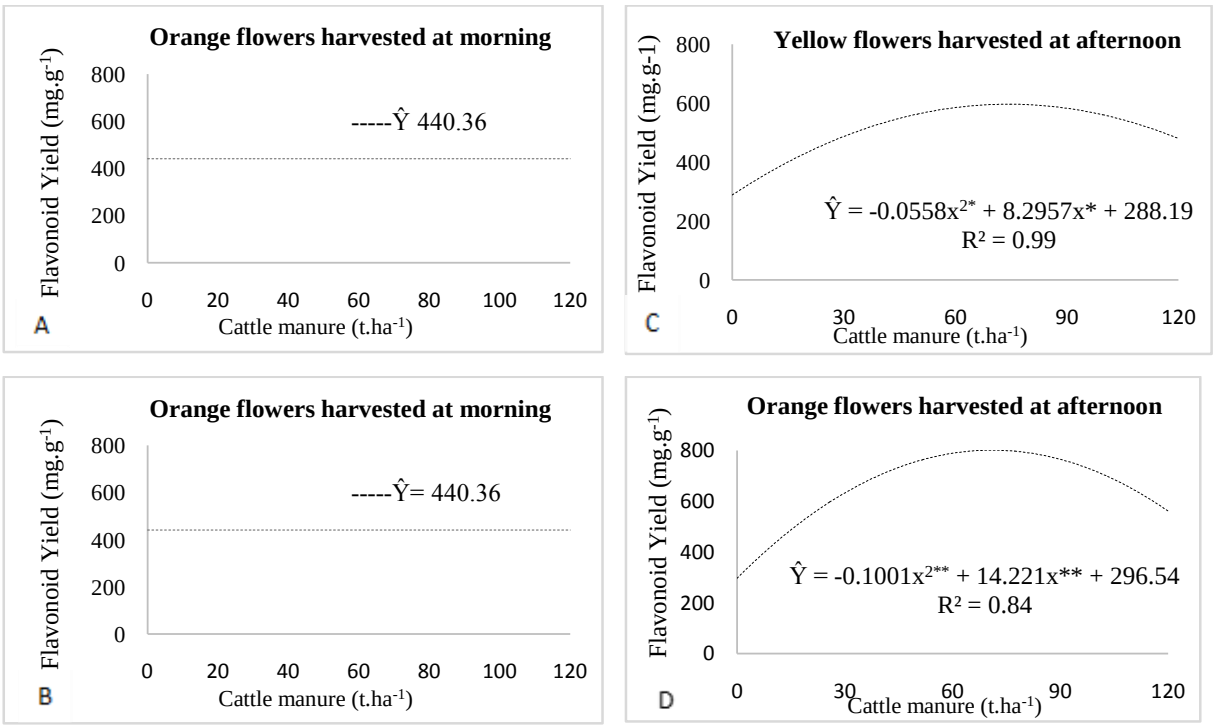

Figure 3: Relationship between cattle manure doses (0, 30, 60, 90 and 120 t/ha) and flavonoids yield in marigold flowers harvested in the morning (A, B) and afternoon (C, D).

Table 1: Variance analysis of phenolic compounds (PC, mg/g dry matter mass), flavonoids (FLAV, mg/g dry matter mass) and antioxidant activity (% AAT t0 and t30) of dried extracts marigold flowers, yellow (Y) and orange (O), cultivated with five doses of cattle manure (0, 30, 60, 90, 120 t/ha).

Treatments	Flower	PC**	FLAV *	AAT t0**	AAT t30**
T0	Y	668.53 C	288.8 A	55.56 E	95.45 ABC
T30	Y	745.200 ABC	482.4 A	58.34 DE	94.44 ABCD
T60	Y	728.53 BC	594.6 A	56.44 E	94.70 ABCD
T90	Y	475.20 DE	574.2 A	51,01 F	92.68 CD
T120	Y	368.53 E	482.4 A	50.63 F	92.42 CD
T0	O	881.86 AB	258.2 B	64.40 BC	97.09 A
T30	O	931.86 A	713.5 A	62.63 C	95.84 AB
T60	O	598.53 CD	778.0A	61.49 CD	95.83 AB
T90	O	638.53 CD	699.8A	67.17 AB	92.05 D
T120	O	755.20 ABC	595.7 A	68.56 A	93.42 BCD
CV (%)	-	9.67	18,28	2.25	1.13

Means followed by the same letter did not vary at the 1% (**) and 5% (*) level of significance by the Tukey test. AAT positive control = BHA (AAT t0 =52.40%, t30=79.80%).

and flavonoids, with the level of fertilization (Borella et al., 2011; Leite et al., 2005).

There was no interaction between the factors: harvest time and flower color. The flavonoid content in orange flowers (713.5 mg/g) was significantly higher than in the yellow flower (482.4 mg/g), which was expected due to the association of this metabolite with flower color. If the goal is to get a higher concentration of flavonoids, the flowers should be harvested separately. The flavonoid content in the afternoon harvest was significantly higher than the morning harvest. These results are in agreement with that of Silva Junior (2006), although there is a morning harvest recommendation (Barboza et al., 2012).

Harvesting in the afternoon is more advantageous because it is possible to harvest a larger quantity of fully opened flowers, besides facilitating the drying process due to the absence of dew present in the morning. The afternoon harvest and fertilization of 30 t/ha promote higher levels of total phenolic compounds and flavonoids. Orange flowers contain a higher content of flavonoids and antioxidant capacity as compared with yellow flowers.

The antioxidant capacity in the BHA control is lower than those used for extracts. All orange marigold flowers treatments were superior to BHA in both times. For yellow flowers, fertilization rates of 30 and 60 t/ha and without fertilization (0 t/ha) were higher than the BHA, at time zero (t0) and at time 30 minutes (t30), in all treatments.

Both the concentration of cattle manure and the color of the flowers (yellow or orange) are not factors that drastically influence the antioxidant capacity. The results of the surveys showed that the average levels of antioxidant capacity for all treatments have an effective antioxidant capacity.

BHA (2,3-tert-butyl-4-hydroxyanisole) is a potent antioxidant of synthetic origin that acts at low concentrations delaying or inhibiting substrate oxidation, but the end of the inhibition reaction was visualized in a few minutes (before 30 minutes). The ideal antioxidant effect is what promotes a slow sweep over time in minimal concentration, as occurred with the calendula extracts. When orange flavonoids (> 713.5 mg/g) and antioxidant activity are associated with orange flower flavonoids (30 minutes), orange flowers (94.85%) are more effective than yellow flowers of calendula (93.94%) and positive control (79.80%), regardless of the dose of fertilization.

Although, it may be thought that BHA, a positive control, was used in low concentration and therefore could be better than calendula extracts, it is emphasized that such compound is not effective in promoting the sweeping effect over time, and in high doses or continuous use, it may be carcinogenic (BHA is permitted in Brazil up to 200 mg/kg and is prohibited in Canada and the European community). Only 40 mg of calendula flower extract, regardless of color, whether orange or yellow, promoted the desired inhibition in the oxidation, slowly and gradually, thus avoiding damage and cellular stress, which makes the use as a functional food much more feasible , and may further be used as an additive, preservative or adjuvant in food or cosmetic preparations.

In summary, the results showed that flavonoids and phenolics were influenced by organic fertilization (cattle manure). Both yellow and orange flowers had good antioxidant capacity and the phytochemical quality of marigold flowers was influenced by organic fertilization and harvest time. The results for these phytochemical compounds show that marigold flowers can be used as an antioxidant and that the consumption of flowers brings nutritional benefits to the body.

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