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# EFFECT OF *SIDA CORDIFOLIA* LINN. LEAF AND ROOT ETHYL ACETATE EXTRACTS ON THE GROWTH AND PHYSIOLOGICAL RESPONSE OF *PANICUM REPENS* AND *PANICUM MAXIMUM*

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***ABSTRACT***

*Due to the incessant application of synthetic herbicides in the agricultural system coupled with a rapid increase in herbicide-resistant weed species across the globe, much attention has been focused on alternative weed management, including allelopathy, which is eco-friendly and reduces reliance on synthetic herbicides. The effect of Sida cordifolia root and leaf ethyl acetate extract was evaluated under field conditions to determine its effects on growth, physiological response, stomatal index and density, and biochemical activity on weed species (Panicum maximum, Panicum repens, and Andropogon gayanus), as well as its effects on soil micronutrients. S. cordifolia root and leaf were collected from the wild in Nigeria and prepared into powder, which was dissolved to obtain the following concentrations 10, 20, 40, 60, and 80%, and distilled water served as the control. The percentage inhibition seems to be correlated with different growth indices as proven by an increase in malondialdehyde (MDA) content, reactive oxygen species (ROS), glutathione transferase (GST), and peroxidase (POD). Physiological growth indices such as plant height, leaf area, number of leaves, dry weight, stomatal density, and stomatal index were inhibited in both weed species while biochemical parameters such as Chlorophyll a, b, total chlorophyll and chlorophyll stability index were slightly reduced, increase in MDA, GST, ROS and POD were also noticed. In general, Andropogon gayanus showed a high inhibition rate compared to tomato. It is therefore recommended that concentrations of the Sida cordifolia extracts be increased to have a robust effect on the physiological processes of the weed species.*

**Keywords**: ethyl acetate extract, biochemical parameters, physiological indices, allelochemicals

## INTRODUCTION

In 1832, the Swiss botanist De Candolle suggested that crop plant exudates were responsible for an agriculture problem called “soil sickness”. He suggested that soil sickness was caused by chemicals released by the crop. Schreiner and Reed (1990) investigated the isolation of several phytotoxic chemicals from plants and soils. In the 1600s, several naturalists noted in the English literature that certain plants do not grow well in the presence of each other. The Japanese literature also shows examples of plants causing injury to others due to the production of extracts of toxic compounds with rainfall, specifically Japanese red pine (*Pinus densiflora*) (Rice, 1984). It is interesting to note that many of the species demonstrated to have powerful medicinal effects on humans also have been subsequently demonstrated to have powerful *allelopathic* effects as well (Chevallier 1996; Rice 1984; Wink 1999). The term ‘*allelopathy’* is derived from the Greek words allelon (meaning "of each other") and pathy (meaning "mutual harm" or "suffering") and was first used in 1937 by Austrian scientist Hans Molisch in the book “Der Einfluss einer Pflanze auf die andere – Allelopathie” (meaning “The Effect of Plants on Each Other”) (Willis 2010). He used the term to describe biochemical interactions that inhibit the growth of neighboring plants, by another plant (Roger *et al*., 2006).

Researchers have expanded the concept of allelopathy to include interactions between plants and higher animals, suggesting it is part of a broader network of chemical communication that aids plant defense. Allelopathy involves plant interference through secondary products added to the rhizosphere. These chemicals are found in nearly all plant tissues and, under suitable conditions, can be released in quantities that impact the growth of neighboring plants (Weston, 1996).

Allelochemicals, as natural agrochemicals, help protect the environment from pollution and maintain ecosystem balance. These secondary metabolites, found in most plant tissues, are released through leaching, decomposition, and volatilization. However, they can negatively affect plant germination and growth by interfering with cell division, energy metabolism, mineral uptake, and biosynthetic processes (Rice, 1984). The readily visible effect of allelopathy includes inhibited germination, seed swollen and darkened, necrosis of root, discoloration, shoot length, radicle/root length, reduced dry weight and reproductive ability (Niakan and Saberi 2009), leading to significant reduction in photosynthesis and other plant functions (Hussain and Reigosa, 2011).

Panicum repens, known by various names like torpedo grass and creeping panic, is a grass species and belongs to the Poaceae family. Its native range is unclear, potentially spanning Africa, Asia, Europe, Australia, the Mediterranean, Israel, and Argentina. Widely introduced and often labeled a noxious weed, it is considered "one of the world's worst weeds." It thrives in tropical and subtropical regions worldwide, spreading primarily through rhizomes as seed production is rare. Its propagules can remain viable for over a year, giving it a high reproductive potential (Hossain *et al*., 1999).

*Panicum maximum*, known as Guinea grass and green panic grass, is a large perennial bunch of grass that is native to Africa and Yemen. It has been introduced in the tropics around the world. It has previously been called *Urochloa maxima* and *Megathyrsus maximus* (Clements, 2011).

*Panicum maximum* is a large tufted, fast-growing perennial grass. It can withstand wildfire and drought and has been listed as an invasive weed in many countries such as South Texas, Sri Lanka, and Hawai'i which suppresses or displaces local native plants and is a fire hazard. It is a major weed in sugar-cane fields, due to its ability to grow under shaded conditions (Dhanesh, 2007).

Invasive species have been estimated globally to cost US$400 million annually due to loss of revenue and expenditures on control measures, while in Africa, an estimated US$60 million is spent annually on the control of invasive species (UNEP, 2006). The main choice for controlling weeds is still the application of synthetic chemical herbicides due to their effectiveness in controlling weeds, however, continuous application tends to create a negative impact on soil, weeds' resistance to herbicides, and Poison to non-target organisms (Inderjit *et al.,* 2011). Disturb ecology as a whole and leave chemical residues on the environment (Annett *et al*., 2014). Thus, this study will demonstrate if the effects on the growth, physiological response, stomatal index, and density are uniform across weed species and on the soil components.

## MATERIALS AND METHODS

### Plant materials collection

Matured healthy leaves and roots of *S. cordifolia* were collected at the vegetative stage in Zaria and the environs of Kaduna State, Nigeria. The leaves and roots were cleaned, air-dried, pulverized, and preserved for further analysis.

**Seeds collection**

Seeds of *Panicum repens* and *Panicum maximum* were obtained from the wild around Ahmadu Bello University, Zaria, Nigeria.

**Soil collection and pot preparation**

A mixture of sandy and loamy soils was collected from the botanical garden of Ahmadu Bello University, Zaria. Planting bags of 15 x 15cm were obtained from an Agrochemical store in Samaru Market Zaria, Kaduna State Nigeria. Planting bags were filled with 3kg of soil.

**Preparation of *S. cordifolia* extract**

One hundred grams (100g) of dried powder of leaves and root of *Sida cordifolia* were weighed and soaked in 1000 ml of 80% ethyl acetate into a conical flask then wrapped with paraffin and shaken on an orbital shaker at 25$^{o}$C for 2 hours (Aslani *et al*., 2013). The extracts were filtered through a cheesecloth, the residue was re-extracted again with 1000ml of ethyl acetate for 24 hours thereafter the two extracts were combined and filtrated using four layers of cheesecloth followed by Whatman No.1 filter paper. The stock was diluted appropriately with sterile distilled water to obtain different concentrations.

Five concentrations were prepared by adding distilled water to 10ml, 20ml, 40ml, 60ml, and 80ml of the stock solution to obtain 10%, 20%, 40%, 60% and 80% (L10, L20, L40, L60, L80, R10, R20, R40, R60, and R80) respectively for both roots and leaves extracts, while distilled water served as control (0%) respectively.

**Pot culture**

Seven (7) seeds of *Panicum repens* and *Panicum maximum* were separately sown in 3kg of soil with 3 replicates each. A total of 66 pots with sown seeds were subjected to the various concentrations. Pots were watered every 48 hours for five (5) weeks.

