Allelopathic Effects of *Guizotia abyssinica* on Nutrient Composition of Seedlings of *Amaranthus tricolor*

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Abstract

The present aim of the work is to assess the allelopathic effect of crop plant Niger on carbohydrate and nutrition composition of seedlings of weed plant *Amaranthus tricolor*. We assessed the effect of aqueous extracts of shoot, root and residues of Niger plant on carbohydrates composition, magnesium, calcium and potassium levels from the seedlings of *A. tricolor.* The results revealed that the higher concentrations of the extracts (10 & 5%) of root and shoot extracts significantly increased the levels of reducing sugars in roots and shoots of *A. tricolor* seedlings to varying degree on fifth day. Where as higher concentrations of both extracts significantly decreased the levels of non-reducing sugars in root and shoots in 5-day old seedlings. The lower concentrations increased the non-reducing sugar levels. Regarding starch the inhibitory pattern was same as that of non-reducing sugar in root and shoots in 5-day old *A. tricolor* seedlings. The data on residual effect on carbohydrate fractions of *A. tricolor* revealed that the similar trend of inhibition observed with extracts treated seedlings. Maximum toxicity was observed in 15day old plants followed by 30 and 45-day old plants. The levels of Magnesium and potassium levels were significantly decreased in roots and shoots of *A. tricolor* seedlings treated with 5 and 2.5% concentrations. While, the Calcium levels were increased in root and shoots of *A. tricolor* seedlings of 15, 30, and 45 day old *A. tricolor* seedlings.

Key words: *Guizotia,* carbohydrate levels, Mg, Ca and K

I. INTRODUCTION

Plants, like all other living things, need food for their growth and development. Plants require 16 essential elements, categorised as primary and secondary elements. Primary elements (Nitrogen, Phosphorus and Potassium) are very crucial for growth and development of leaf and root. while secondary elements are important for physiological functions of plant such as cell structure, photosynthesis and protein synthesis. If any imbalance in the nutrient uptake it leads to abnormal features in plant growth and development. Several scientific investigations have shown that compounds having the allelopathic nature and are water soluble can alter several aspects of plant metabolism [1]. These include mitochondrial respiration, photosynthetic rate, chlorophyll content, synthesis of proteins, balance of carbon flow from glucose onto the organic compounds and water potential. While alterations in any of these aspects could contribute to growth reductions in a susceptible species, other physiological disturbances may also be implicated. Nutrient imbalance may be one among the physiological disturbances and the altered nutrient levels in weed plants [1].

For the present study we selected Niger plant (*Guizotia abyssinica*) to test its allelopathic effect on carbohydrate (reducing, non-reducing and starch) composition and uptake of nutrients (Mg, Ca and K) of weed plant *Amaranthus tricolor*. Niger plant is a herbaceous plant belongs to the family Asteraceae, cultivated for edible oil obtained from its seed. The species of genus Amaranthus widely distributed in tropical and sub-tropical and temperate regions of the world. Few species of *Amaranthus* are grown as green leafy vegetables and some are serious weeds in agriculture crops. *Amaranthus tricolor* (Syn. *A. gangeticus)* known as Chinese spinach, grown in India and China [2]. Several *Amaranthus* species such as *A. tricolor* [3]. *A. retroflexus* L. reported as potent weeds in agriculture crops.

II. MATERIALS AND METHODS

Plant material

The seeds of crop plant *Guizotia abyssinica* were procured from Andhra Pradesh Seed Development Corporation, Ananthapuramu. The plant material was collected from the field grown *G. abyssinica*, surface sterilized with 0.1% mercuric chloride and air dried in the laboratory. Dried plant material was separated into roots and shoots, chopped into fine pieces and stored in air tight containers at 4 ºC.

Preparation of Aqueous Extract

The extracts were prepared by taking 10 g of chopped part in 100ml distilled water in sterilized beakers. The beakers were kept for 72h at 10 – 15 ºC. Aqueous extracts thus obtained were filtered through Whatman No.1 filter papers and the volume were made up to 100 ml. This was labelled as pure extracts or stock solutions. A part of this stock solution was further diluted (0.1, 10 times) with distilled water to get 10, 5 and 1 per cent solutions.

Residue Incorporation Studies

For the residue incorporation studies earthen pots (6.5” x 5.5” size) containing 1 kg of sterilized silica sand and 5, 2.5 and 1 % of finely ground root and shoot residue of niger seed were maintained under natural photoperiod of about 12-14h with a temperature of 28± 4 ºC in the botanical garden. A control was maintained without residue. The pots were irrigated once a day with distilled water. The pots were incubated for 15 days for decomposition of the residues. The seeds of horse gram were surface sterilized and raised in pots. After the seedlings emerged, the plants were thinned to 3 per pot and the plants were maintained for 45 days from the day of seeding emergence. The pots were irrigated once a day with distilled water and twice a week with Hoagland’s nutrient solution [4]. Maximum care was taken to ensure that the amount of water added was slightly less than field capacity. The pots were arranged in a randomized complete block experimental design with three triplicates to avoid drainage of leachates during experimentation.

