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Allelopathic effects of *Androstachys johnsonii* Prain on germination and growth of *Zea mays* L. and *Vigna subterranea* (L.) Verdc.

Mokganya Mokgaetji Georginah[1], Ligavha-Mbelengwa Hadzhi Maanda[2]

[1]Department of Science Foundation, University of Venda, Private Bag X5050, Limpopo Province, Thohoyandou, 0950, South Africa, [2]Department of Botany, University of Venda, Private Bag X5050, Limpopo Province, Thohoyandou, 0950, South Africa

molotja@univen.ac.za[1], watshemale@gmail.com[1], maanda.ligavha@univen.ac.za[2]

Abstract. Allelopathic effects of the donor plants are mostly regarded as harmful to target plants such as traditional crops. Traditional crops play pivotal role as staple food for rural communities and hence ensuring food security. Study of the allelopathic effects of *Androstachys johnsonii* Prain on the germination and growth of *Zea mays* L. and *Vigna subterranea* (L.) Verdc was conducted. Different plant leachates concentrations of 0.675%, 1.25%, 2.5% and 5% were prepared through serial dilutions. Germination bioassays were arranged in a complete randomized setup of three replicates for each treatment for seven days. Germination rates were calculated, radicle and hypocotyl lengths were also measured. Germination rates of *Z. mays* L. and *V. subterranea* (L.) Verdc. ranged from 50 - 100% and 18 - 76%, respectively. Significant differences of $P< 0.005$ were noticed from the germination rates of *V. subterranea (L.) Verdc* subjected to high concentrations of different extracts used. Statistically there was no significant differences between germination rates *of Z. mays L*. treated with all concentrations of leaf leachates against germination rates of the seeds treated with distilled water. Outstandingly, the germination rate and early growth of *Z. mays* L. were promoted by less concentrated extracts of leaf, root, stem bark and soil which then suggest future use of allelochemicals from *Androstachys johnsonii* Prain for promotion of maize plant productions. Based on the beneficial attributes of allelochemicals from *Androstachys johnsonii* on the germination and growth of *Z. mays* L., planting of *Androstachys johnsonii* Prain in maize fields is highly recommended.

Keywords. Allelopathy, *Androstachys johnsonii*, Food security, Rural communities, Seed germination, Staple Food, Traditional crops

1. Introduction

Allelopathy is defined as the direct harmful or beneficial effects of the donor plant on the target through the production of chemical compounds that escape into the environment.[1] It includes both detrimental and beneficial interactions between plants through chemical release by the donor. However, in practice, the term allelopathy is generally used to refer to detrimental plant -plant interactions.[2] Furthermore, Kohli et al.[2] alluded that allelopathy is exploited for weeds control in agricultural practices. Findings of other researches indicated allelopathy of tropical tree species as detrimental to germination and growth of paramount crop plant species.[3] Additionally, Abugre et al.[4] concur that inhibitory effects of allelochemicals pose a serious crop reduction.

Several weed and crop species are reported to possess negative allelopathic effects on growth of other plant species.[1] Normally chemicals with inhibitory characteristics are present in many plants more especially in many organs including leaves, flowers, fruits and buds.[5] Most importantly, clear knowledge of surroundings of tree species is vital for proper selection regarding farmlands tree species incorporation practices. Uncleared leaf litter of some plants from gymnosperms group qualifies the role played by allelochemicals in determining the understorey vegetation, hence maintenance of weeds or unwanted invasive plants.[6]

Zea mays L. is an important staple cereal crop that naturally accumulates provitamin A and carotenoids in the endosperm of seed.[7] Additionally, Phokane et al.[8] alluded maize plants as a staple food produced by subsistence farmers in rural areas of South Africa. According to Masekoameng and Molotja [9] inhabitants of the rural areas of the Limpopo province also consider maize as a staple food to assist combat food insecurity. Maize plant is found in Poaceae family, commonly known as the grass family.[10] This important crop is commonly referred to as Indian corn, corn or maize.[11]

