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ALLELOPATHIC EFFECTS OF *GUIZOTIA ABYSSINICA* ON BIOCHEMICAL COMPOSITION OF SEEDLINGS OF HORSE GRAM

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ABSTRACT

The present aim of the work is to assess the allelopathic effect of crop plant Niger on biochemical composition of seedlings of another crop plant horse gram. We assessed the effect of aqueous extracts of shoot, root and residues of Niger plant on protein content, chlorophyll content and total free amino acids from the seedlings of horse gram. The results indicated that protein content of roots and shoots of the test plant seedlings decreased significantly by treatment with higher concentrations of root and shoot water extracts. The results on amino acid content revealed that the higher concentrations (10 & 5%) of extracts increased the amino acid levels of cotyledons significantly in 5 day old plants. However, the seedlings treated with 1% concentration of both extracts showed a decrease in amino acid levels. The same inhibitory pattern was also observed in 7 day old seedlings. The chlorophyll content results revealed that the higher concentrations significantly inhibited the total chlorophyll content in horse gram seedlings. However, stimulation was observed with lower concentrations on 5th day. In conclusion, the tested crop Niger plant significantly reduced the biochemicals like protein content, chlorophyll content and free amino acid contents of crop plant horse gram.

KEYWORDS: Niger plant, horse gram; biochemicals.

INTRODUCTION

Crop plants protect themselves from weeds by producing certain chemical components into their surrounding environment. These are called as allelochemicals. Allelochemicals are categorized into different groups such as water-soluble organic acids, simple lactones, phenolics, long chain fatty acids, quinones, coumarins, tannins, flavonoids, and terpenoids. These chemicals are released into the soil through leaching, decomposition of plant material, washed down

by rain and root exudates (Soltys et al., 2013).[1] Allelopathy is a natural phenomenon that happen in the environment, involves inhibitory or stimulatory effects of allelochemicals released by the donor plant to the receiver plant. Allelopathy plays an important role in regulating plant diversity and sustainable agriculture through releasing allelochemicals.

For the present study we selected Niger plant (*Guizotia abyssinica*) to test its allelopathic effect on seed germination of crop plant Horse gram. Niger plant is a herbaceous plant belongs to the family Asteraceae, cultivated for edible oil obtained from its seed. Horse gram [Scientific name: *Macrotyloma uniflorum* (Lam.) Verdc.] is one of under exploited legume crop with great potential in sustainable agriculture. Horse gram having better yield potential, quality of produce, adaptability, tolerance to biotic and abiotic factors and also adopting proper nutrient management practices (Rathiya and Neelam, 2021).[2]

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 was 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 (Hoagland and Arnon, 1950).[3] 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.

Protein Estimation

The total protein content of different parts like root and shoots of both treated and non -treated seedlings of 5 and 7 days and 15, 30 and 45days old plants using Lowry et al., (1951) method.

Estimation of total free amino acids

The extraction and estimation of amino acids was done according to Moore and Stein (1948).[4] Different organs of both treated and control seedlings harvested at different time intervals (5,7, 15,30 and 45 days old). Five hundred milligrams of plant material was placed in 80% ethanol and refluxed for 30 min over a boiling water bath for complete extraction of alcohol soluble compounds. One ml of sample and 1 ml of ninhydrin reagent were taken in a test tube and incubated in water bath for 20 min. After incubation period the contents were diluted with 5 ml of diluent solution (equal volumes of n-propanol and distilled water). The absorbance of the contents was measured at 70 nm in a spectrophotometer against a blank reagent.

Estimation of chlorophyll pigments

The total chlorophyll content of the treated and control samples was estimated using Arnon method (1949).[5] Five hundred milligrams of plant material were homogenized in a pre-chilled mortar using 80% acetone. The homogenate was centrifuged at 3000 rpm and supernatant was collected. The step was repeated twice and the supernatant was pooled. The absorbance of the supernatant was measured at 645 nm and 663 nm using 80% acetone as blank. Total chlorophyll content, chlorophyll a and chlorophyll b contents were calculated using the following formula.