All the plants were harvested at an interval of 15 days. The pots were flooded with water, the sand was loosened slowly and the plants were uprooted carefully. After each harvest (i.e. 15, 30 and 45 day – old plants), the plants were thoroughly washed with water and separated into root and shoot. Prior to this, seed germination was recorded. Morphological parameters such as seedling growth (root and shoot length) leaf area, dry mass production and some physiological parameters were studied. The results were averaged of three replicates.

Estimation of carbohydrate fractions

Two hundred milligrams (200 mg) of oven dried plant material from different harvested parts (5 and7 day old seedlings treated with extracts and 15, 30 and 45day-old plants treated with residue) was extracted with 80% ethanol according to the method [5]. The alcoholic extract was evaporated to 10 ml on water bath and cooled to room temperature. It was centrifuged and the supernatant (alcoholic extract) was used for the estimation of reducing sugars and non-reducing sugars, while the sediment was used for the estimation of starch.

Estimation of reducing sugars

The reducing sugars were estimated by Nelson method (1944) [6] as modified by Somogyi (1952) [7]. In brief, one ml of alcoholic extract was taken into a test tube. To this 1 ml of freshly prepared mixture of 25 parts of reagent A and 1 part of reagent 2 B were added and the contents were mixed. The test tubes were kept in a boiling water bath for 20 min and cooled and running tap water. 1 ml of arsenomolybdate reagent was added and the tubes were allowed to stand for 15 min. the mixture was then diluted to 10 ml. the absorbance of the solution was measured at 500 nm using spectrophotometer. Reagent blanks were used to adjust to zero. Reducing sugar content was estimated by using a standard curve prepared with dextrose.

Estimation of Non-reducing sugars

From the alcoholic extract, non -reducing sugars were estimated by following Scott (1960) [8] method. 10 ml of original alcoholic extract was hydrolysed with 10 ml of N HCl for 30 min. at 100 °C in water bath. Later it was cooled and neutralized with 30% NaOH. The total soluble sugars in the hydrolysed extract were estimated by Nelson method as described above.

Estimation of Starch

The sediment left after the alcoholic extraction of original material was taken for starch estimation according to the Mc Cready et al., (1960) [9]. Starch was solubilized with 25% perchloric acid for 30 min, filtered and was made up to known volume. To 1 ml of the perchloric acid extract, 4 ml of freshly prepared anthrone reagent was added in cold. The test tubed were heated for 75 min at 100 °C in water bath. The test tubes were cooled rapidly to room temperature and the colour intensity was measured at 630 nm using spectrophotometer. Reagent blanks were used to adjust the absorbance to zero.

Plant Nutrient Analysis

Digestion of Plant Material

15, 30- and 45-day old plants of *A. tricolor* treated with residue s were harvested and digested by wet digestion according to the method of Humphries (1956) [10] described below.

Wet digestion

One gram of oven dried powdered plant material was transferred into a 300 ml Kjeldahl flask and 4 ml of perchloric acid and nitric acid (about 7ml per every gram material) were added to ensure compete oxidation of organic matter. Then 5 ml of sulfuric acid (at least 2 ml should be always be present) was added to ensure the presence of sufficient amount of acid of high boiling point to digest in the later stages. After adding the three acids the contents were mixed gently at low heat for 5 min until the appearance of dense brown fumes. Then the flask was again placed on the heater and digestion was continued slowly at low heat until the appearance of dense fumes of sulfuric acid. The digestion was further continued for 10 min after the appearance of dense white fumes for 2 min at increased heat. Then the liquid was colourless. The contents were diluted with distilled water to a known volume. Potassium and Calcium were estimated in flame photometer and Magnesium was estimated in Atomic Absorption Spectrophotometer a appropriate wavelength. Standard graph was prepared individually with KCl. $CaCO_3$ and Mg ribbon. The amounts of these minerals were expressed as mg/gram dry weight.

III. RESULTS AND DISCUSSION

The toxicity of *Guizotia* extracts of root and shoot and residues of root and shoot on carbohydrate fractions such as reducing, non-reducing sugars and starch composition of *A. tricolor* seedlings was presented (Figure 1, 2, 3, and 4) respectively. The data reveals that the higher concentrations of the extracts (10 & 5%) of root and shoot extracts significantly increased the levels of reducing sugars in roots and shoots of *A. tricolor* seedlings to varying degree on fifth day (Figure 1 and 2). However, decrease in levels of reducing sugars was associated with 1% concentration of root and shoot extracts. The same trend to a less toxicity level was also observed in 7day old seedlings. The higher concentrations of both extracts significantly decreased the levels of non-reducing sugars in root and shoots in 5-day old *A. tricolor* seedlings. The lower concentrations increased the non-reducing sugar levels. Regarding starch the inhibitory pattern was same as that of non-reducing sugar in root and shoots in 5-day old *A. tricolor* seedlings. Same trend was observed in 7-day old seedlings.