**Chemical analysis**

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and chlorophyll stability index were estimated following the procedure of Lichenthaler and Bushman (2001) and Amin (2012). Fresh leaves (0.2g) of each sample were ground using pure acetone (10 ml) in a glass bottle covered with aluminum foil and kept for 48 hours. The absorbance of the solution was measured at 663nm, 646nm, and 470nm and quantified using a spectrophotometer (Thermo Scientific™ GENESYS™ 150 UV-Visible) for the Chl *a*, Chl *b*, and total chlorophyll contents and expressed using the following relationships:

Chlorophyll *a* (µg/ml) = 12.7 ($A_{663}$) – 2.69 ($A_{645}$)
Chlorophyll *b* (µg/ml) = 22.9 ($A_{645}$) – 4.68 ($A_{663}$)
Total chlorophyll (µg/ml) = 20.2 ($A_{645}$) + 8.02 ($A_{663}$)

Chlorophyll stability index: $\frac{A_{652} \text{ of treated sample}}{A_{652} \text{ of the control sample}} \times 100$

The concentrations of available copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe) in the soils after application of *S. cordifolia* were determined using Atomic Absorption Spectroscopy (AAS) model number LAAS-210 after harvest. Colorimetric determinations of exchangeable acidity ions were conducted with the aid of a Beckman model DU Spectrophotometer according to the procedure outlined by Robertson (1950). About 20 g of dry soil was weighed and 100 mL of water was added to the soil in a conical flask. A glass rod was used to stir and was allowed to swirl in an orbital shaker for a few minutes. The flask was removed and the pH of the solution

was measured with a well-calibrated pH meter. This was measured before and after the experiment.

**Determination of stomatal density and stomatal index**

Leaves of the *Panicum repens* and *Panicum maximum* were collected and washed thoroughly with water and placed in sodium hypochloride for 1 hour and a 1mm square section of the upper and lower epidermis of the leaves was taken for the microscopic examination.

The Stomatal Index and number of stomata on both surfaces of leaves were carefully observed and recorded. The stomatal density was determined as the number of stomata per field of view of the leaf. The stomatal index was determined as the number of stomata per field of view divided by the number of stomata plus number of epidermal cells per field of view multiplied by 100 (Ruzin, 1999).

$$\text{Stomatal index} = \frac{S}{E + S} \times 100$$

Where S = donates the number of stomata per field of view and
E = the number of epidermal cells in the same field of view.

**Antioxidants enzyme determination**

An assay of Reactive oxygen species (ROS) was done according to the method of Jana and Choudhuri (1982) was employed for measuring internal $H_2O_2$ formation with some modifications. Assay of Peroxidase (POD) activity was assessed by the method of Reddy *et al*., (1995) with some modifications. An assay of Glutathione S-Transferase (GST) activity was performed by the method of Habig *et al*., (1974) with some modification. Assay of Lipid peroxidation (MDA) was determined by measuring the concentration of malondialdehyde (MDA) according to the method of Heath and Packer (1968).

**Data analysis**

Germination percentage was monitored on a daily basis. Shoot length, number of leaves, leaf breadth and leaf length was measured using a meter rule. Growth parameters were measured on weekly basis and experiment was terminated on the sixth week after planting.

Percentage inhibition was calculated using method by Sundra and Pote (1978). On termination of the field experiment, plants of garden crops and weed species were harvested, roots were washed thoroughly with water and oven dried at 60$^{o}$C for 72 hours and dry weights was determined using digital scale balance.

Percentage weed control efficiency was determined using the formular below:

$$\text{Weed control efficiency (\%)} = \frac{WDMc - WDMt}{WDMc} \times 100$$

Where: WDMc= weed dry matter of ontrol
WDMt= weed dry matter of treatment

All obtained results will be statistically analyzed to determine the degree of significance ($p \leq 0.05$) between the different treatments and plant variables. Results will be subjected to ANOVA and where there is significance, Duncan Multiple Range Test (DMRT) will be used to separate the means using R statistical analysis program version 4.2.2.

## RESULTS AND DISCUSSION

There was significant decrease in plant height, leaf number and leaf area of *Panicum maximum* and *Panicum repens* with application of different concentrations of root and leaf extracts of *S. cordifolia* compared to the control. The control gave the highest plant height, leaf number and leaf area in *Panicum maximum* and *Panicum repens* at 5 weeks after planting (figure 1, 2 and 3). *S. cordifolia* ethyl acetate root and leaf extract reduced plant height as the level of concentrations increased across species although the species respond independently. The inhibitory result of *S. cordifolia* on plant height, leaf number and leaf area of the weed species may be related to the presence of allelochemicals including aldehydes and some mono unsaturated fatty acids present in both the roost and leaf extracts of *Sida cordifolia*. Wasan (2020) stated that ethyl acetate extracts of corn, wheat and barley showed effect on plant height and leaf area, but had no effect on the number of leaves in *P. repens* with respect to high concentrations which may be due to the presence of allelopathic compounds which interfered with the various growth mechanisms and inhibited other physiological processes of plant growth. Ahmed *et al*. (2017) reported that high concentrations of methanolic extracts of *S. cordifolia* tend to influence the shoot height and leaf area of tomato and carrot plants.