Total chlorophyll = 20.2 X O.D. at 645nm + 8.02 X O.D. at 663 nm

Chlorophyll a = 12.7 X O.D. at 663 nm – 2.69 X O.D. at 645nm

Chlorophyll b = 22.9 X O.D. at 645nm – 4.68 X O.D. at 663 nm

RESULTS AND DISCUSSION

The results on allelopathic effect of *Guizotia* root and shoot extracts and residue on protein content of horse gram seed lings was depicted in Figure 1 and Figure 2. It revealed that the protein content of roots and shoots of the test plant seedlings increased significantly by treatment with higher concentrations of both extracts on 5th day to varying degree. The lower concentration, however, decreased the cotyledonary protein content. Root protein content decreased drastically compared to shoot. However, the protein content of root and shoot in both the plants increased when treated with 1% concentration. The same trend followed up to 7th day and the toxicity was achieved thereafter.

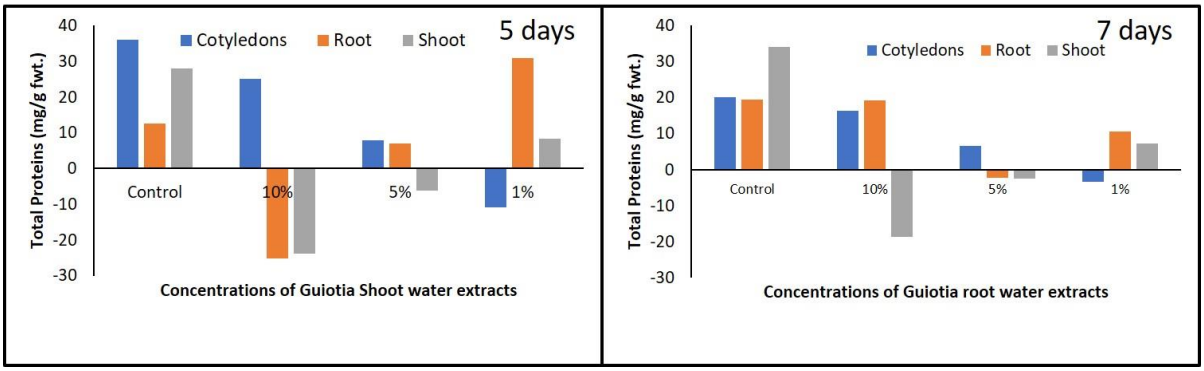

Figure 1: Effect of *Guiotia abyssinica Sh*oot water extract on total proteins of cotyledons, roots and shoots of Horse gram seedlings.

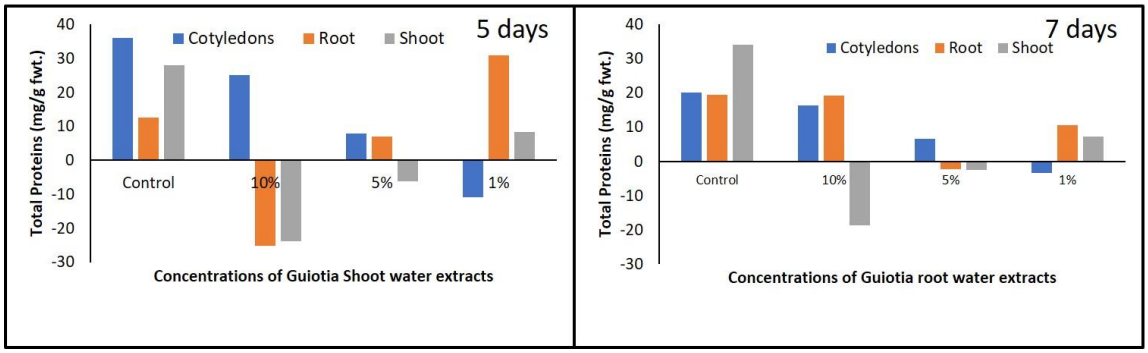

Figure 2: Effect of *Guiotia abyssinica Root* water extract on total proteins of cotyledons, roots and shoots of Horse gram seedlings.

The higher concentrations of root and shoot residues of *Guizotia* significantly decreased the root and shoot protein content of *horse gram* in 15, 30- and 45-day old plants to varying degrees (Figure 3,4). However, there was significant increase in root and shoot protein content of 15, 30- and 45-day old plants of *horse gram* treated with lower concentration (1%) of root and shoot residues (Figure 3,4). Thus, the inhibitory activity was concentration dependant and maximum inhibition was observed in 15 day-old plants followed by 30 day-old and the inhibition was almost alleviated in 45 day old plants.