Figure 1. Effect of aqueous extracts of *Guozotia absynnica* root on carbohydrate fractions of roots and shoots of *Amaranthus tricolor* seedlings

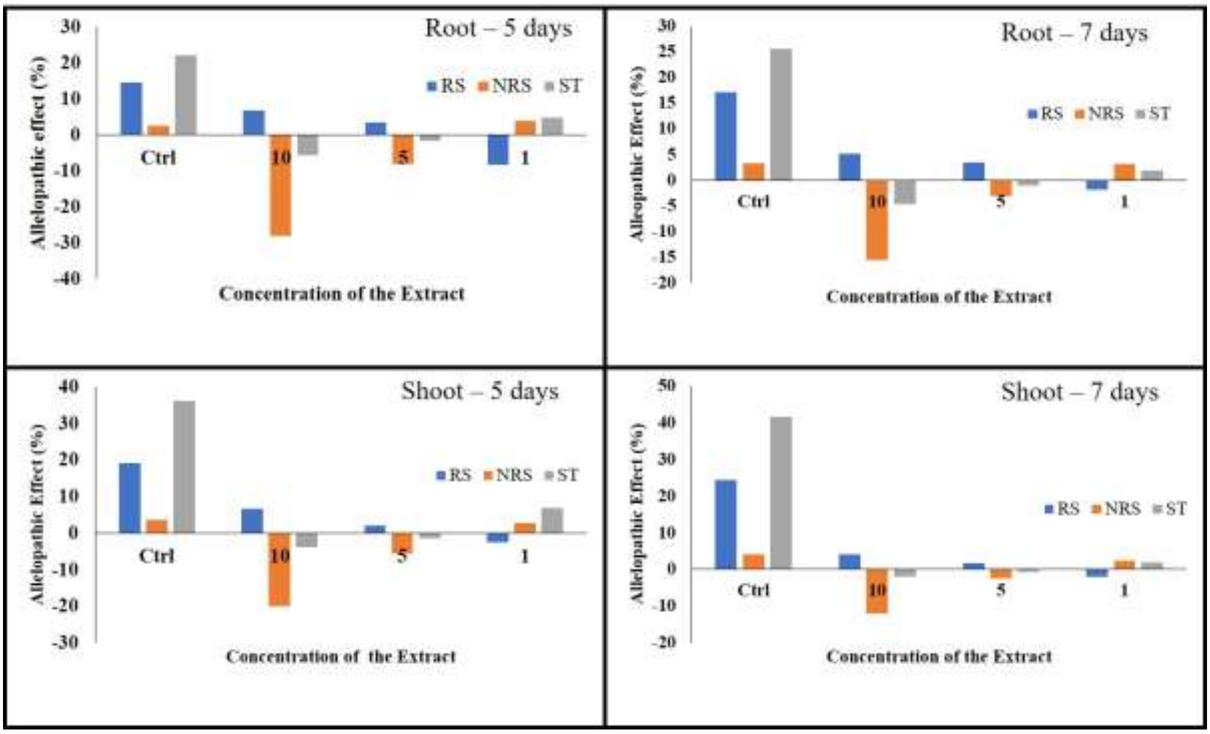
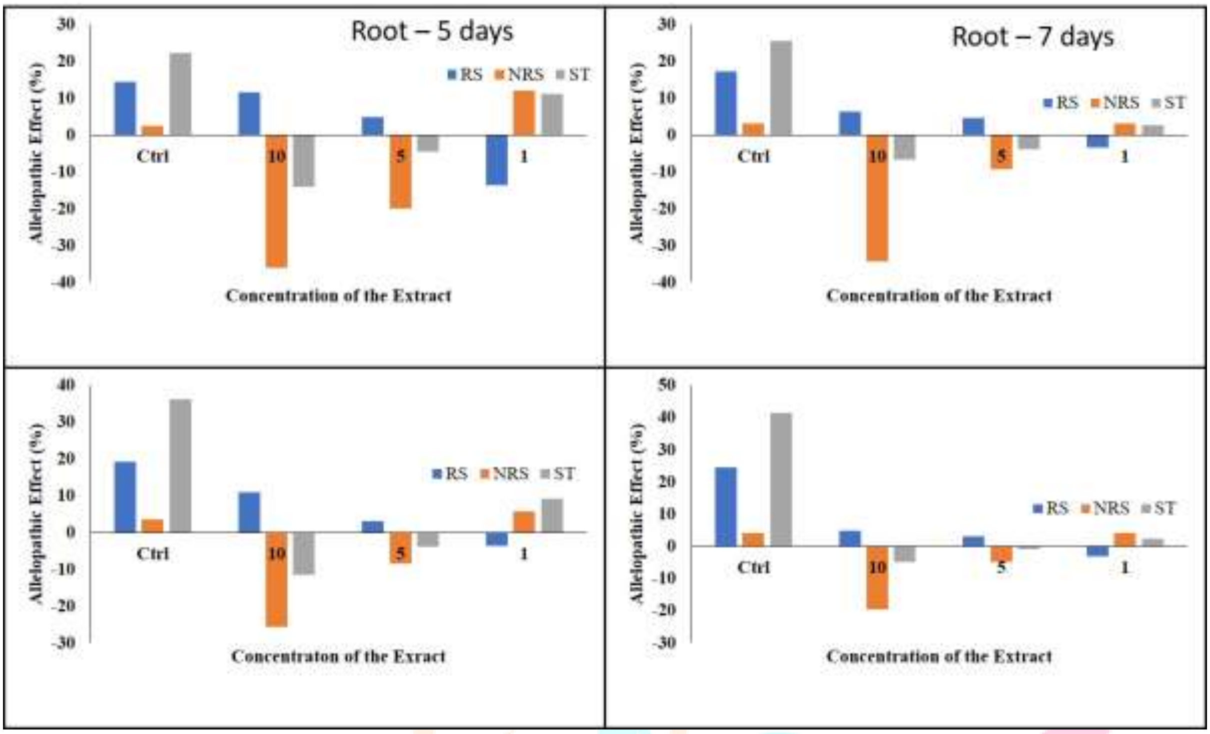