Vigna subterranea (L.) Verdc. is a legume commonly known as Bambara groundnut or round beans.[12] It has various local names depending on different local languages.[12] For example, it is called " Phonda" in Tshivenda. *Vigna subterranea* (L.) Verdc. is related to cow pea and it belongs to the Fabaceae family. Its importance includes being a good source of essential amino acids, fatty acids and minerals such as Fe.[12] Naturally, Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is a nutritionally rich grain legume crop indigenous to Africa.[13]

Androstachys johnsonii Prain (Euphorbiaceae) is commonly known as Lebombo ironwood.[14] It is an evergreen tree with a maximum height of about 15m.[14] The evergreen characteristic provides important forage and shade for wildlife at a time of drought.[15] Results of the study conducted in the Makuya Nature Reserve, Limpopo Province, South Africa illustrated negative interaction between *Androstachys johnsonii* Prain and its understories (Molotja and Ligavha-Mbelengwa)[16] with the suspicions that it might be allelopathic. Numbers and weights of plant species growing under *Androstachys johnsonii* Prain were less as compared to understories of *Colophospermum mopane* (J. Kirk ex Benth.) J. Leornard. On the other hand, people of the far Limpopo Province rely on *Androstachys johnsonii* Prain for shade in their crop fields, therefore this might be dangerous to the wellbeing and production of their crops . The study looked at the allelopathic effects of leaf and root leachates of *Androstachys johnsonii* Prain on germination, plumule and radicle elongation of *Zea mays* L. and *Vigna subterranea* L. Verdc.

2. Materials and methods

2.1 Description of the study area

Leaf, stem bark, soil and root samples of *Androstachys johnsonii* Prain were collected from Thengwe (indicated with a blue arrow in Figure 1) and Makuya (indicated with an orange arrow in Figure 1) villages of the Vhembe District Municipality and the laboratory experiments were conducted at the University of Venda, School of Mathematical and Natural Sciences. The two study areas are found in the Vhembe District Municipality within the Vhembe Biosphere Reserve. Vhembe District is situated about 184 km north of Polokwane.[17] Thengwe Village is located at 650 m above the sea level.[17] Latitude and longitude of the Thengwe village are $22^0$40'22.325"S and $30^0$87'41.030" E respectively [18,19]. Soil type is characteristically darkish

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loamy to reddish brown and in other cases coarse clayey. Thengwe and Makuya are situated at about 50 km from Thohoyandou (Figure 1). The warmest period is from October to February with a cool climate period between April and August. The average temperature ranges from 30°C to 40°C.