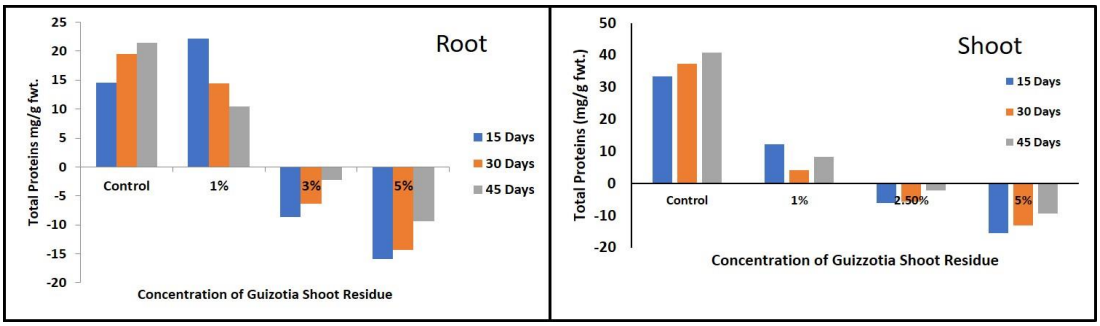

Figure 3: Effect of *Guiotia abyssinica Sh*oot residue on total proteins of roots and shoots of Horse gram.

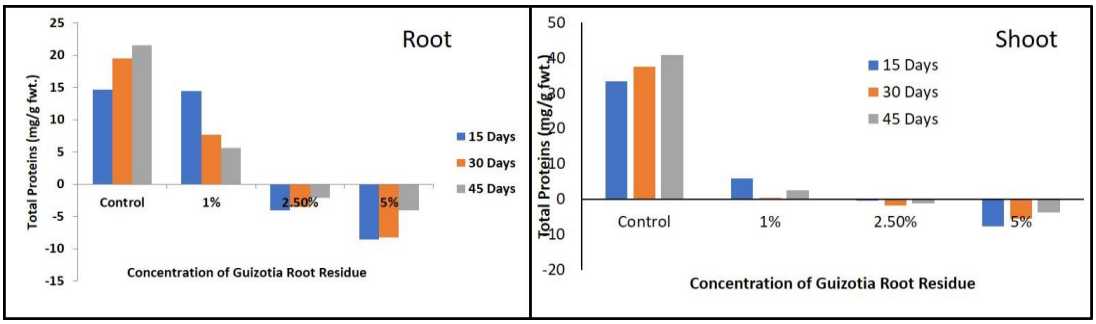

Figure 4: Effect of *Guiotia abyssinica* Root residue on total proteins of roots and shoots of Horse gram.

Proteins are one of the important and major reserve food materials in the seeds. The storage proteins of most seeds are found in well-defined organelles, the protein bodies. These storage proteins are hydrolysed into the constituent amino acids by proteolytic enzymes and are mobilized to the developing embryonic axis and are utilized for the synthesis of proteins or for providing energy by oxidative deamination. So, the proteolysis following germination is important for further growth of the seedling. The protein content in cotyledons is important because they are the primary sites of

protein reserves (Diaz-Mendoza et al., 2019). Since the seeds contain reserve proteins, their metabolism is nevertheless of considerable importance during germination.

The significant accumulation of proteins in cotyledons of treated seedlings may be due to the non-degradation of storage proteins (Poljakoff-Mayber, 1953).[6] This might be due to the inhibition of Protease which is responsible for the breakdown of endogenous trypsin inhibitor. Breakdown of this inhibitor seems to be involved in permitting the formation of trypsin-like enzyme required for the treatment of storage proteins (Shain and Mayer, 1963).[7] The decreasing pattern of protein levels in root and shoots of test plants closely agree with the reports of Duke et al., (1987).[8], Eyini et al., (1989).[9], and Suseela Devi et al., (1992).[10] The leaf extract (5%) of *Ipomoea carnea* decreased the leaf protein content of green gram. However, lower concentrations increased the protein content. Root extract did not inhibit the parameter and have more promotary effect than the leaf extracts (Suseela Devi et al., 1992).[10] Eyini et al., (1989)[9] observed the decrease in protein content of the leaves of *Arachis hypogaea* by the treatment of different concentrations of bamboo leaf extract.