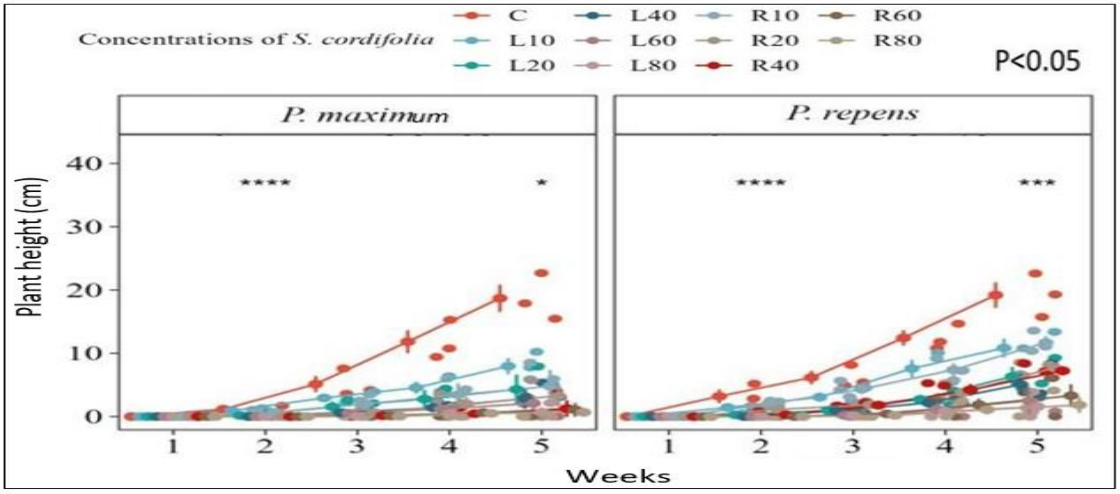


**Figure 1: Plant height of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

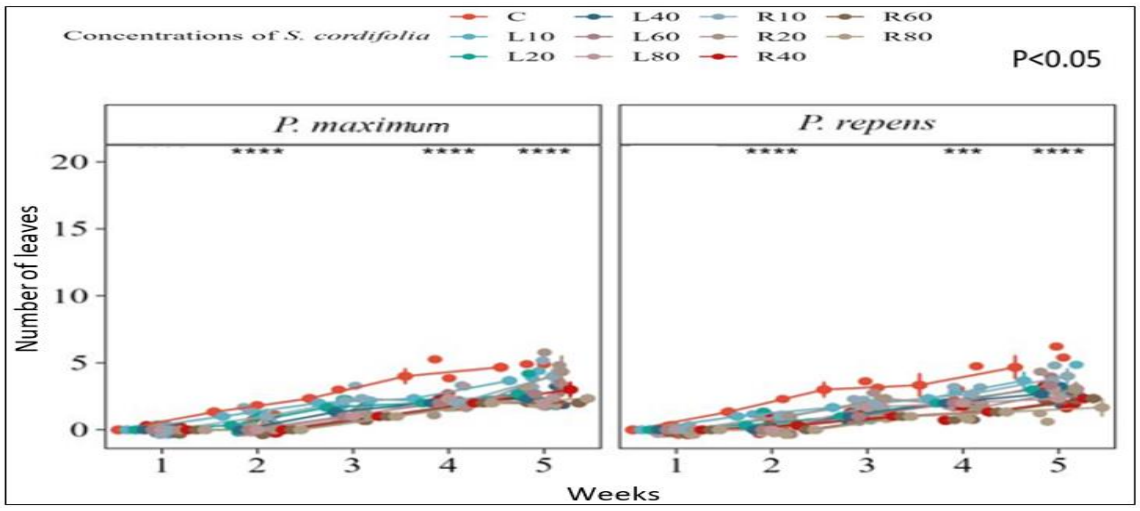


**Figure 2: Number of leaves of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

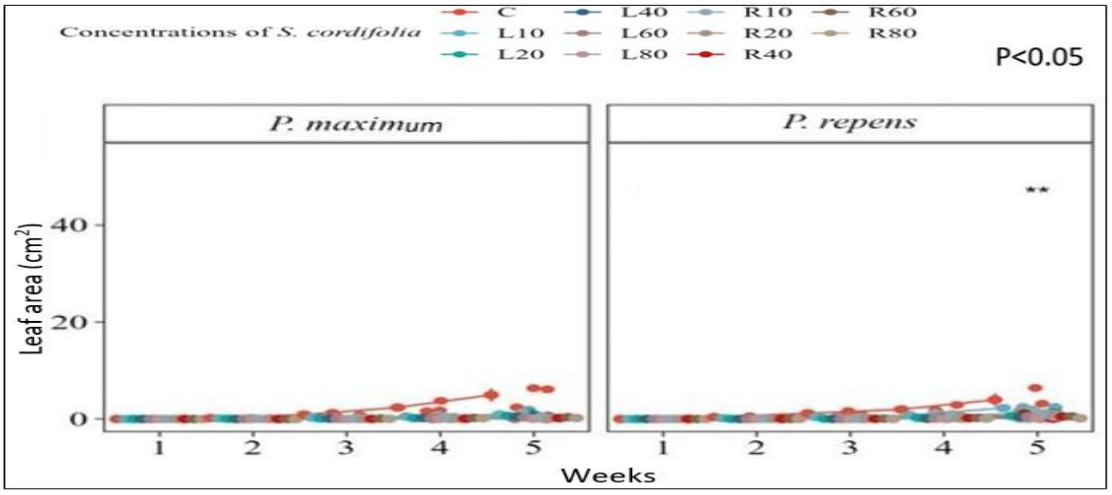


**Figure 3: Leaf area of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

As the concentration of *S. cordifolia* root and leaf extracts increased so the percentage inhibition and dry weight of *Panicum maximum* and *Panicum repens* significantly increased and decreased respectively. Least percentage inhibition and highest dry weight were noticed with the application of the lowest concentration of leaf extracts of *Sida cordifolia* (L10) on all the test plants (Figures 4 and 5). The inhibitory effect of organic solvent plant extract is generally stronger than that of water extract (Kazinci *et al*., 2013). Adewale *et al*. (2019) reported that ethyl acetate solvent tends to show a significant impact on the growth and inhibition of plants as it is able to extract vital active ingredients in plants. A similar result was reported in *Sida acuta* and *S. rhombifolia* (Stephen, 1983). Germination of the tested weed species was significantly (P <0.05) inhibited at all concentrations of the root and leaf extract of *S. cordifolia.* Our findings are by Ahmed *et al*. (2017), who tested the influence of *S. cordifolia* on the germination of carrots, tomatoes, and lettuce and found that the percentage inhibition and dry weight were greatly reduced as the concentration went higher.

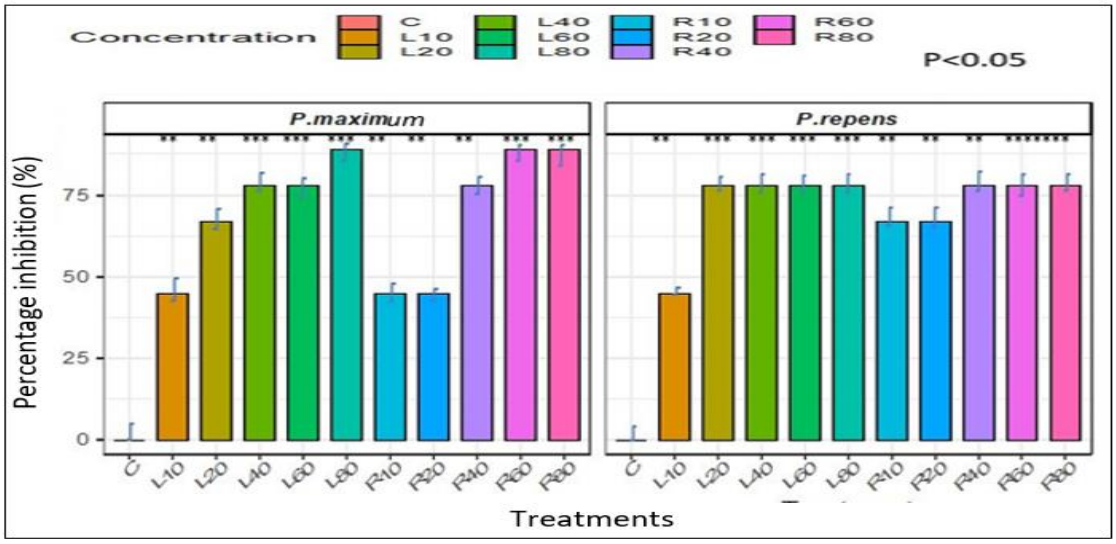


**Figure 4: Percentage inhibition of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

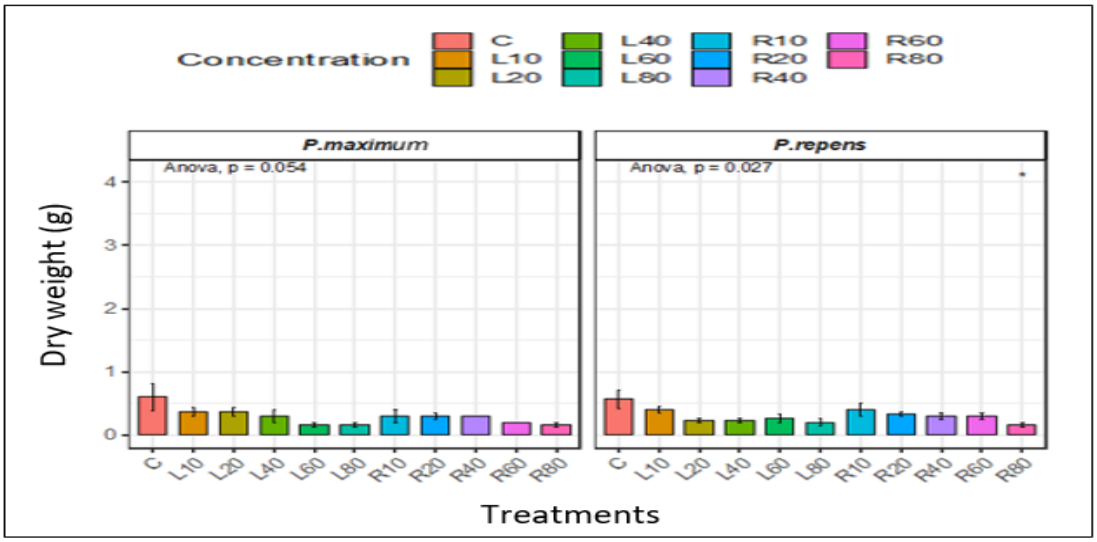


**Figure 5: Dry weight of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

There was significant difference in weed control efficiency in *P. repens* and *P. maximum.* Highest percentage weed control efficiency was found with application of L60, L80 and R80 of root and leaf extracts of *S. cordifolia* to the three weed species. *Panicum maximum* showed highest weed control efficiency (71.67%) compared to *Panicum repens* (figure 6). Our present findings present *Sida cordifolia* as an ideal candidate for the control of invasive species such as *Panicum repens* and *Panicum maximum.*