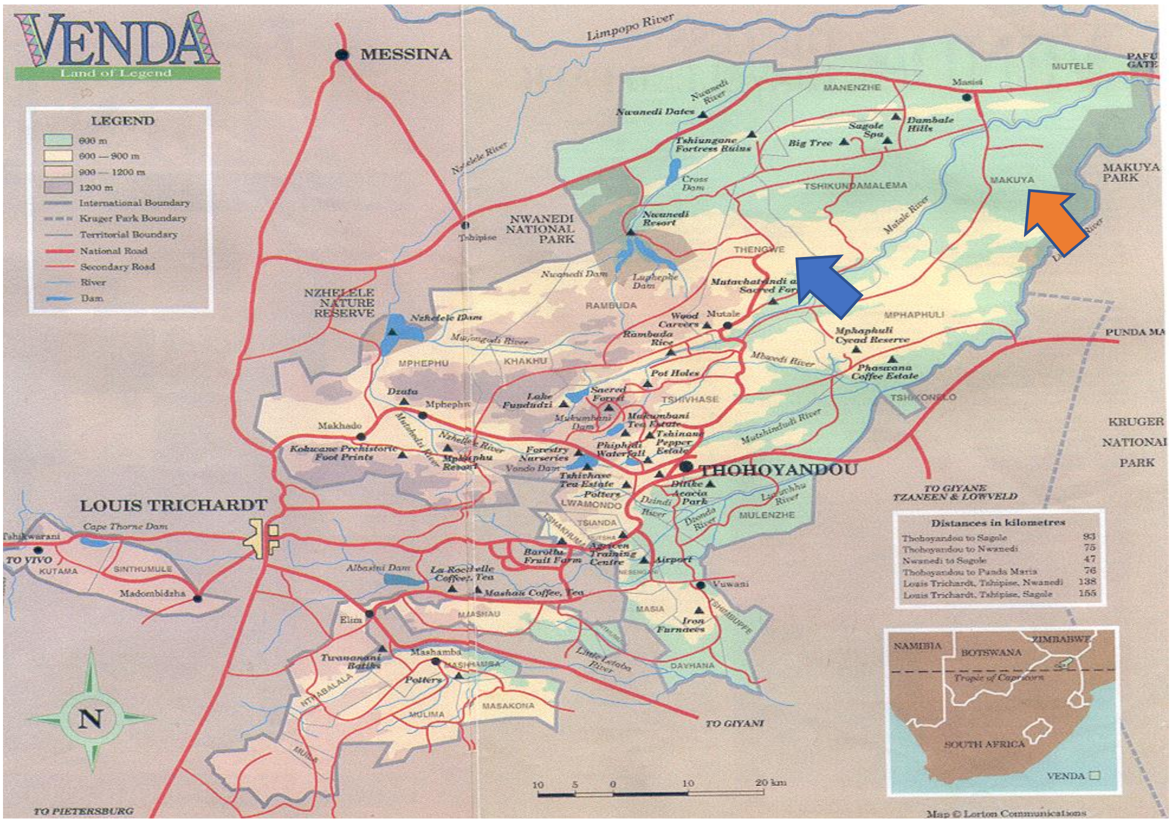

Figure 1: A map of Venda region showing two study areas (Lorton communications).

2.2 Sample collection

Since the plants in the study sites were clumpishly distributed, line and quadrat methods were used to select the trees for leaves and roots collection. These methods were thought suitable in randomizing the sampling. Spades and picks were used to excavate the roots and an axe was utilized to cut the roots where necessary. Defoliation was done manually. The collected samples were carefully cleaned and dried in the shade before crushed into fine powder with the pestle and mortar. Spades were used to collect soil from the selected *Androstachys johnsonii* Prain sites.

2.3 Extraction bioassay

Extract bioassays are simple, rapid and straightforward and can be used preliminarily to determine residue allelopathy for weed control or impacts of weed residues on crops. Aqueous extract bioassays have been widely employed to evaluate residue allelopathy of a suspected donor species. In this study, 20g dried powders of leaves, stem barks and roots of *A. johnsonii* Prain were added to 200 ml distilled water and kept for 48 hours at room temperature (25^0C).

The mixture was filtered and kept in the dark until used. Different concentrations were obtained by serial dilution: (5%, 2.5%, 1.25% and 0.675%).

2.4 Germination bioassay

In general, germination bioassays are conducted in petri dishes by placing seeds of receiver species on substrata (often filter paper) moistened with aqueous plant extracts of donor species [20,21,22]. Seeds of two selected crops, namely, *Zea mays* L. (maize) and *Vigna subterranea* (L.) Verdc. (Bambara groundnut) purchased from the local market were used as most cultivated field crops in the study areas. The seeds used in the study were steeped in water to determine their viability; those that floated were not used. Ten seeds each of *Zea mays* L. and *Vigna subterranean* (L.) Verdc. were placed in separate petri dishes (9 cm in diameter) lined with one layer of Whatman No.1 filter paper for germination. Ten milliliters of different prepared concentrations of leaf, stem bark, soil and root extracts of *Androstachys johnsonii* Prain were transferred each to petri dishes with ten seeds in three replicates. Replicates were placed in the Botany Laboratory of the University of Venda in a Randomized Complete Block Design (RCBD) layout [4]. In separate control series of three replicates seeds were saturated with 10 ml of distilled water. Germination of seeds of two selected crops was observed by checking emergence of radicle and therefore, number of germinated seeds was recorded, and germination percentages calculated while radicle and hypocotyl lengths were measured with a measuring tape after seven days. Seeds germination percentages for all treatments and the control were calculated by considering average of germinated seeds against the total number of seeds sown using the following formula (adopted from Mustafa et al. [23] with some modifications):