The decrease in protein content of test plant seedling may be due to inhibition of protein synthesis by the allelochemicals present in the extracts and residues. Another effect may be due to the non-degradation of reserve material in the cotyledons since the proteolysis followed by germination is important for further growth of seedlings. Another possible reason may be the non-incorporation of amino acids into protein. This view may be strengthened by the reports of Van Sumere et al., (1972)[11] that ferulic acid inhibited the incorporation of phenyl alanine -1-C^{14} into lettuce seed and barley embryos and whole seeds. In another experiment, most of the 17 phenolic aldehydes, benzoic and cinnamic acids, coumarins and naphthoquinone tested were found to be inhibit the incorporation of phenyl alanine -1-C^{14} into protein.

The effect of aqueous and residues of *Guizotia* on total free amino acid content of *horse gram* was evaluated. The results revealed that the higher concentrations (10 & 5%) of shoot and root extracts decreased the amino acid levels of cotyledons significantly in 5 day old plants (Figure 5 and 6). The lower concentrations increased the amino acid content. The trend was also observed on 7th day but to a less degree of toxicity. The root and shoot residues of *Guizotia* also increased the amino acid content in 15, 30 and 45 day-old plants of *horse gram* at higher concentrations (Figure 7 and 8). However, the increase at 2.5% was not significant in some cases. The lower concentration (1%) of all treatments depleted the amino acid levels. the increase in amino acid levels was observed to be maximum in 15day old plants followed by 30- and 45-day old plants. The accumulation of amino acids was high in roots compared to shoots of treated plants.

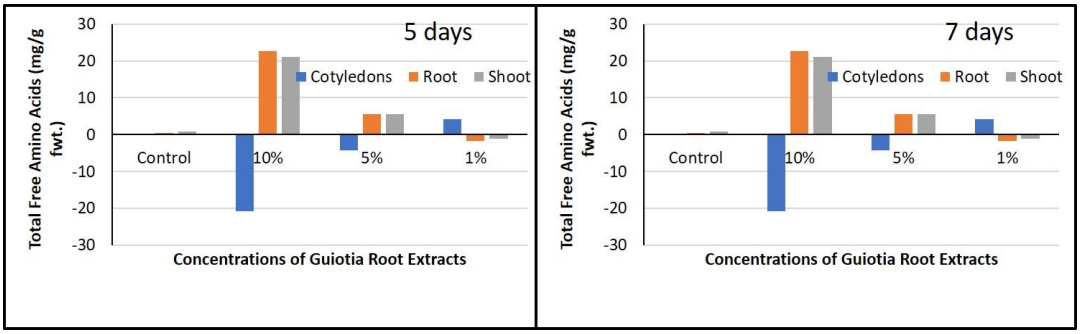

Figure 5: Effect of *Guiotia abyssinica* Shoot water extract on total free amino acids of cotyledons, roots and shoots of Horse gram seedlings.

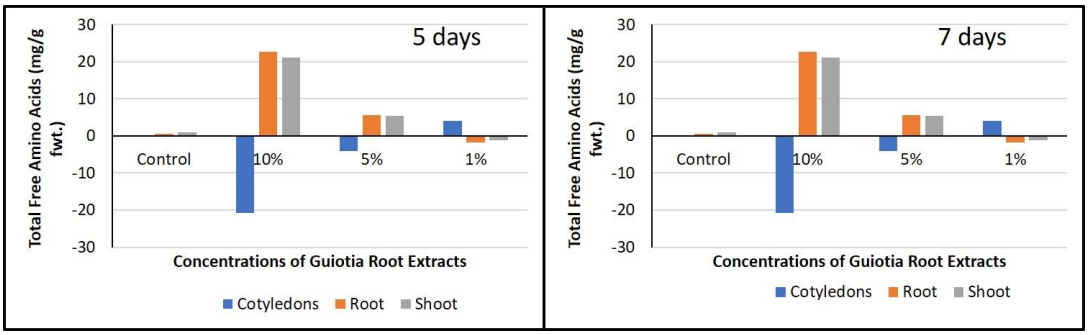

Figure 6: Effect of *Guiotia abyssinica* Root water extract on total free amino acids of cotyledons, roots and shoots of Horse gram seedlings.