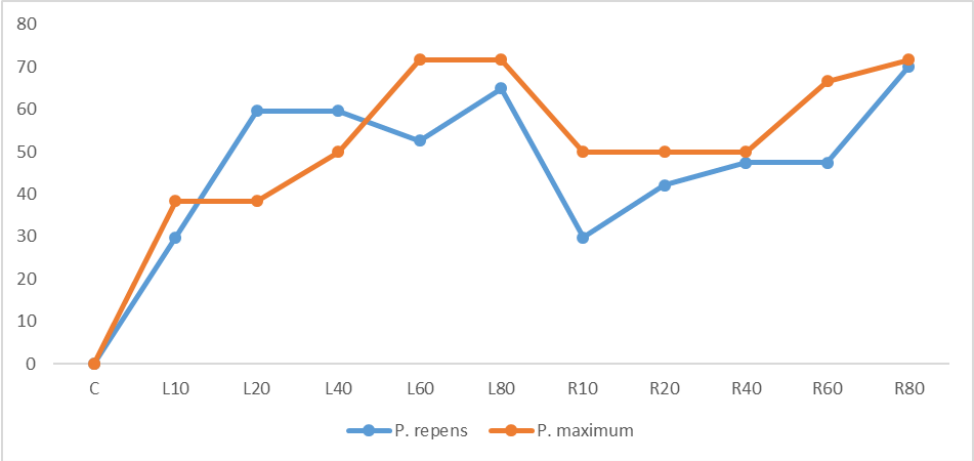


**Figure 6: Weed control efficiency percentage of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

There was no significant difference in stomata density and stomata index of the abaxial and adaxial surfaces of *P. maximum* and *P. repens* compared to the control as the concentration of *S. cordifolia* root and leaf extracts increases (figure 6, 7, 8 and 9). Stomata are small pores located on the leaf surface that allow plants to exchange gases with the environment. They play an essential role in the intake of $CO_2$ for photosynthesis, moisture regulation and temperature control, but at the same time, they allow water loss by transpiration (Gudesblat *et al.*, 2009).

Allelochemicals compounds have been reported to inhibit the growth of other plants through inhibition of cell division (Li *et al*., 2010). Despite the present results showed no significant decrease, but lower stomata index and density were noticed with higher concentrations of the leaf and root extracts of *S. cordifolia.* This result may lead to a decrease chloroplast activity in cells and can affect the synthesis of chlorophyll. Allelochemical affects the absorption of ion and water concentrations which then affect the opening of the stomata, the number of stomata and photosynthesis processes (Sharma *et al*., 2012).

