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Accumulation of Secondary Metabolites and Osmotica in Different Parts of *Tagetes erecta* L. and its Ecophysiological Relevance.

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ABSTRACT

A field experiment was conducted to quantitatively analyse different phytochemical and osmotic constituents in 30 days old seedlings of African Marigold (*Tagetes erecta*L.) cultivar Pusa Narangi Gainda. Different parts of *Tagetes erecta* L. exhibit considerable variation in antioxidant components (phenols, tannins, phytic acid, flavonoids) and osmolytes (free sugars, free amino acids, free proline and potassium). Leaves showed higher contents of phenols, tannins, phytic acid and flavonoids followed by stem and root. Greater concentration of free amino acids and free proline was found in leaves as compared to stem and root whereas free sugars were more in stem and starch was maximum in leaves. Potassium and nitrogen were greater in leaves as compared to stem and root. On the other hand, higher concentration of sodium was restricted to roots. Higher contents of above reported osmotica may probably help plants withstand the extreme environmental conditions and secondary metabolites like phenolic compounds may impart the medicinal value to *Tagetes erecta* L.

KEY WORDS:*Tagetes erecta* L., Secondary metabolites, Osmolytes.

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INTRODUCTION

Plants frequently encounter unfavourable growth conditions. Climatic factors, such as extreme temperatures, drought and soils contaminated by high salt concentration are major abiotic environmental stressors limiting plant growth, development and thus agronomic yield, reflecting on the geographic distribution of a plant species. These adverse conditions are generally referred to as stresses. Environmental stresses can disrupt cellular structures and impair/damage key physiological functions[1]. Plants respond by activating tolerance mechanisms at different levels of organization while facing adverse or growth limiting conditions. Plants alter metabolism in different ways which include production of compatible solutes that are able to stabilize proteins and contribute to maintain cell turgor by osmotic adjustment[2-3]. The chemical nature of these small molecular weight organic osmoprotectants is diverse which includes amino acids, amines, and γ-amino-N-butyric acid (GABA). Carbohydrates including fructose, sucrose, trehalose, raffinose, and polyols (myo-inositol, D-pinitol) [4, 5], and anti-oxidants such as glutathione (GSH) and ascorbate[6, 7] may accumulate in response to osmotic stress. Accumulation of compatible solutes in response to stress is considered a defense mechanism triggered not only in plants but also in animal cells, bacteria, and marine algae, possibly as an evolutionarily conserved trait[8, 9]. Scavenging of reactive oxygen species (ROS), preservation of cellular turgor by establishment of osmotic balance are protective functions of compatible osmoprotectants during environmental stress[10].

Plants are rich source of a variety of phenolic compounds which contribute to the sensory and nutritional quality of plants[11]. Phenolic compounds comprise a variety of molecules having a polyphenol structure. Polyphenols include flavonoids, phenolic acids, tannins etc[12]. Different species and cultivars of the same species exhibit diverse contents of phenolics, flavonoids and antioxidant activity[13,14]. Nowadays, naturally occurring antioxidants have been drawing considerable interest because of their potential in health promotion and disease prevention, safety and consumer acceptability[15].

Marigold (*Tagetes erecta* L.) is a common ornamental plant bearing bright yellow and orange flowers. Its natural range extends from southwestern United States into Argentina, and the area of their greatest diversity is in south-central Mexico[16]. Marigold is widely used as a medicinal herb for its valuable properties which are useful in dermatology and cosmetology[17, 18]. The pharmacological activities of marigold are attributed to several secondary metabolites like terpene, essential oils, flavonoids, sterols, carotenoids and sesquiterpenes[19]. The objective of this study was to investigate the variation in the contents of such phytochemicals/ secondary metabolites and osmolytes in different parts of*Tagetes erecta* L which could be useful for its medicinal application.

MATERIALS AND METHODS

Seeds of *Tagetes erecta* L. were procured from Indian Agriculture Research Institute (IARI) New Delhi and were sown in Botanical Garden of School of Studies in Botany, Jiwaji University, Gwalior. Plants were uprooted 30 days after sowing. Different parts were separated and oven dried at 60 °C for 48 hours and the analysis of different phytochemical constituents was done using following methods.