Figure 2. Effect of aqueous extracts of *Guozotia absynnica* Shoot on carbohydrate fractions of roots and shoots of *Amaranthus tricolor* seedlings



The data on residual effect on carbohydrate fractions of *A. tricolor* was represented in (Figure 3 & 4). The results revealed that the similar trend of inhibition observed with extracts treated seedlings. Maximum toxicity was observed in 15day old plants followed by 30 and 45-day old plants. The increase in reducing sugars, decrease in non-reducing sugars and starch was associated with roots and shoots of treated plants. The carbohydrate fractions of roots were significantly altered in terms of per cent inhibition followed by shoots. Shoot residue was found to possess maximum toxicity followed by root residue. The alteration in the levels of carbohydrate fractions in root and shoots of treated plants agree with the reports of [11, 12, 13]. Though there is decrease in non-reducing sugar levels and total sugar levels (reducing and non-reducing).

Figure 3. Effect of aqueous extracts of *Guozotia absynnica* Root residue on carbohydrate fractions of roots and shoots of *Amaranthus tricolor* seedlings.

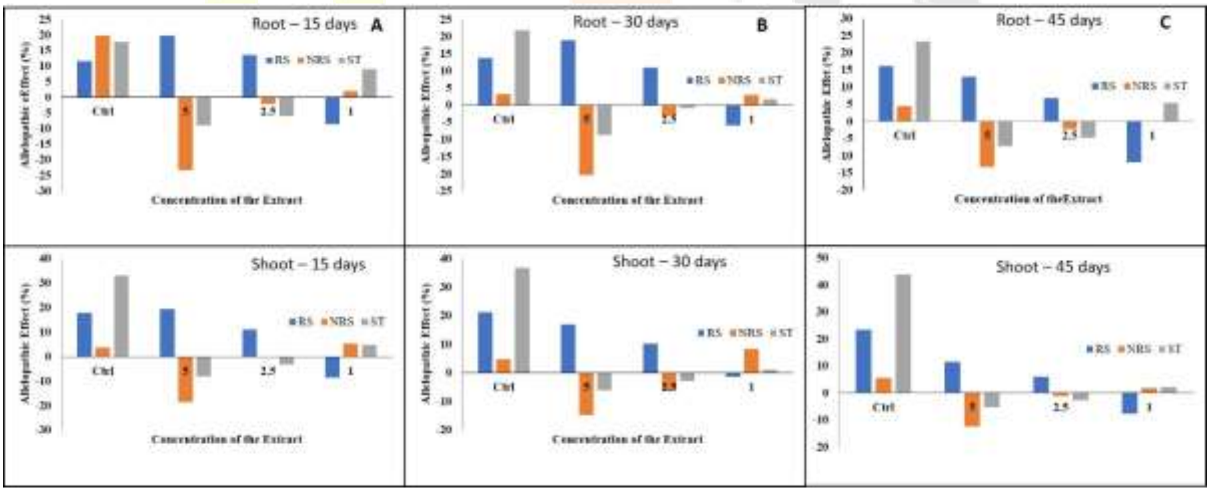
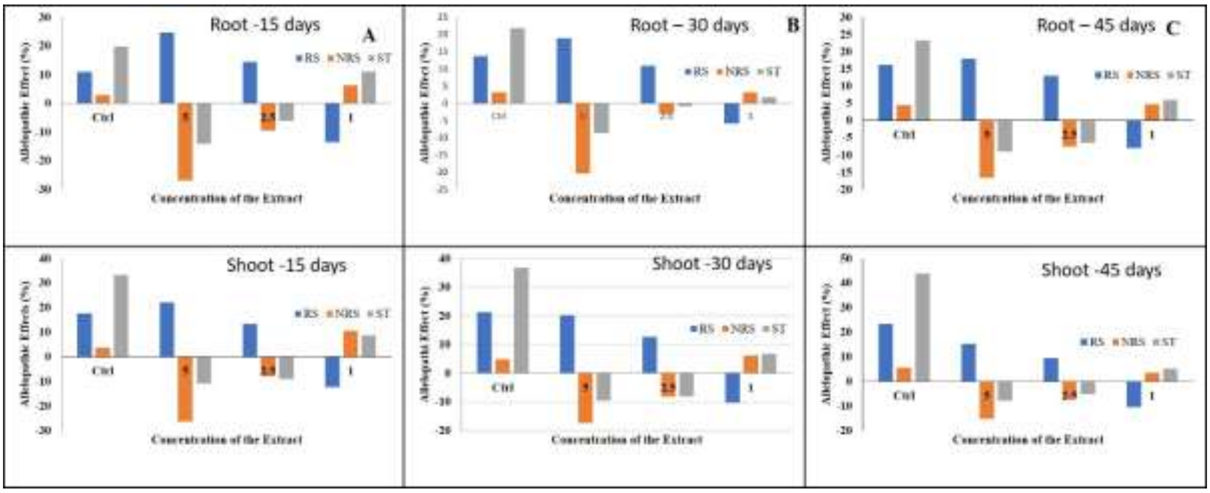