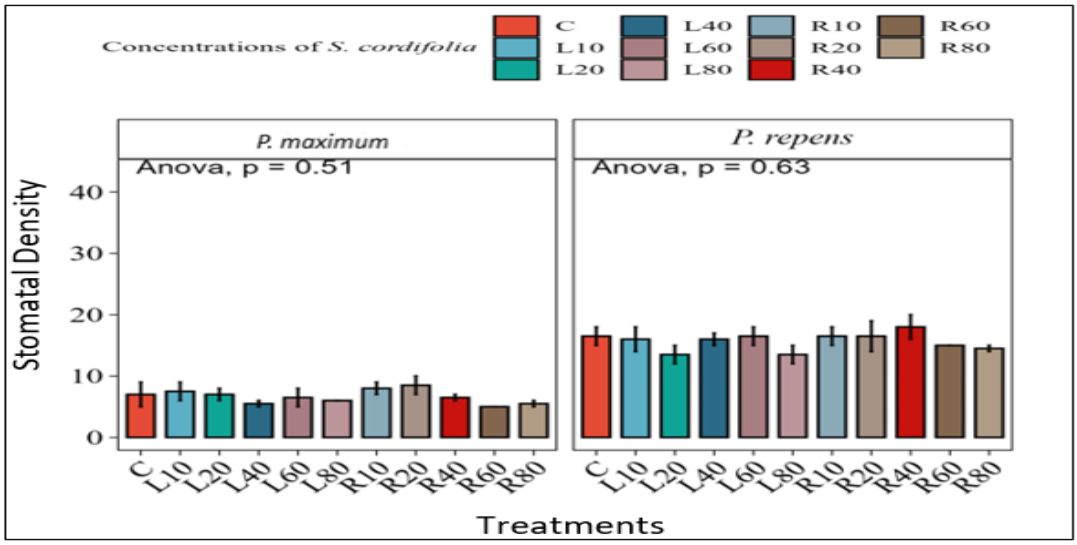


**Figure 6: Stomatal density of the abaxial surface of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

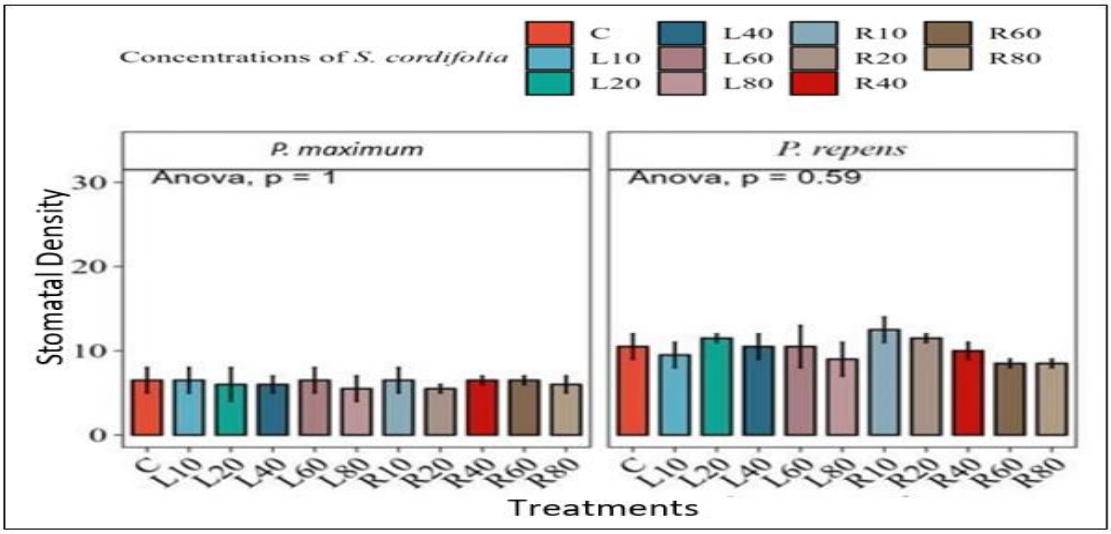


**Figure 7: Stomatal density of the adaxial surface of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

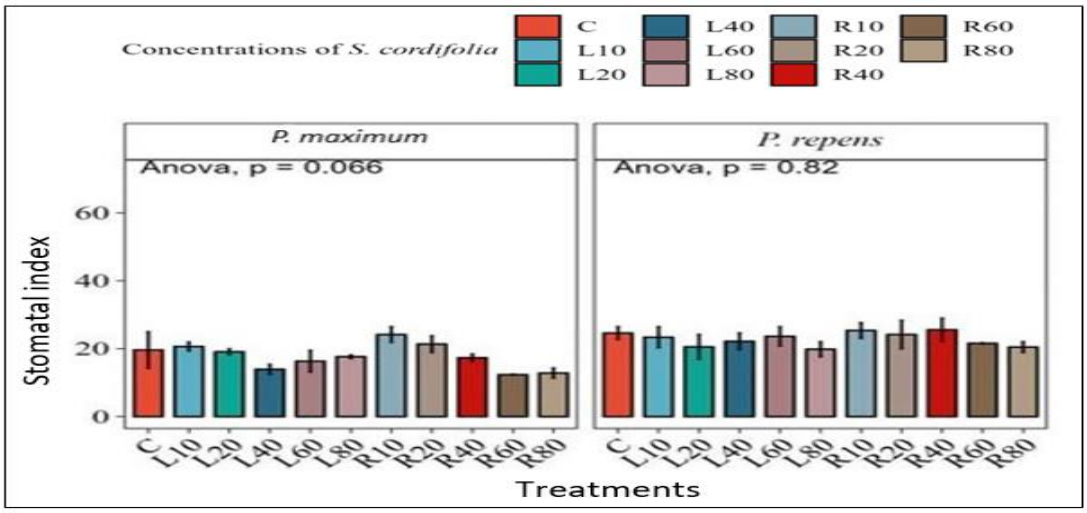


**Figure 8: Stomatal index of the abaxial surface of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

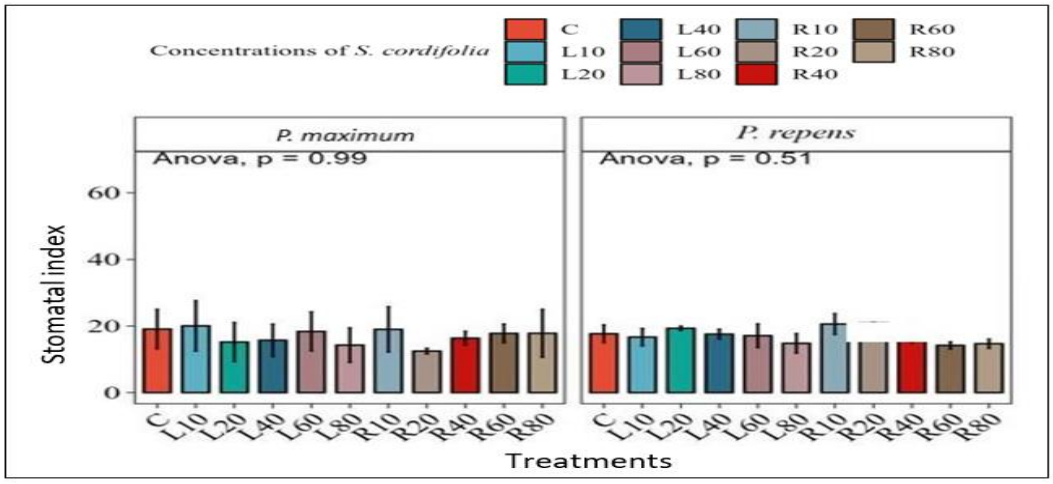


**Figure 9: stomatal index of the adaxial surface of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

There was no significant decrease in chlorophyll A, chlorophyll B, total chlorophyll and chlorophyll stability index in all the test plants with application of different concentrations of *S. cordifolia* root and leaf extracts compared to the control (figure 10, 11, 12 and 13). Chlorophyll is a determinant factor in photosynthesis, chlorophyll molecules play an important role in photosynthesis and serve as core element pigment embedded in photosynthetic membranes hence a chlorophyll reduction usually resulted to decrease in photosynthesis. This tallies with the findings of Ding *et al.* (2016) who observed high concentration of allelochemicals inhibits photosynthesis and plant growth by reducing chlorophyll content. Chlorophyll stability index is an indicator used to judge the tolerance of plant against stress (Mohan *et al.*, 2000). Several reports have shown that allelochemicals cause reduction in chlorophyll and inhibit photosynthesis processes (Liu *et al*., 2009; Abu-Romman *et al*., 2010; Ding *et al*., 2016).