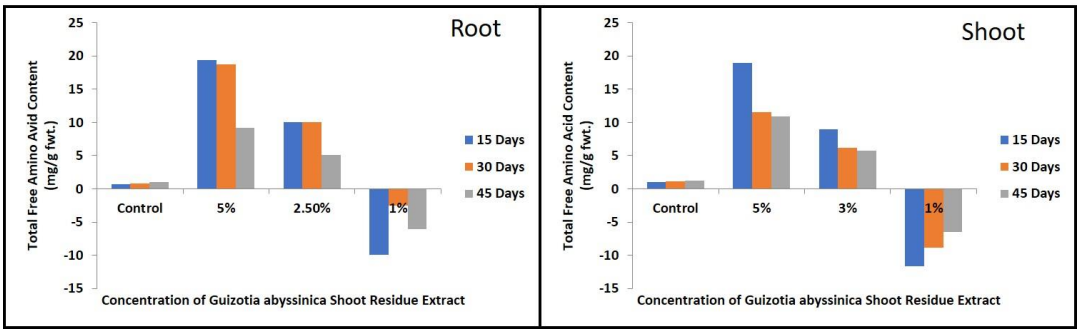

Figure 7: Effect of *Guiotia abyssinica Sh*oot residue on total free amino acids of roots and shoots of Horse gram plants.

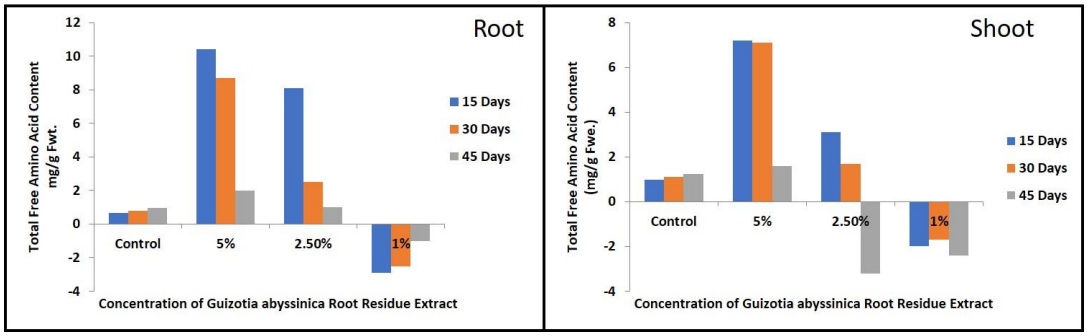

Figure 8: Effect of *Guiotia abyssinica* Root residue on total free amino acids of roots and shoots of Horse gram plants.

The decrease of amino acid levels in cotyledons and their increase in roots and shoots of treated seedlings may be due to reduced protein synthesis which indirectly leads to nn-incorporation of amino acids into proteins. This view agrees with the work of Van Sumere et al., (1972).[11] and Gu et al., (1983).[12] The decreasing levels of amino acids in cotyledons may be attributed to the fact that there may be slow breakdown of storage proteins and inhibition of protease activity. This might have resulted in low levels of free amino acids in cotyledons of treated seedlings. Thus, the allelopathic complex associated with the extracts possess a negative effect and may be responsible for the altered metabolism. Thus there exists well marked correlation between the protein content and amino acid levels. Gu et al., (1983).[12] found that artemisinin rapidly inhibited incorporation of radiolabelled leucine into protein by *Plasmodium falciparum* when the protozoan was in an erythrocyte. Of course, rapid inhibition of protein synthesis can be due to indirect effect of inhibition of any of several other processes.

The photosynthetic pigments such as total chlorophylls and chlorophyll a and chlorophyll b were estimated in the first pair of leaves of *horse gram*. Inhibitory pattern of *Guizotia* extracts on chlorophyll pigments of *horse gram* is presented in (Figure 9 and 10). The data reveals that the higher concentrations significantly inhibited the total chlorophyll content in *horse gram* seedlings. However, stimulation was observed with lower concentrations on 5th day (Figure 9 and 10). The same trend was observed with chlorophyll a in the test plant. Similar to chlorophyll a, the higher concentrations (10 & 5%) of root and shoot extracts decreased the content of chlorophyll b.Whereas lower concentrations stimulated the synthesis of the pigments. The same trend was observed but to a less inhibitory level on 7th day.