Figure 4. Effect of aqueous extracts of *Guozotia absynnica* Shoot residue on carbohydrate fractions of roots and shoots of *Amaranthus tricolor* seedlings



Plant nutrients like Magnesium, Calcium and Potassium were estimated in *A. tricolor* seedlings treated with root and shoot residues of *Guizotia*. The levels of Magnesium were significantly decreased in roots and shoots of *A. tricolor* seedlings treated with 5 and 2.5% concentrations. However, the lower concentrations increased the Mg content (Figure 5A &B). Significant decrease was associated with shoots followed by roots of treated plants. Shoot residue appeared to be more potent. The inhibitory activity seemed to be concentration dependant and was maximum in 15 -day old plants followed by 30 and 45-day old plants.

Figure 5A. Effect of aqueous extracts of *Guozotia absynnica* Root residue on magnesium levels of roots and shoots of *Amaranthus tricolor* seedlings.

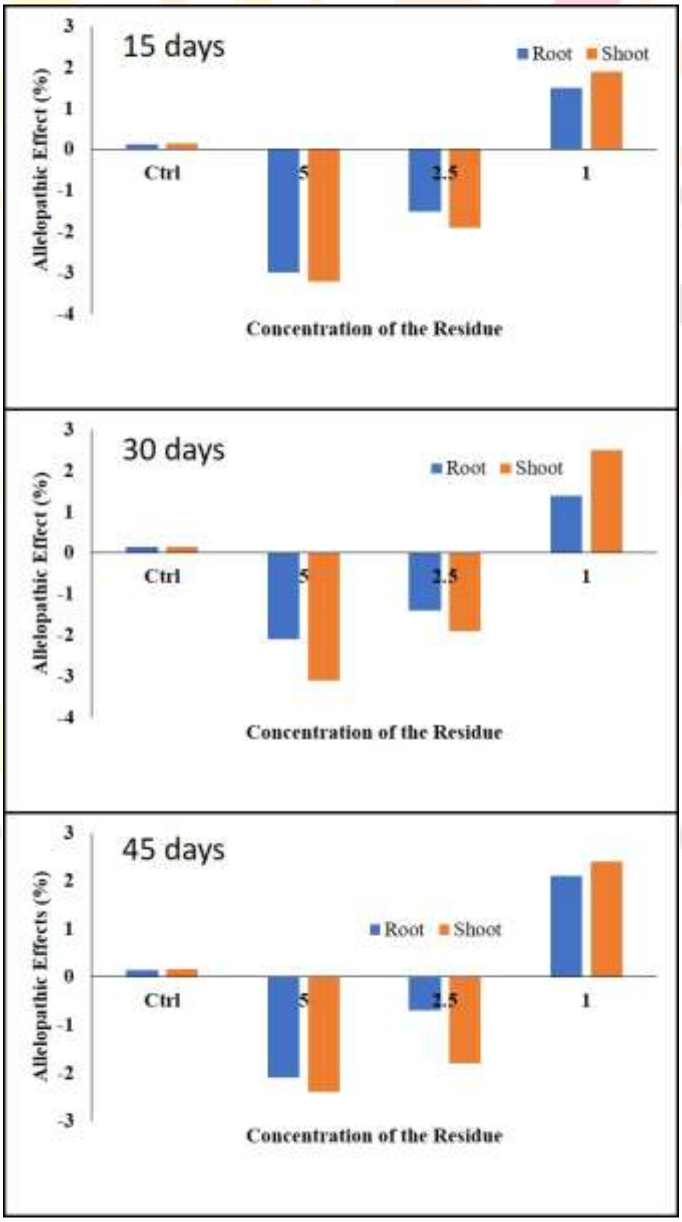
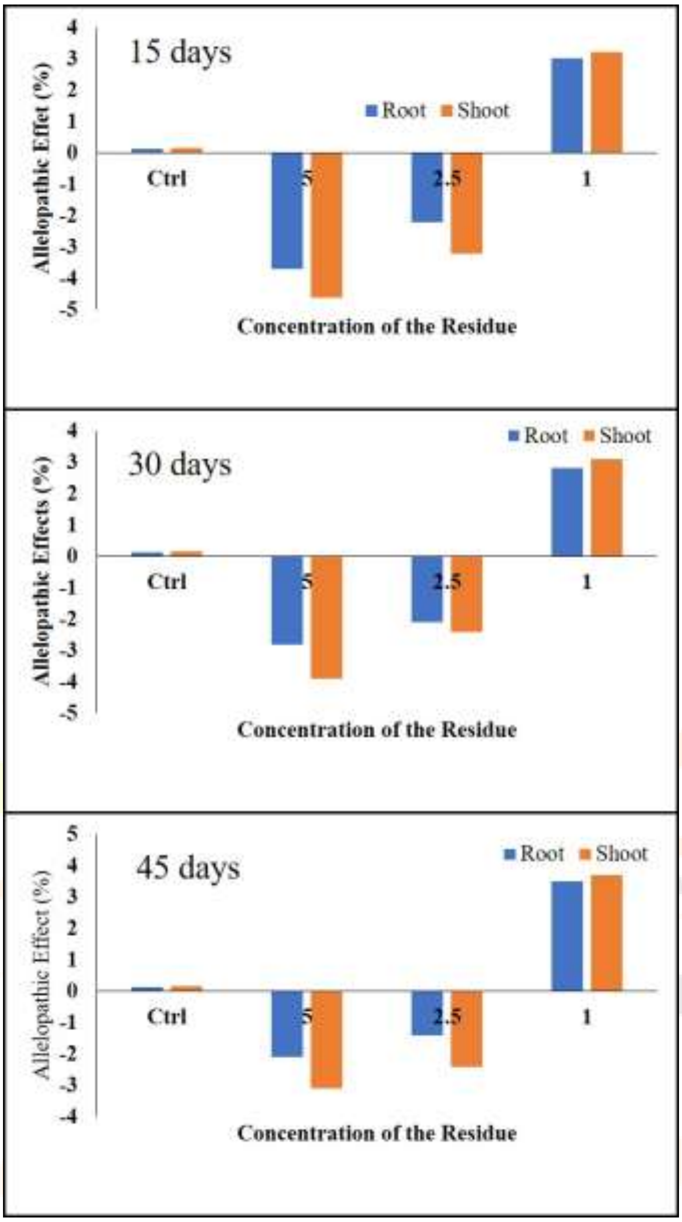