$$\text{Germination percentage}(\%) = \frac{\text{Average of germinated seeds per treatment}}{\text{Total number of seeds sown per treatment}} \times 100,$$

Where average was determined by total number of seeds germinated per replicate divide by number of replicates per treatment.

One ml of leachates and distilled water were added to the appropriated dishes daily up to the seventh day for the maintenance of the moisture in petri dishes. Data were subjected to one-way analysis of variance using STATISTICA software where significance was established ($p < 0.05$), the mean values were separated using Duncan's multiple range test.

3. *Results and discussion*

Germination of *Zea mays* L. and *Vigna subterranea* (L.) Verdc. varied across all the treatments employed (Figure 3 and 4). Aleem et al.[3] provided evidence that germination of Cowpea seeds planted both in the field and in pot experiment was significantly affected by the tree species which are allelopathic to their understories or neighbours. Further evidence attested availability of allelochemicals in all parts of plants, for example, in the alfalfa plant: they are found in leaves, stems, flowers, seeds and roots[24]. Similarly, results of the present study alluded availability of allelochemicals in leaves, stem bark and roots of *Androstachys johnsonii* Prain and from the soil collected from the *Androstachys johnsonii* Prain stand. Clearly, germination, elongation of radicles and elongation of hypocotyls of *Zea mays* L. and *Vigna subterranean* (L.) Verdc. were suppressed by the used parts. However, all ten seeds of *Zea mays* L. and nine *Vigna subterranea* (L.) Verdc. treated with distilled water germinated (Figure 2a and 2b).

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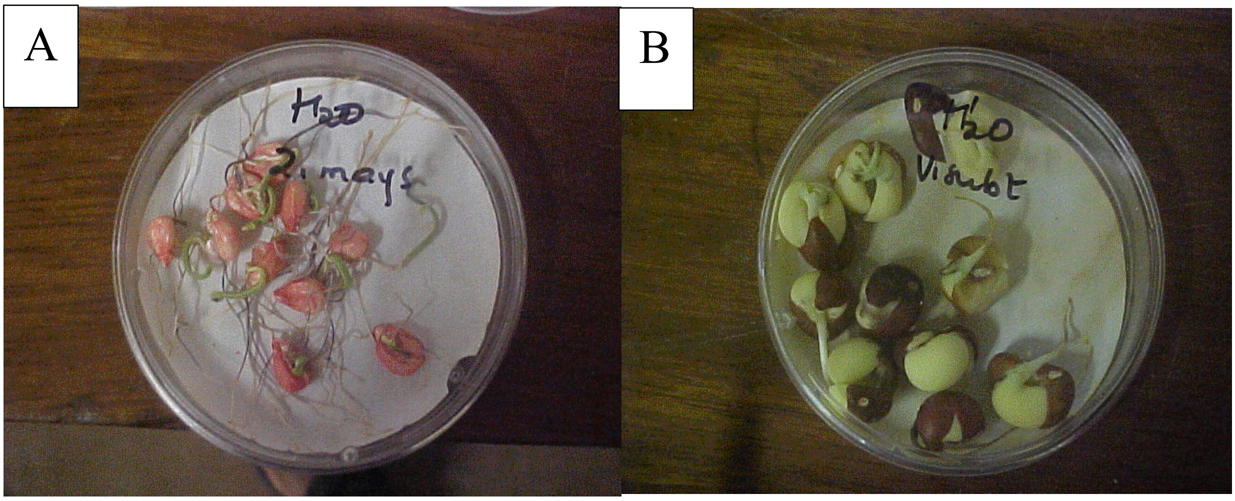

Figure 2: Control treatments of the germination bioassay.
Figure 2a is the control bioassay for *Zea mays* L. and Figure 2b is the control bioassay for *Vigna subterranea* (L.) Verdc. Note the germination and elongated radicles in both 2a and 2b.