Total Phenols

Total phenols were estimated in accordance with Malick and Singh[20]. 0.5 g dry sample was homogenized in 5 mL ethanol (80%) and extract was centrifuged at 10,000 g for thirty minutes. Supernatant was saved and residue was re-extracted in 80% ethanol. Supernatant was collected and evaporated to dryness and subsequently dissolved in 10 mL distilled water. 0.1 mL aliquot was made 2 mL using distilled water and 1 mL Folin-Ciocalteau's reagent was added. Thereafter 2 mL sodium bicarbonate solution was added and kept in dark for thirty minutes, the solution was boiled for one minute and absorbance was recorded at 650 nm. Concentration of total phenols was expressed in mg g^{-1} dry weight equivalent to catechol.

Tannins

100 mg dry sample was extracted in 7.5 mL distilled water in a water bath for thirty minutes at boiling temperature. The contents were cooled and centrifuged at 2000 g for 20 minutes. To 0.1 mL extract 1 mL Folin-Denis reagent was added followed by addition of 2 mL sodium bicarbonate (35%) and the resultant was incubated for 45 minutes in dark at room temperature. Optical density was recorded at 700 nm and calculations were done using tannic acid standards (Swain and Hills)[21].

Total Flavonoids

Total flavonoids were measured according to Zhishenet al.[22]. Dry plant sample was homogenized in 80% ethanol and homogenate was centrifuged at 10,000g for 20 min. $NaNO_2$ (5%) was added to supernatant and after 5 minutes $AlCl_3$ 10% was added and incubated for 6 min followed by addition of NaOH (1M). After 15 minutes optical density was measured at 510 nm. Flavonoid contents were computed employing quercetin standards.

Phytic acid

Phytic acid was determined using the method of Wilcox et al.[23]. Dry powdered plant sample was extracted in 1 mL HCl (0.4 mM) and the extract was centrifuged at 10,000 g for 20 minutes. To 0.1 mL supernatant 1 mL of colorimetric reagent was added and the resulting solution was kept for

one hour in dark at room temperature. Absorbance was recorded at 650 nm and phytic acid was expressed in mg g^{-1} dry weight equivalent to phytic acid.

Free Amino Acids

Free amino acids were determined following Sadasivam and Manickam[24]. 0.5 g plant material was extracted in 10 mL of 80% ethanol and centrifuged at 2000 g for 20 minutes. 0.1 mL aliquot was made to 2 mL by adding distilled water followed by addition of 1.0 mL ninhydrin reagent and incubating it for thirty minutes at 100 °C. Thereafter, 5 mL diluent was added and left for fifteen minutes and absorbance was recorded at 570 nm. Free amino acids were expressed in mg g^{-1} dry weight equivalent to glycine.

Free Proline

Extraction was done in sulphosalicylic acid and was reacted with acid ninhydrin followed by the addition of equal amount of distilled water, the resultant was kept at 100 ^{0}C for one hour. The reaction was terminated by keeping tubes in ice. Free proline was separated using toluene, optical density was recorded at 520 nm and computation was done using proline standards (Bates et al.)[25].

Total Free Sugars

Total free sugars were determined using anthrone method[26, 27].Plant sample was boiled in 80 % ethyl alcohol followed by centrifugation at 3000 g for five minutes. The residue was again crushed in 80 % ethyl alcohol and centrifuged. The alcoholic extract (1ml) so prepared was evaporated to 0.2 ml in a water bath and the volume was made up to 1 ml with distilled water. 1 ml HCl (1 N) was added and the tubes were kept in a boiling water bath for 40 minutes for hydrolysis. 0.5 ml of hydrolyzed extract was mixed with Anthrone reagent and the tubes were kept in boiling water bath for 10 minutes. After bringing it to room temperature, optical density was recorded at 620 nm. Computation of total free sugars was done using glucose standards.

Starch

Starch was determined by anthrone method[24] as followed by Pandey et al.[28]. Residue (pellet) of the sample from which free sugars were extracted was dried in a boiling water bath followed by the addition of 2 ml distilled water and then keeping on a water bath for 15 minutes. Subsequently cooled, 2 ml 9.2 N perchloric acid was added and stirred for 15 minutes, followed by centrifugation at 3000 g for five minutes. Supernatant was collected in 50 ml volumetric flask. 2 ml of 4.6 N perchloric acid was added again to the residue and stirred for 15 minutes then centrifuged. Supernatants were combined, volume made up to 7.5 ml with distilled water and 4 ml anthrone reagent was added to an aliquot (0.1 ml). The tubes were then kept in a boiling water bath for 10

minutes followed by cooling on ice. It was brought to room temperature and optical density recorded at 620 nm. Computation was done using standard curve.