Figure 5B. Effect of aqueous extracts of *Guozotia absynnica* Shoot residue on magnesium levels of roots and shoots of *Amaranthus tricolor* seedlings.



The higher concentrations (5 and 2.5%) of root and shoot residues of *Guizotia* significantly increased Calcium levels in root and shoots of *A. tricolor* seedlings of 15, 30 and 45 day old (Figure 6A & B). on the contrary, lower concentration of both residues decreased the levels of Calcium in treated plants. However, more accumulation was observed in roots followed by shoots. As with magnesium, a similar trend of decrease was observed with the levels of Potassium in roots and shoots of *A. tricolor*. The higher concentrations significantly decreased the Potassium levels with a pronounced decrease in roots followed by shoots (Figure 7 A & B). However, there was an increase in Potassium levels of roots and shoots associated with the 1% concentration of both residues. The toxicity was found to be concentration – dependent and shoot residue was more potent in altering the nutrient balance. Maximum inhibitory activity was observed with 15 day- old plants which was alleviated with the increase in age of the plants.

Figure 6A. Effect of aqueous extracts of *Guozotia absynnica* Root residue on Calcium levels of roots and shoots of *Amaranthus tricolor* seedlings

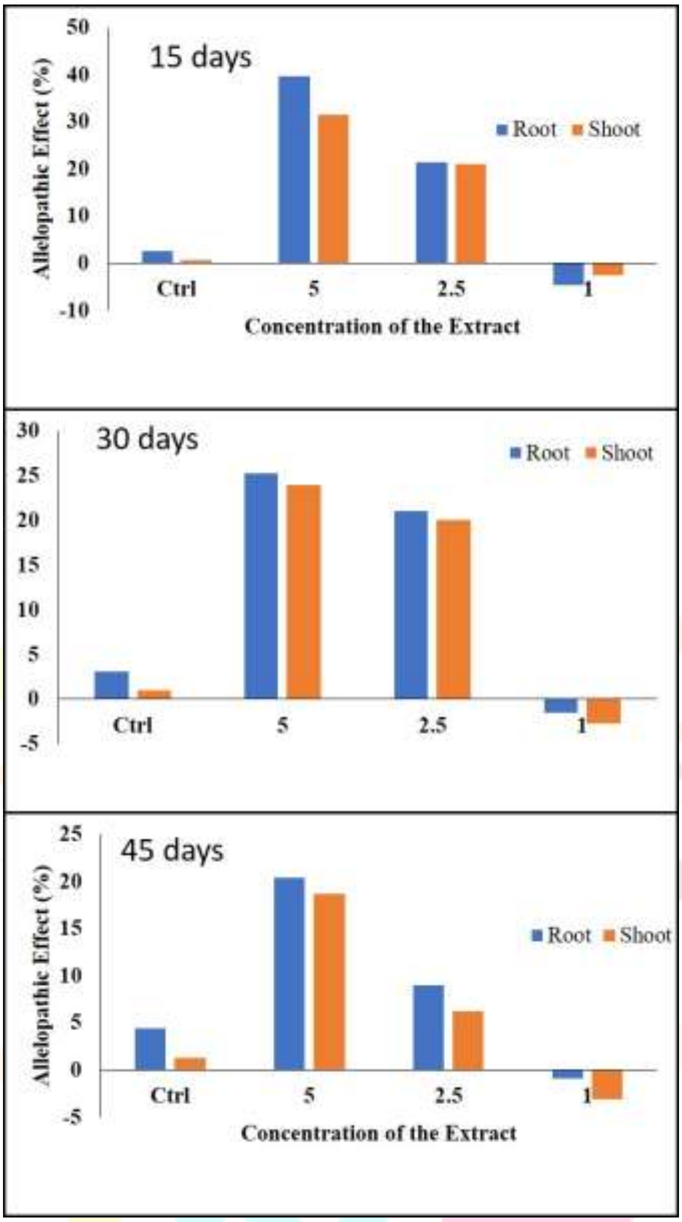


Figure 6B. Effect of aqueous extracts of *Guozotia absynnica* Shoot residue on calcium levels of roots and shoots of *Amaranthus tricolor* seedlings.

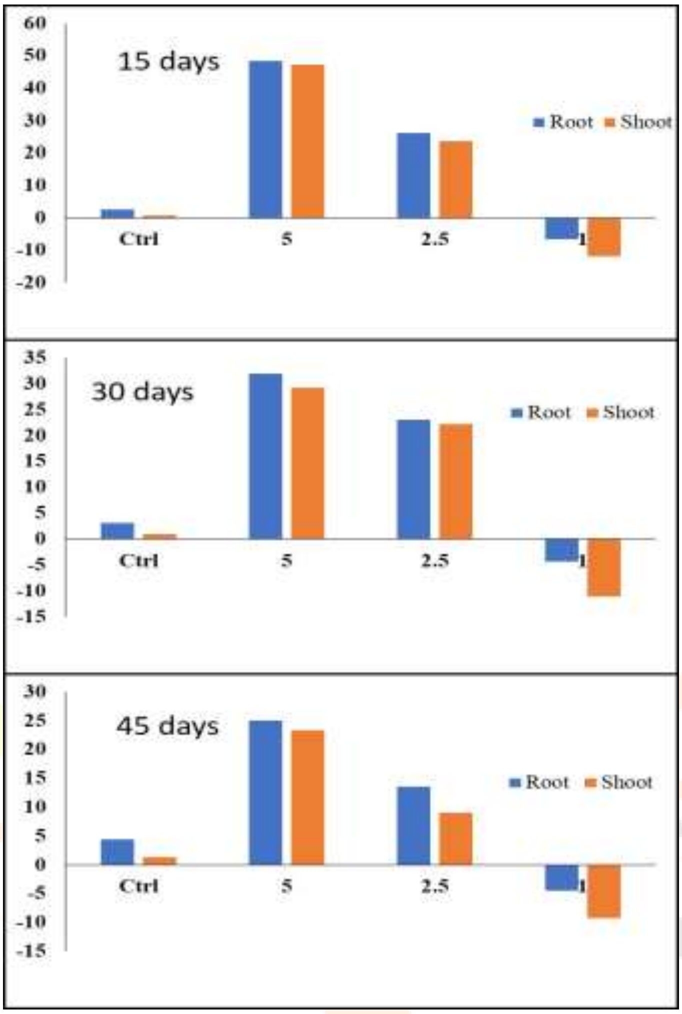


Figure 7A. Effect of aqueous extracts of *Guozotia absynnica* Root residue on levels Potassium of roots and shoots of *Amaranthus tricolor* seedlings.