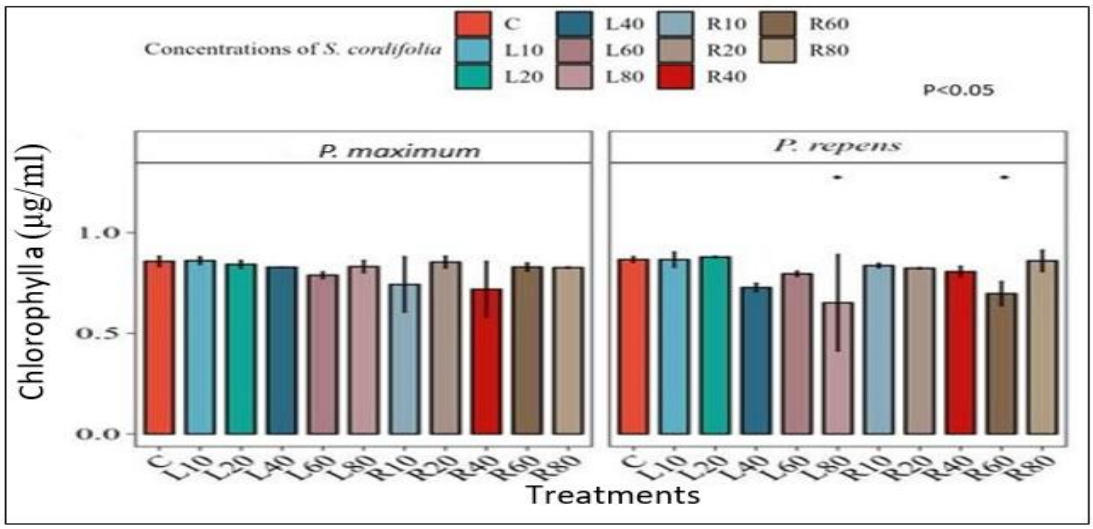


**Figure 10: Chlorophyll a of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

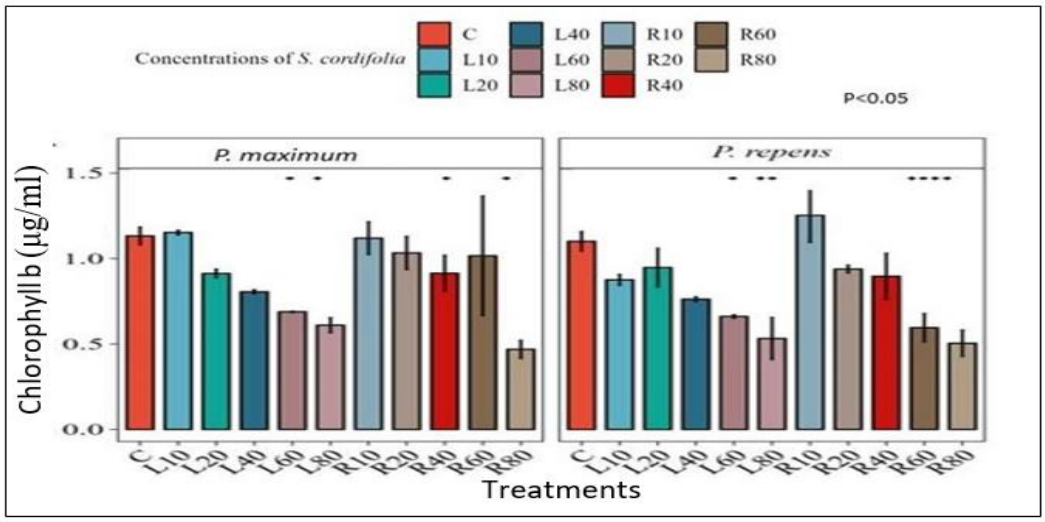


**Figure 11: Chlorophyll b of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

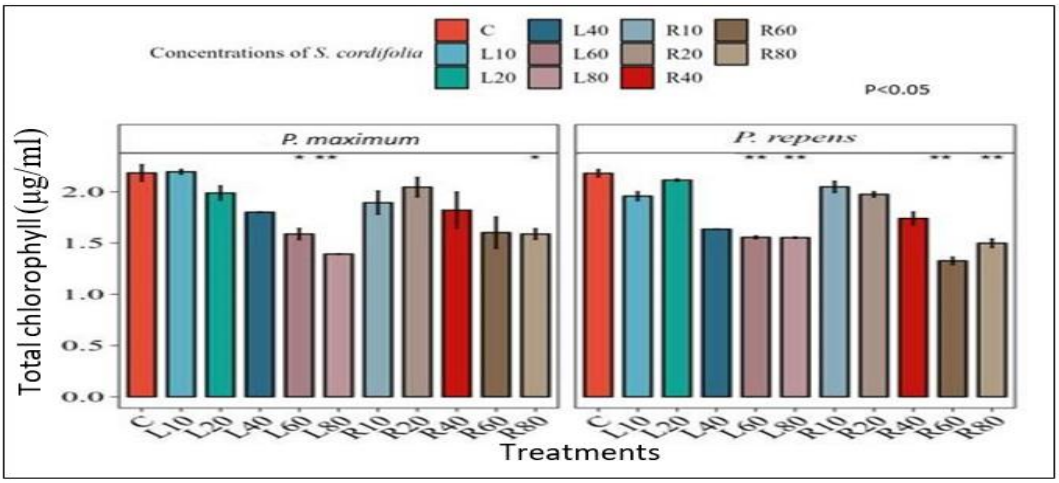


**Figure 12: Total chlorophyll of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

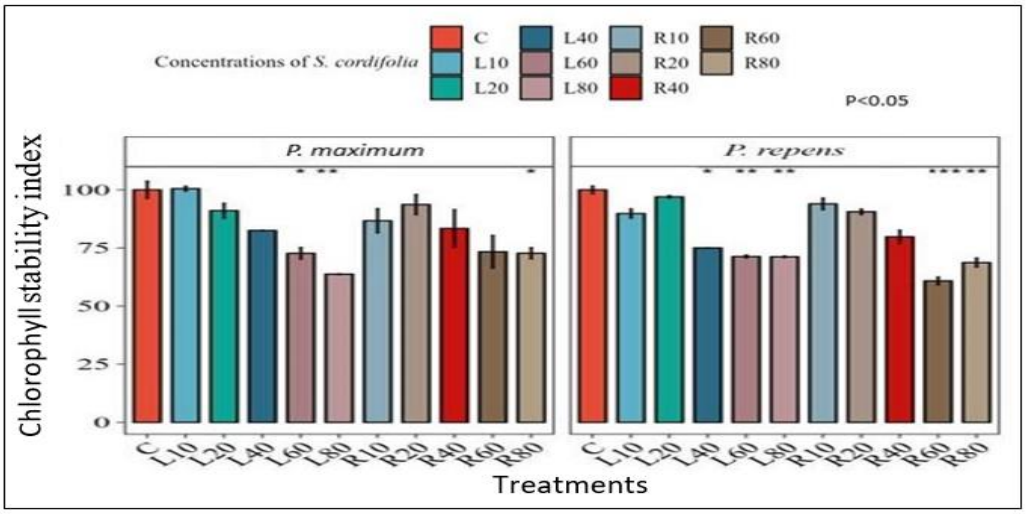


**Figure 13: Chlorophyll stability index of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

There was significant increase in peroxidase, glutathione s-transferase, malondialdehyde and reactive oxygen species levels in all the test plants with application of different concentrations of root and leaf extracts of *S. cordifolia. Panicum repens* showed more increase in the various anti-oxidants compared to *Panicum maximum* with the highest concentrations of the root and leaf extracts of *Sida cordifolia* giving the highest levels (figure 14, 15, 16 and 17). A reduction of peroxidase (POD) and activity causes a mass accumulation of active $O_2$ in plant leaves, which leads to membrane lipid peroxidation and results in destruction of membrane systems (Salah *et al*., 2019). One of the effects of allelochemicals on a target plant is uncontrolled production and accumulation of reactive oxygen species (ROS), which causes peroxidation of membrane lipids and membrane damage (Yu *et al*., 2003; Gniazdowska and Bogatek, 2005).