3.1 Effects of leaf, root, stem bark and soil extracts on the germination of Z. mays L.

The results shown in figure 3 indicate that germination of *Z. mays* L. was slightly promoted by aqueous extracts of leaves, roots and stem bark of *A. johnsonii* Prain and aqueous extracts of soil collected under *A. johnsonii* Prain at the concentrations of 0.675 and 1.25. At the concentration of 2.5, the germination rate began to decrease. At the concentration of 5%, germination was significantly inhibited by the aqueous extracts of roots, stem bark and soil. Most remarkably, germination rate of *Z. mays L.* treated with all different concentrations of extracts ranged from 50 to 100%.

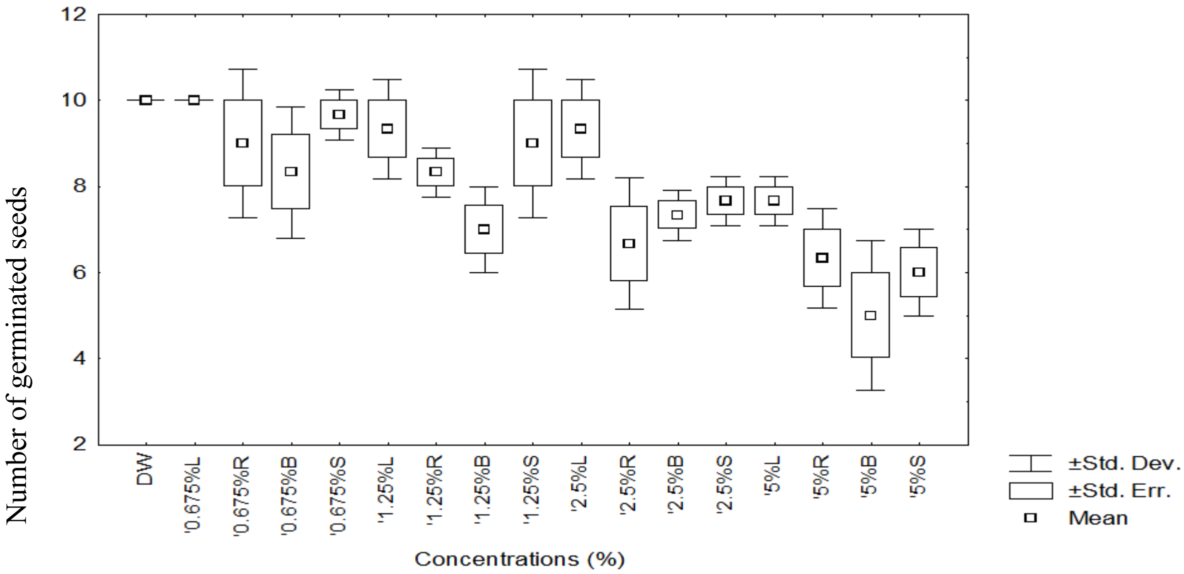

Figure 3: Number of germinated seeds of *Z. mays* L. Standard error is indicated by a ± standard deviations above and below the mean.