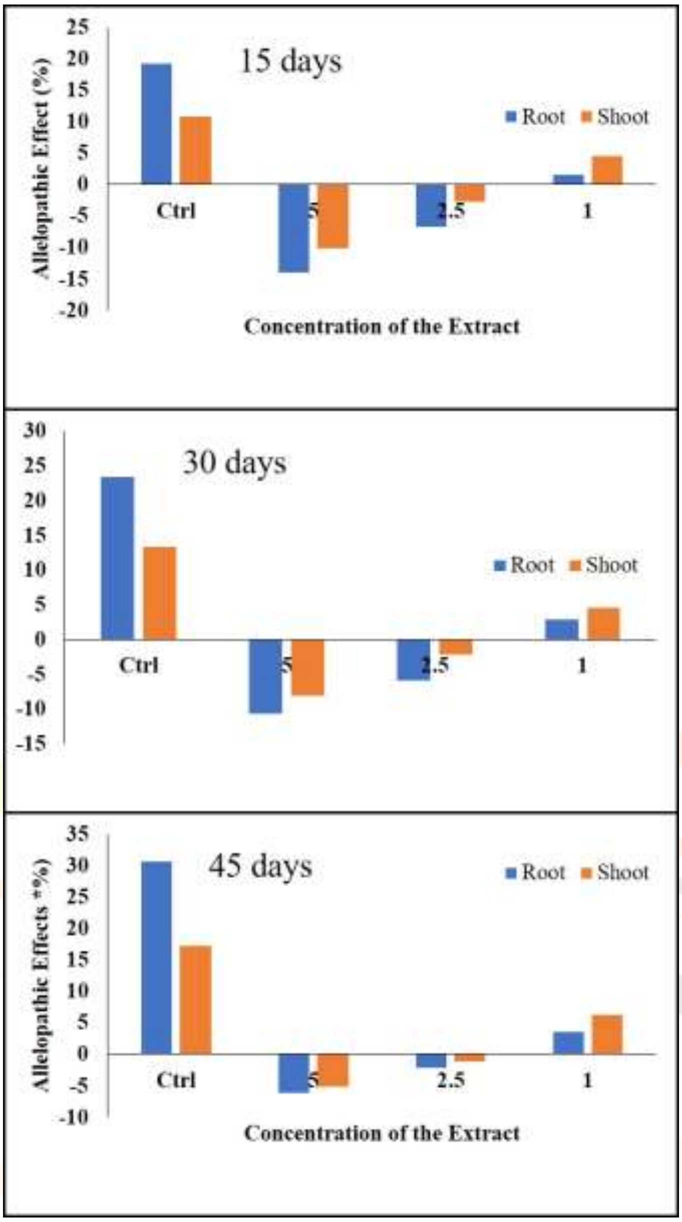


Figure 7B. Effect of aqueous extracts of *Guozotia absynnica* Shoot residue on levels Potassium of roots and shoots of *Amaranthus tricolor* seedlings.

Relatively few studies have been conducted to test the effects of allelochemicals associated with extracts / residues of donor plants on the mineral content of test plants. The findings of present investigation clearly signify the alteration in nutrient balance compared to control. The evidences documented in literature regarding allelopathy indicate that higher plants subjected to inhibitory allelochemicals have a reduction in one or more phases of growth, ranging from seed germination to overall seedling growth. However, it has been difficult to determine the biochemical and physiological causes for these reductions in growth.

A variety of investigations have shown that compounds having the allelopathic nature and are water soluble can alter several aspects of plant metabolism [1]. These include mitochondrial respiration, photosynthetic rate, chlorophyll content, synthesis of proteins, balance of carbon flow from glucose onto the organic compounds and water potential. While alterations in any of these aspects could contribute to growth reductions in a susceptible species, other physiological disturbances may also be implicated. Nutrient imbalance may be one among the physiological disturbances and the altered nutrient levels in *A. tricolor*, correlates with the works of Hall et al., [14, 15]. The data from the study of Kobza and Einhellig (1987) [15] indicate that the ferulic acid interferes with the nutrient uptake (K, Ca, Mg) in sorghum seedlings. The reduced Mg content found in roots may also be a primary effect.

However, it is not possible to suggest how suppression of root Mg might affect the growth since shoot tissue of these plants had a higher Mg level than controls. However, our results clearly showed that reduction in Mg levels of both roots and shoots of treated plants. Thus, it appears that Mg, Ca and K concentrations in plant tissues are directly affected by residual treatment. These alterations parallel growth reductions and can be implicated as a casual factor in growth inhibition. The reduction in Mg levels as well as the increase observed for Ca might have resulted from a slower rate of leaf expansion in treated plants. There is a possible correlation between leaf area, dry weight and nutrient balance in treated