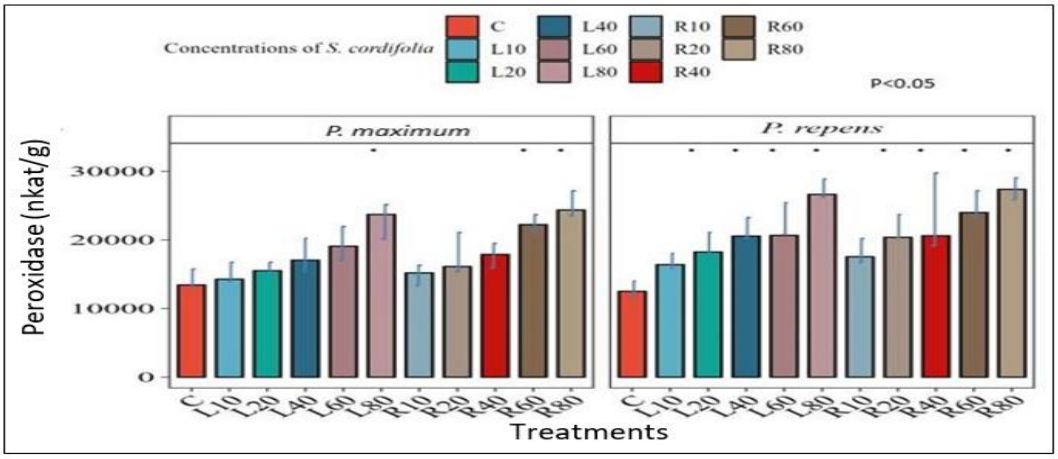


**Figure 14: Peroxidase levels of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

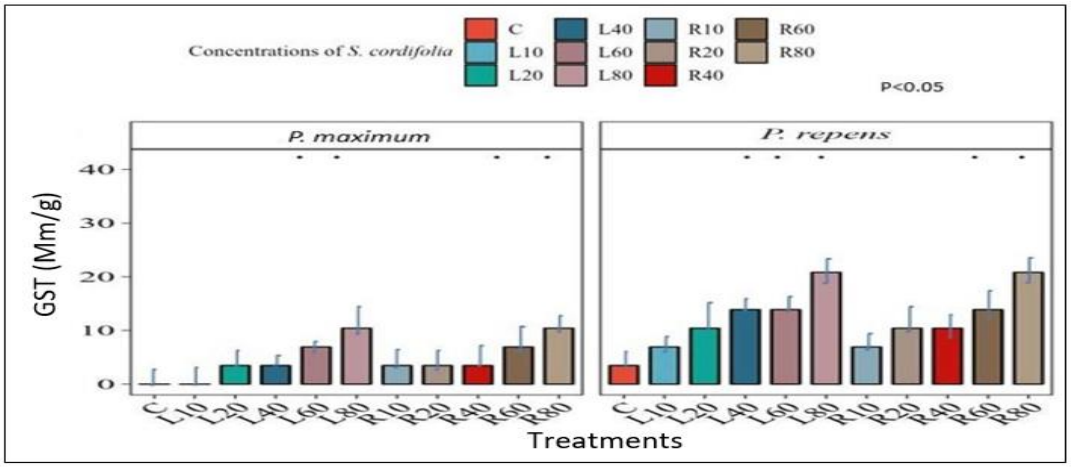


**Figure 15: Glutathione s-transferase levels of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

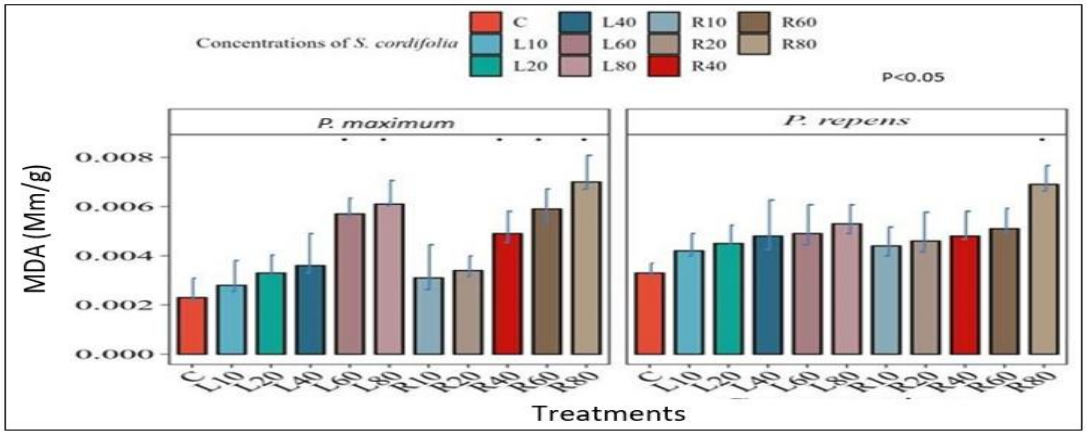


**Figure 16: Malondialdehyde levels of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

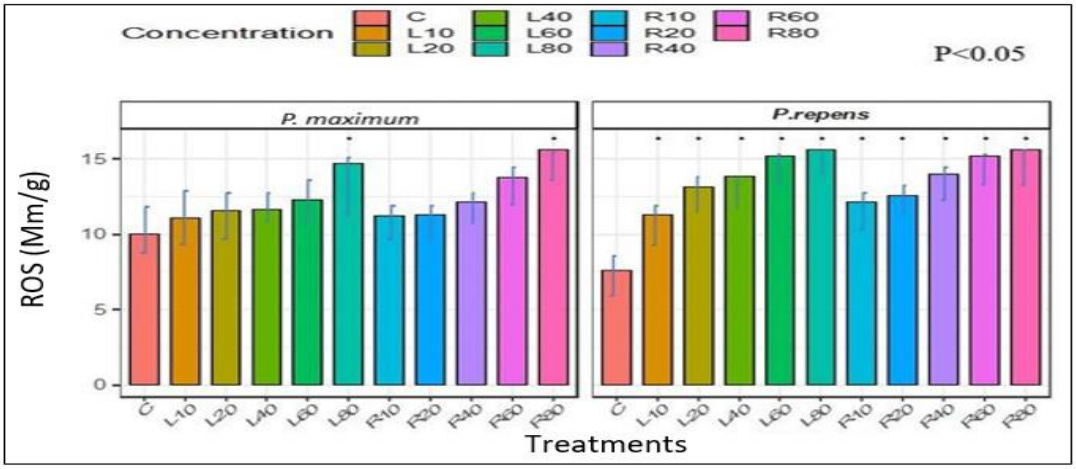


**Figure 17: Reactive oxygen species levels of *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

There was no significant decrease in iron (Fe), copper (Cu) and zinc (Zn) concentration, but a significant difference was noticed in manganese (Mn) concentration in the soil with application of higher concentration of *S. cordifolia* root and leaf extracts in all the test plants (figure 18, 19, 20 and 21). There was no significant decrease in acidity ion and pH of the soil after planting in all the test plants with application of root and leaf extracts of *S. cordifolia* (figure 22 and 23). Iron and manganese have been reported to play a vital role in the formation of the chloroplast in plants, while copper aids formation of vitamins and zinc is a component of enzymes (Sambo *et al*., 2017). The pH (figure and acidity ion (figure 8) showed no significant difference in all the soils after planting the test plants. Growth problems in plants can be caused by nutrient deficiencies, possibly due to the soil's pH. Optimal pH-levels are between 6 and 7. Low pH-levels (an acidic environment) stunt nutrient uptake. This is because $H^+$ ions have a negative effect on root growth, and subsequently, on nutrient uptake. Furthermore, low pH-levels hinder the availability of nutrients.