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At the concentration of 0.675, the germination rate of *Z. mays* L. treated with leaf and soil extracts was equal to that of the control, apart from root and stem bark extracts, which were slightly inhibitory. This suggests that leaf and soil extracts have the weakest inhibitory effect on germination of *Z. mays* L. Purification of allelochemicals and their derivatives can be employed as novel agrochemicals for sustainable management in an eco-friendly manner.[25]

3.2 Effects of leaf, root, stem bark and soil extracts on the germination of V. subterranea (L.) Verdc.

Extracts concentration of 0.675% from both leaf, root, stem bark and soil were not effective on the inhibitory of germination and growth of *Vigna subterranea* (L.) Verdc. The germination rates for other concentrations were as follows: 1.25% leaf extracts (65%), 1.25% root, stem bark and soil extracts (70%), 2.5% leaf and soil extracts (70%), 2,5% root extract (40%), 2.5% stem bark extract (60%), 5% leaf, stem bark and soil extracts (60%) and 5% root extract (20%) (Figure 4) .

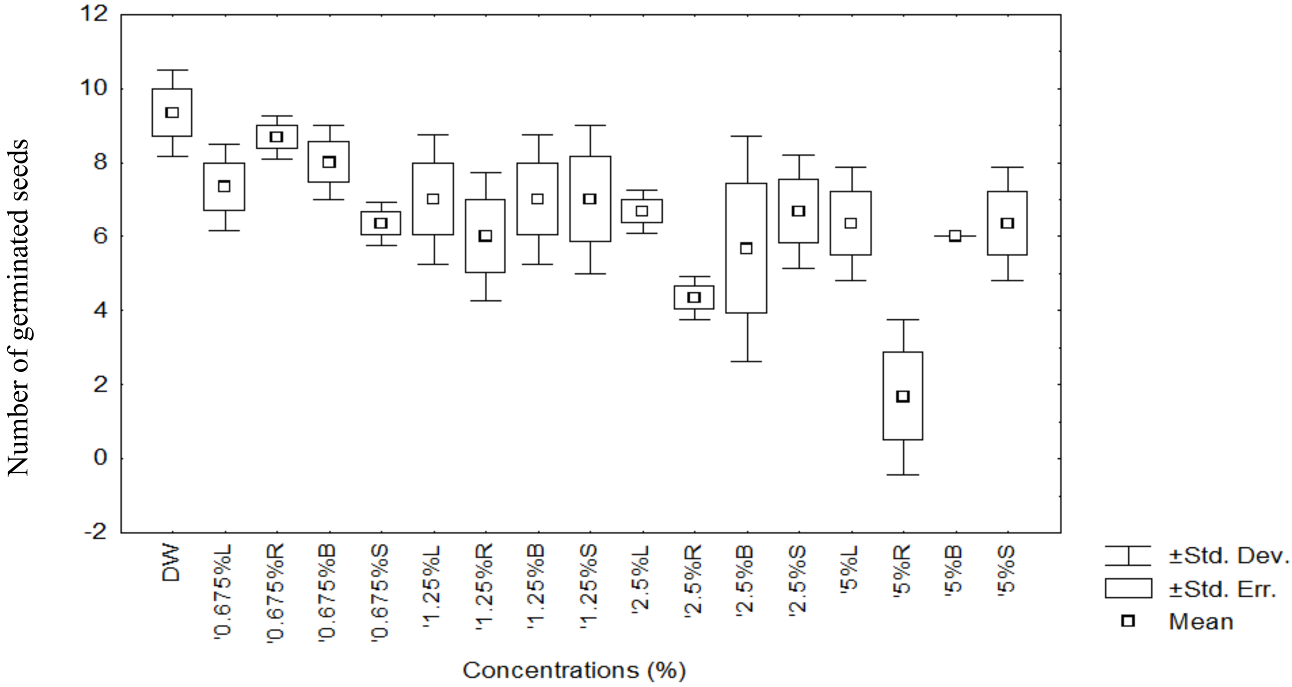

Figure 4: Number of germinated seeds of *V. subterranea* (L.) Verdc. Standard error is indicated by a ± standard deviations above and below the mean.