plants. Recently, it was reported that cowpeas inhibited by syringic, caffeic and protocatechuic acids had less N, P, K, Fe and Mo than untreated plants [16]. Evidence from excised root tissue indicates the first site of interference by the benzoic acid, cinnamic acid is the membrane and the magnitude of this early action on mineral uptake is correlated with their lipid solubility [17]. The mechanisms that mediate phenolic acid-induced changes in membrane permeability are vague [18]. It is even possible that reduced ion uptake by phenolic acids over an extended period is produced by a general inhibition of metabolism [19].

Blake (1985) [18] reported both increase and decrease in the content of nutrient elements in plants subjected to a variety of allelopathic conditions where the specific inhibitors have not been identified. Causes of mineral imbalance in test plant by extracts [20] by residue [21, 22] indicated that the extent and nature of changes in N, P and K content of receiving species have often differed according to the residue or leachate sources. It is interesting to note that the effects of allelochemical complex on mineral nutrition appear to be one of the important factors in growth inhibition.

IV CONCLUSION

In present study we studied the allelopathic effects *Guizotia abyssinica* aqueous extracts of root and shoot and residues on carbohydrate composition and nutrient levels in seedlings of *Amaranthus tricolor* using different methods. The results revealed that aqueous extracts root and shoot of Niger plant significantly increased the levels of reducing sugars and decreased the non-reducing sugars levels in root and shoots *Amaranthus* seedlings. The data on residual effect on carbohydrate fractions of *A. tricolor* revealed that the similar trend of inhibition observed with extracts treated seedlings. Maximum toxicity was observed in 15day old plants followed by 30 and 45-day old plants. The levels of Magnesium and Potassium levels were significantly decreased in roots and shoots of *A. tricolor* seedlings. While, the Calcium levels were increased in root and shoots of *A. tricolor* seedlings of 15, 30, and 45-day old *A. tricolor* seedlings. The results indicated that Guizotia extract expressed significant allelopathic effect on *A. tricolor* seedlings Further studies are required to identify the active principle responsible for Allelopathic effects of Niger plant.

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