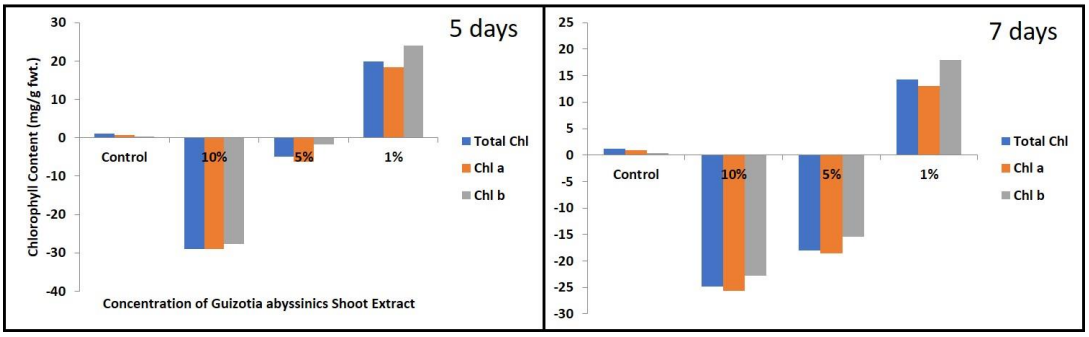

Figure 9: Effect of *Guiotia abyssinica Sh*oot extract on chlorophyll content of Horse gram plants.

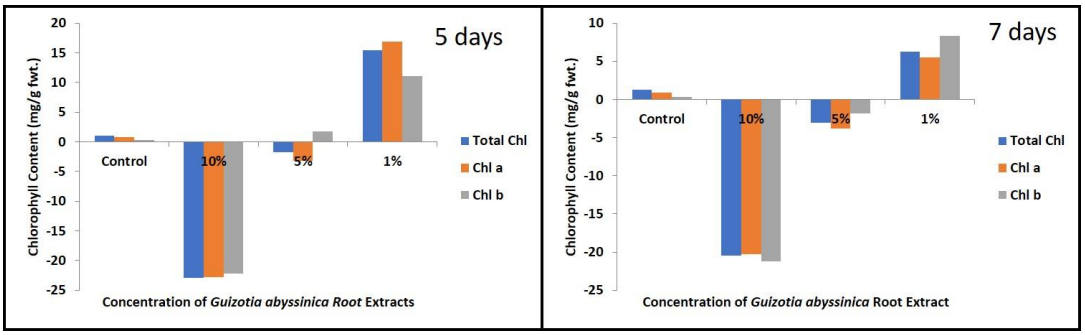

Figure 10: Effect of *Guiotia abyssinica* Root extract on chlorophyll content of Horse gram plants.

The higher concentrations (5 & 2.5 %) of root and shoot residue of *Guizotia* significantly inhibited three pigments i.e. total chlorophyll, chlorophyll a and chlorophyll b in 15th day old plants of *horse gram* (Figure 11 and 12). But in 30- and 45-day-old plants only 5% of residues reduced the chlorophyll pigment while the other two residue levels stimulated the pigment synthesis. The higher concentrations of shoot residue decreased the total chlorophylls, chlorophyll a and chlorophyll b, whereas stimulation was observed with 1% of shoot residue.

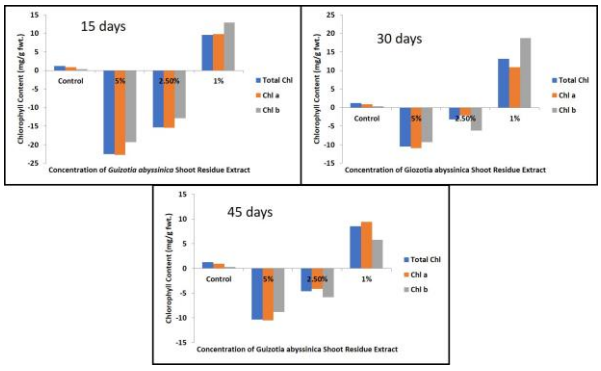

Figure 11: Effect of *Guiotia abyssinica* Shoot residue on chlorophyll content of Horse gram plant.