Chloride

Chloride was estimated in accordance with the method of Eaton et al.[29]. One gram dry plant sample was boiled in distilled water (100 ml) on a water bath for 30 minutes. The extract was filtered and titrated against 0.1 N $AgNO_3$ till a permanent brick red precipitate persists.

Potassium, Sodium and Calcium

The estimation was done flame photometrically. One gram dry plant material was taken in a conical flask and digested in tri-acid mixture H_2SO_4 + HNO_3 + $HClO_4$ in 9:3:1 ratio. The colorless digested material was filtered through Whatman filter paper number 1 making up the total volume to 100 ml. Aliquot (10 ml) was made up to 25 ml with distilled water and read on a digital flame photometer Systronic flame photometer-128 employing K, Na and Ca filters separately and standard curves of K, Na and Ca were used for computation.

Total Nitrogen

Nitrogen was estimated following micro-Kzeldahl's method as suggested by Jackson[30]and modified by Iswaran and Marwaha[31].Dry plant sample (1 gm) was taken in a 100 ml Kzeldahl digestion flask moistened with 5 ml distilled water and 15 ml concentrated H_2SO_4 was added to it followed by thorough shaking. A few drops of 1N $KMnO_4$ were added till pink colour appeared followed by the addition of catalyst mixture 3gm K_2SO_4 + 0.3 gm $FeSO_4.5H_2O$ + 0.15 g $CuSO_4.5H_2O$. The whole mixture was digested at low flame for half an hour till the colour of mixture turns brown to yellowish green. Digested mixture was cooled and final volume made up to 100 ml with distilled water. Ammonia in the digested solution was then estimated using micro-Kzeldahl's method.

Data presented is the mean of four replicates with standard error (±SE) calculated.

RESULTS AND DISCUSSION

Increased contents of total phenols and flavonoids were observed in leaf and stem of *Tagetes erecta* L. as compared to root (Figure 1 A). Phenolic compounds are a diverse group of phytochemicals widely distributed in plants apparently providing defence against herbivores, microbes, viruses or competing plants etc and also protecting plants from ultraviolet radiation and oxidants[32, 33]. Synthesis of phenolic compounds increases when plants are subjected to insecticides, fungicides and herbicides[34].The pattern of secondary metabolites in plants is complex feature and it can be tissue/organ specific and different developmental stages can also show variation[35-37]. Plant

Polyphenols are divided into several classes depending on the number of phenol rings that they contain and the structural elements binding these rings to one another. These include: flavonoids, phenolic acids, tannins, stilbenes and lignans[38]. Flavonoids are especially important antioxidants due to their high redox potential which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also have a metal chelating potential[39] which can protect the plant against UV light, herbivores, pathogens and oxidative cell damage[40].

Tannins and phytic acid contents were also found more in leaf and stem of *Tagetes erecta* L. as compared to root (Figure 1 A). Tannins are relatively high molecular compound phenolics subdivided into hydrolysable and condensed tannins[41]. Tannins are potential metal ion chelators, protein precipitating agents and biological antioxidants[42]. The plants with more phenolic compounds reportedly exhibit good antioxidant activity[43]. Phytic acid is an anti-nutrient having strong ability to complex multi-charged metal ions, especially Zn, Ca and Fe making them unavailable to body and found in most of the cereals[44]. A high concentration of phytic acid is undesirable as it hinders the bioavailability of some essential nutrients such as Fe, Mg, Ca, Zn and Cu[45].

The concentration of free amino acids was highest in leaves (Figure 1 B), stem showed greater accumulation of free sugars as compared to root (Figure 1 B). Accumulation of compatible osmotic constituents is an important adaptation strategy for better growth of plants under different environmental stresses[46]. Greater amount of proline was recorded in marigold leaves as compared to stem and root (Figure 1 C). Proline is one the important organic osmolytes utilized in osmotic adjustment leading to maintenance of turgor, through better extraction of water from the soil[47].Starch was found maximum in leaves (Figure 1 D). In stem and root there is continuous decrease of starch (Figure 1D). As plants are usually physiologically active at earlier stages of growth and carbon assimilation is high. The excess carbon is stored as starch in leaf and stem before being transported to sink (Figure 1D).