Findings from Dornbos et al.[26] concluded potential availability of allelochemicals from all parts of alfalfa. The similar trend was noticed with results of this study as extracts from all parts of *Androstachys johnsonii* Prain had some inhibitory potentials on the growth of *V. subterranea* (L.) Verdc.

Five percent root extract had the strongest inhibitory effect as compared to other treatments. Based on the results of Zubay et al.[27] a clear indication outlined that allelopathic effects of plants can vary from one concentration to the other.

3.3 Elongation of radicle and hypocotyl of Z. mays L.

As showed in figure 5, elongation of *Z. mays* L. hypocotyl was significantly inhibited in the concentrations 2.5% - 5% of aqueous extracts of root and stem bark of *A. johnsonii* Prain.

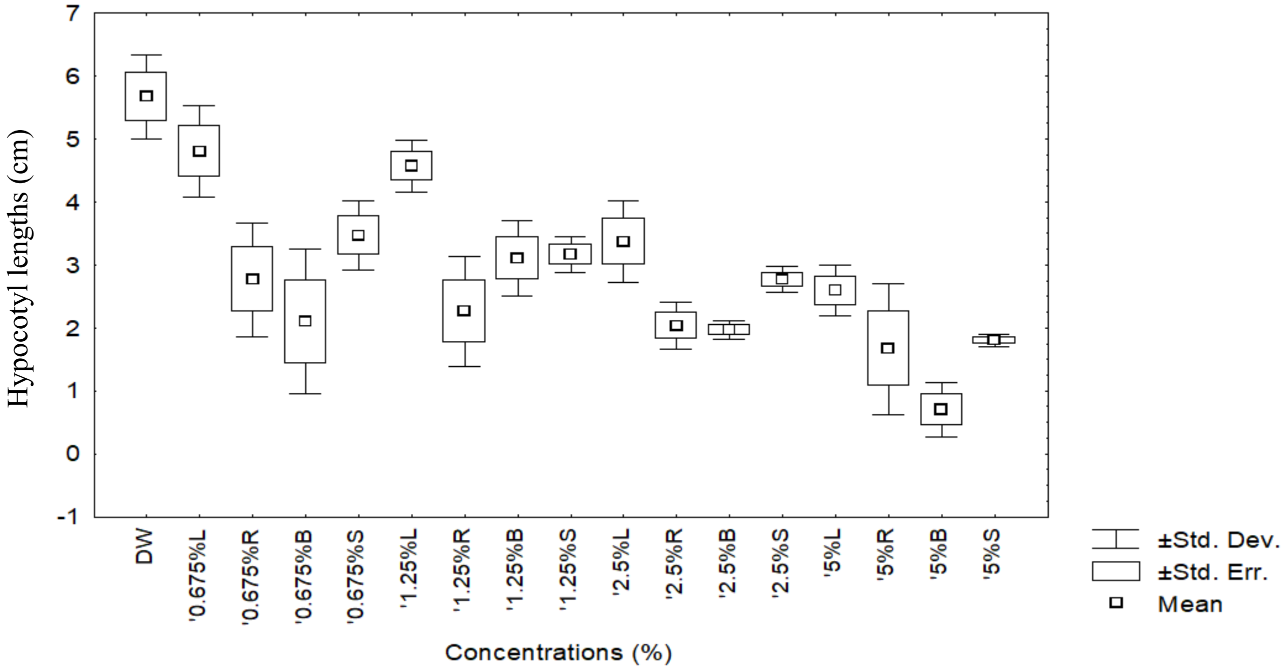

Figure 5: Lengths of *Z. mays* L. hypocotyl. Standard error is indicated by a ± standard deviations above and below the mean.