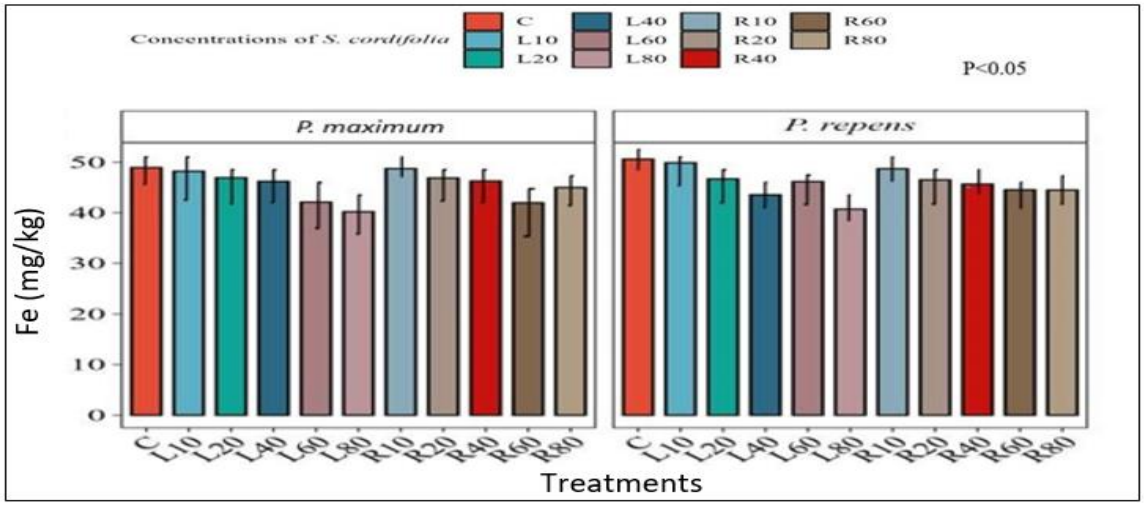


**Figure 18: Iron levels in the soil after planting *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

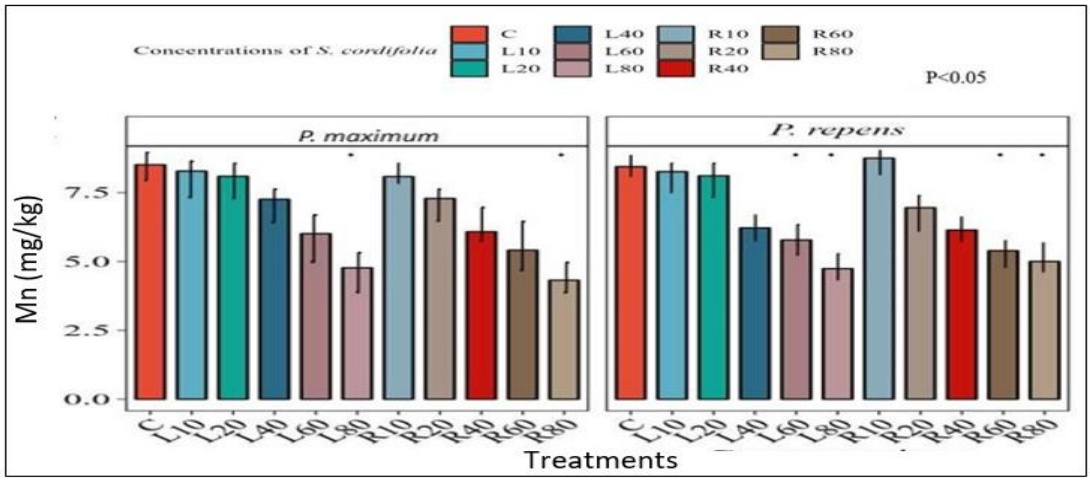


**Figure 19: Manganese levels in the soil after planting *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

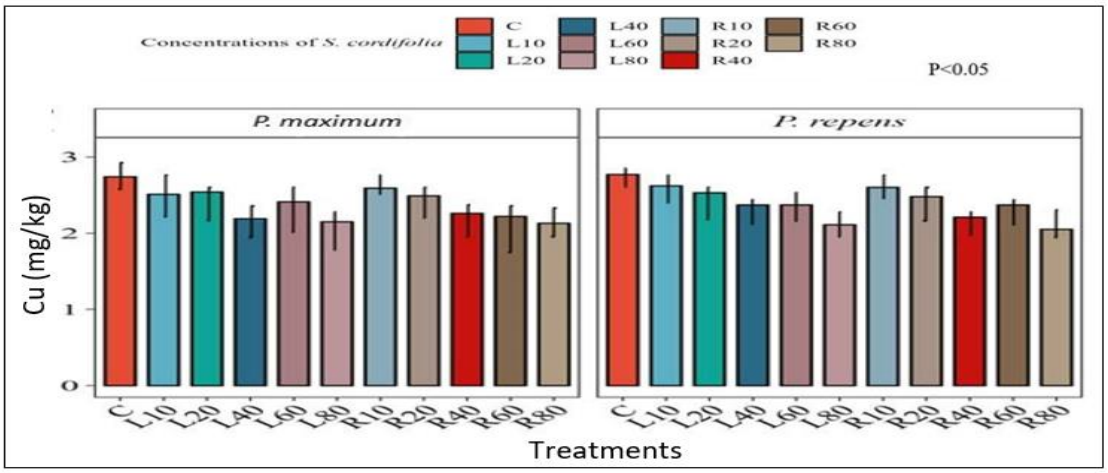


**Figure 20: Copper levels in the soil after planting *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

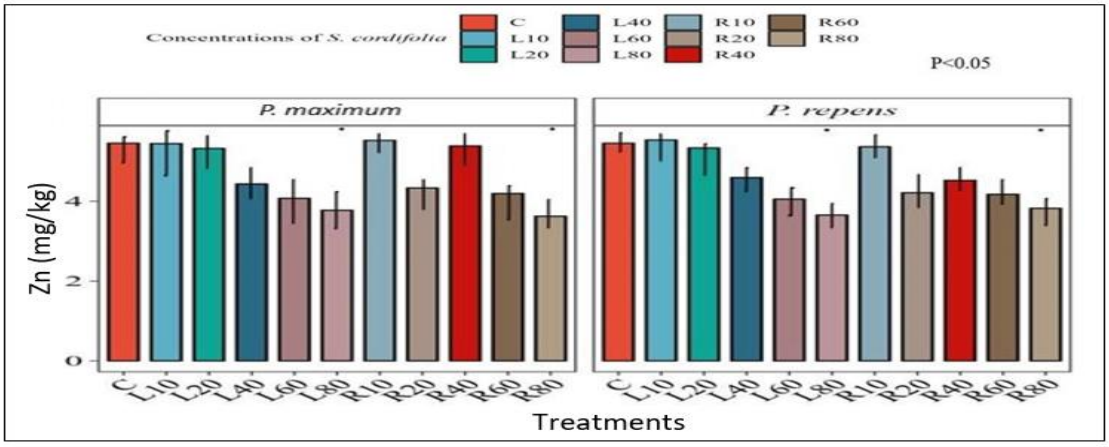


**Figure 21: Zinc levels in the soil after planting *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

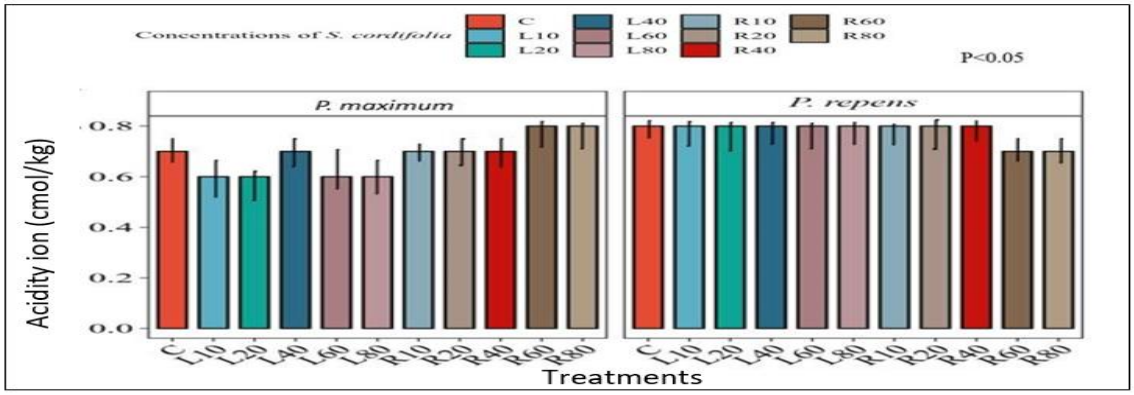


**Figure 22: Acidity ion of soil after planting *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

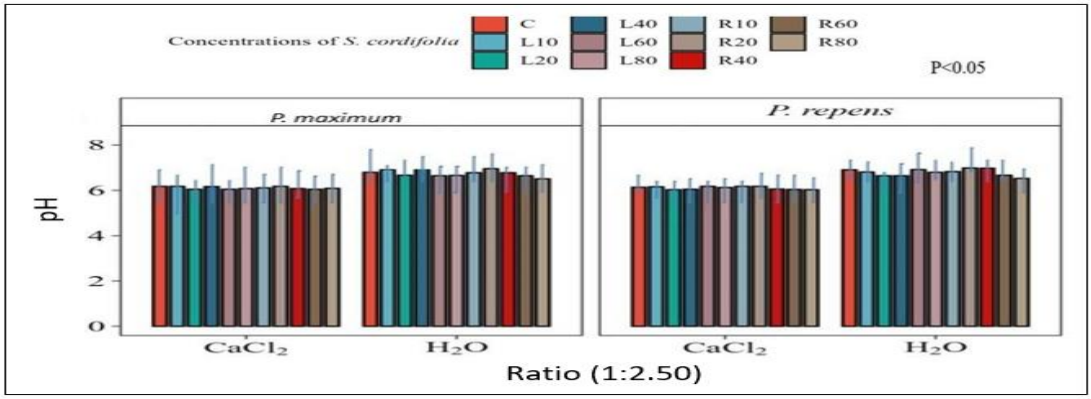


**Figure 23: pH levels of soil after planting *P. maximum* and *P. repens* under different concentrations of ethyl acetate root and leaf extracts of *S. cordifolia***

## CONCLUSION

The present study showed that *Sida cordifolia* inhibited the rate of growth in *Panicum repens* and *Panicum maximum* by reducing their plant height, leaf area and number, stomata index and density and also subjecting them to stress as a result of increasing their various antioxidant levels without having a negative impact on the soil properties. Foliar spray should be conducted on both monocot and dicot weed species to see which among is *Sida cordifolia* extracts is best effective on.

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