Leaves of *Tagetes erecta* L. showed significant increase in contents of potassium and calcium whereas, maximum concentration of sodium was restricted to roots (Figure 2). Nitrogen and chloride were more in the leaves of marigold (*Tagetes erecta* L.) followed by stem and root (Figure 3 A and B). Accumulation of potassium, sodium and chloride contents in greater quantity by plants shows well adaptation / tolerance to saline soils by reducing the toxicity [48].Potassium induces enhancement of amino acid accumulation[49] nitrogen uptake and the nitrogen containing secondary metabolites thereby, imparting tolerance to stresses. K accumulation leading to reduced Na/K ratio, accumulation of osmolytes including proline and sugars resulting in greater WUE[50].

Accumulation of considerable amounts of phenolic compounds and organic osmolytes by *Tagetes erecta* L. observed in the present study may impart better stress tolerance thus helping them flourish under extreme environmental constraints.

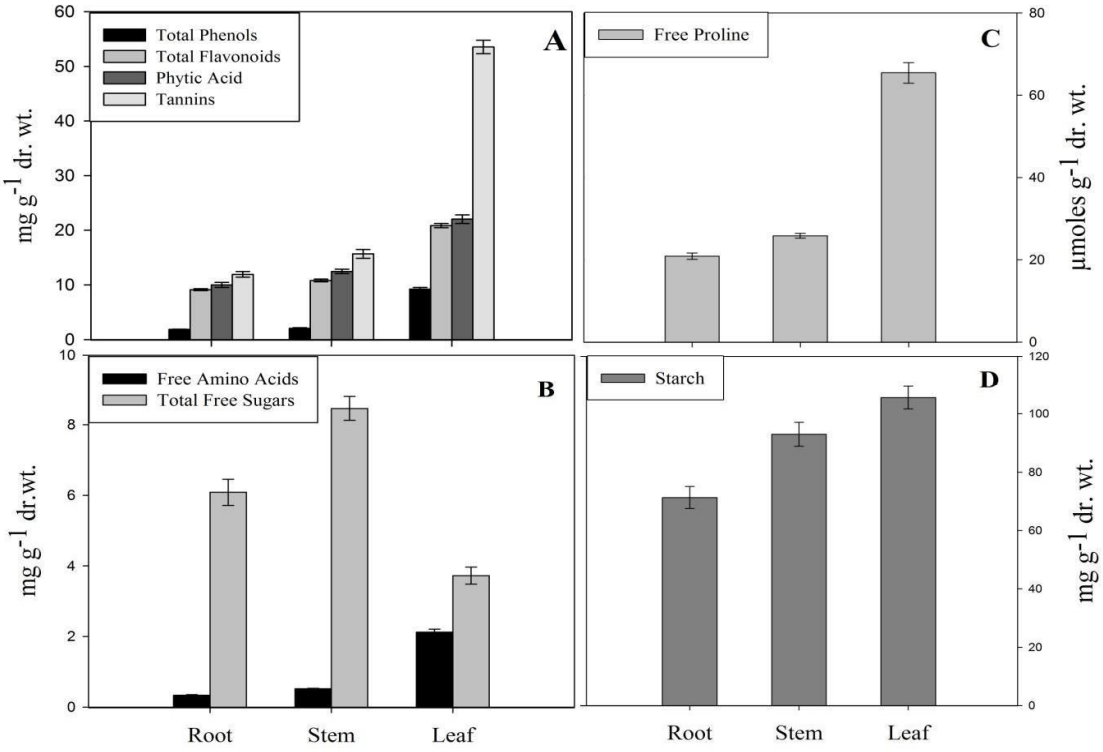

Fig 1: A Total phenols, flavonoids, phytic acid and tannins, **B** Free amino acids and total free sugars, **C** Free proline and **D** Starch in different parts of 30 days old seedlings of *Tagetes erecta*L.