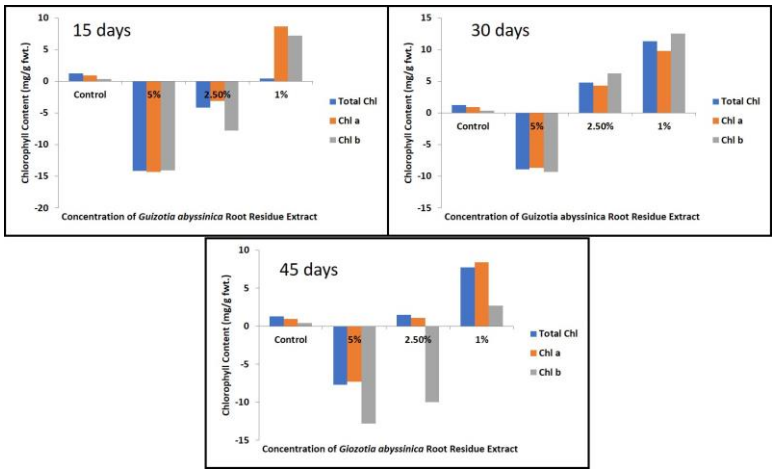

Figure 12: Effect of *Guiotia abyssinica* Root residue on chlorophyll content of Horse gram plants.

Total chlorophylls, chlorophyll a and chlorophyll b of *horse gram* treated with extracts and residues of *Guizotia* decreased significantly at higher concentrations. Further, in most cases the lower concentration elevated the pigment levels. Many investigators (Kamal, 2011;[13], Khaliq et al., 2012;[14] Elisante et al., 2013;[15] Siyar et al., 2019;[16] Li et al., 2021).[17] found that the weed extracts/ residues decrease the chlorophyll pigments of different crop plants. The reduction in chlorophyll pigments in the present study agrees with the report of Eyini et al., (1989).[9] Suseela Devi et al., (1992).[10] and Rajangam et al (1992).[18] who found that the decrease in chlorophyll content was proportional to the increase in concentration of the extract. Leaf extract of *Croton sparsiflorus* was found to be more inhibitory towards pigment synthesis in paddy cultivars followed by stem and root extracts (Rajangam et al., 1992).[18] Similar observations were also made by Colton and Einhllig (1980).[19] with the velvet leaf extract on soybean leaf chlorophyll content. They further reported that all extract dilutions of velvet leaf extract reduced chlorophyll a and chlorophyll b and total chlorophylls. The present data, however, do not indicate whether the reductions in leaf chlorophyll are due to degradation or reduced synthesis.

The decreasing pattern in the chlorophyll pigments of test plants is also in accordance with the report of Soni and Mohnot (1988).[20] that different concentrations of leaf extract of *C. argentea* inhibited the synthesis of chlorophyll a and chlorophyll b and total chlorophylls except by the very low concentrations of the extract like 0.05% in which time dependent specific effect on chlorophyll a, b ratio was noticed. It is quite evident that there is a significant decrease in chlorophyll content of test plant. The correlation of dry matter production and photosynthesis rate with chlorophyll content is well established (Buttery and Buzell, 1977).[21] But the information about the correlation of growth and chlorophyll is sporadic. In the present study, a positive correlation was found to exist between the chlorophyll content and shoot growth with the treatment of different extracts and residues.

CONCLUSION

In the present study we assess the allelopathic effect of crop plant Niger on biochemical composition of seedlings of another crop plant horse gram. The results indicated that protein content of roots and shoots of the test plant seedlings decreased significantly by treatment with higher concentrations of both extracts. The results on amino acid content revealed that the higher concentrations (10 & 5%) of extracts increased the amino acid levels of cotyledons significantly in 5day old plants. The chlorophyll content results revealed that the higher concentrations significantly inhibited the total chlorophyll content in horse gram seedlings. However, stimulation was observed with lower

concentrations on 5th day. In conclusion, the tested crop Niger plant significantly reduced the biochemicals like protein content, chlorophyll content and free amino acid contents of weed plant horse gram.

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