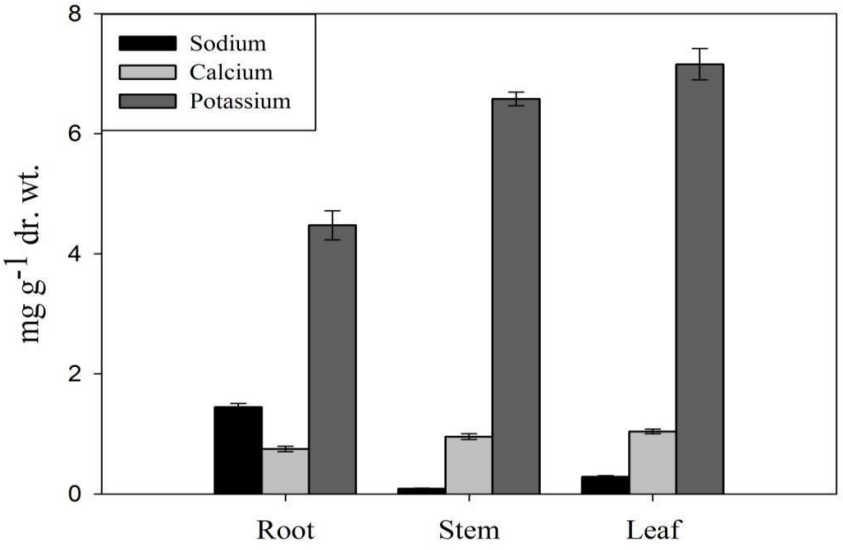

Fig 2: Sodium, Calcium and Potassium in different parts of 30 days old seedlings of *Tagetes erecta* L.

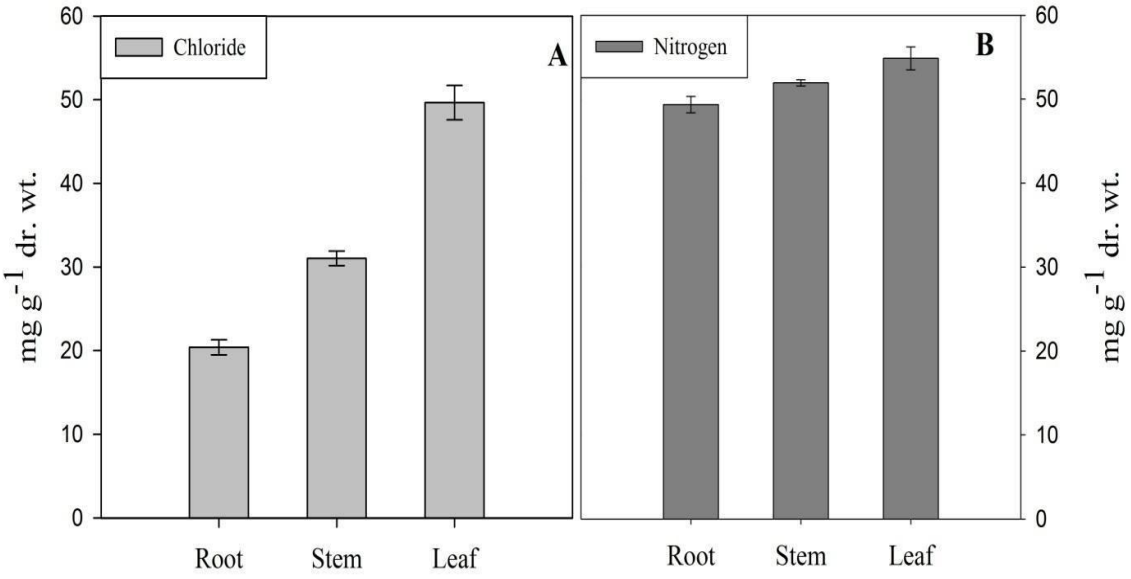

Fig 3: A Chloride and **B** Nitrogen in different parts of 30 days old seedlings of *Tagetes erecta* L.

CONCLUSION

The present study indicates the accumulation of considerable amount of secondary metabolites by *Tagetes erecta* L. including phenols, flavonoids, tannins and phytic acid which is organ based, the information so gathered can be of pharmaceutical application. Accumulation of organic osmolytes can be useful for the growth of *Tagetes* under adverse environmental conditions as promising plant for reclamation of wastelands.

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