In concentrations 0.675 to 1.25% of both root, leaf, stem bark and soil aqueous extracts, elongation of hypocotyl was significantly promoted. In some instances, germination and seedling growth of species can be inhibited by different concentrations applied whereas on the other hand for some plants germination and growth can be enhanced.[28] From these results, it is apparent that lesser concentrations of allelochemicals contained in roots, leaves, stem bark and soil had the smallest inhibitory effects whereas the higher concentrations had the greatest inhibitory effects on elongation of *Z. mays* hypocotyls.

Elongation of *Z. mays* L. radicle responded like *Z. mays* L. hypocotyl. As the extracts become less concentrated, the inhibitory effects also become less. As depicted in figure 6, the aqueous extracts of root and stem bark seem to have strong inhibitory effect on the elongation of *Z. mays* L. radicle for almost all concentrations. This might be an indication that root and stem bark of *A. johnsonii* Prain have certain allelochemicals that cause great harm to the elongation of *Z. mays* L. radicle and are not present in other parts.

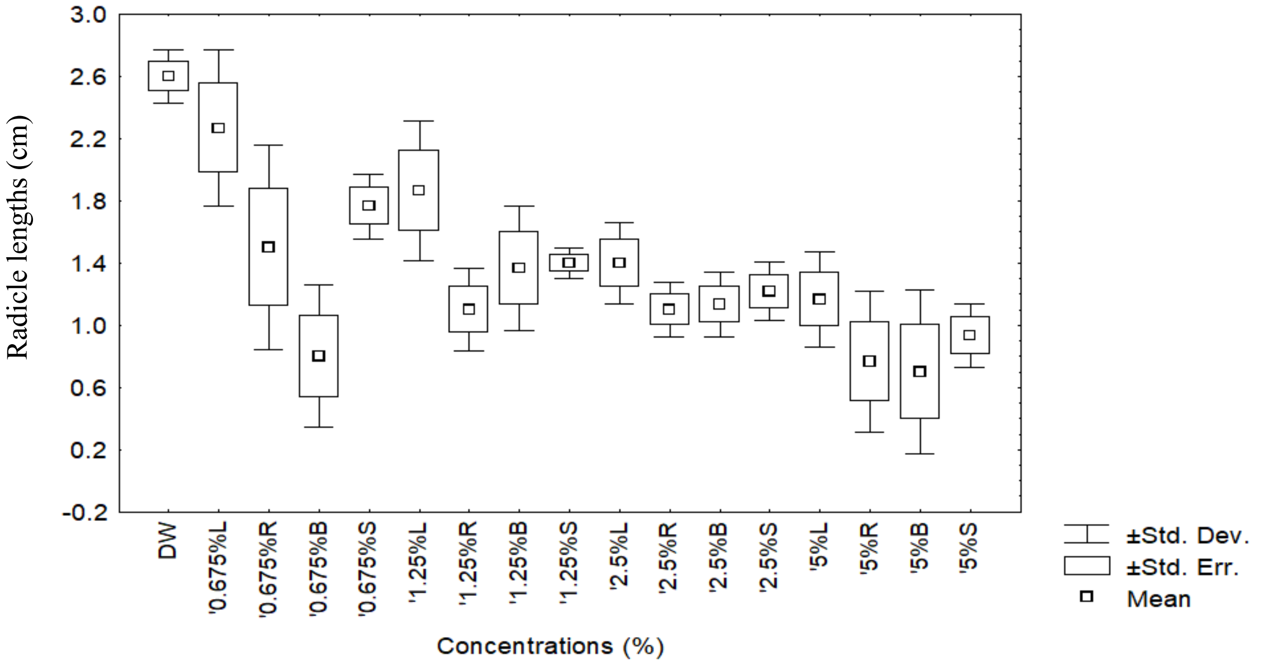

Figure 6: Lengths of *Z. mays* L. radicle. Standard error is indicated by a ± standard deviations above and below the mean.

3.4 Elongation of radicle and hypocotyl of V. subterranea (L.) Verdc .

Figures 7 and 8 report the elongation of radicle and hypocotyl of *V. subterranea* (L.) Verdc.

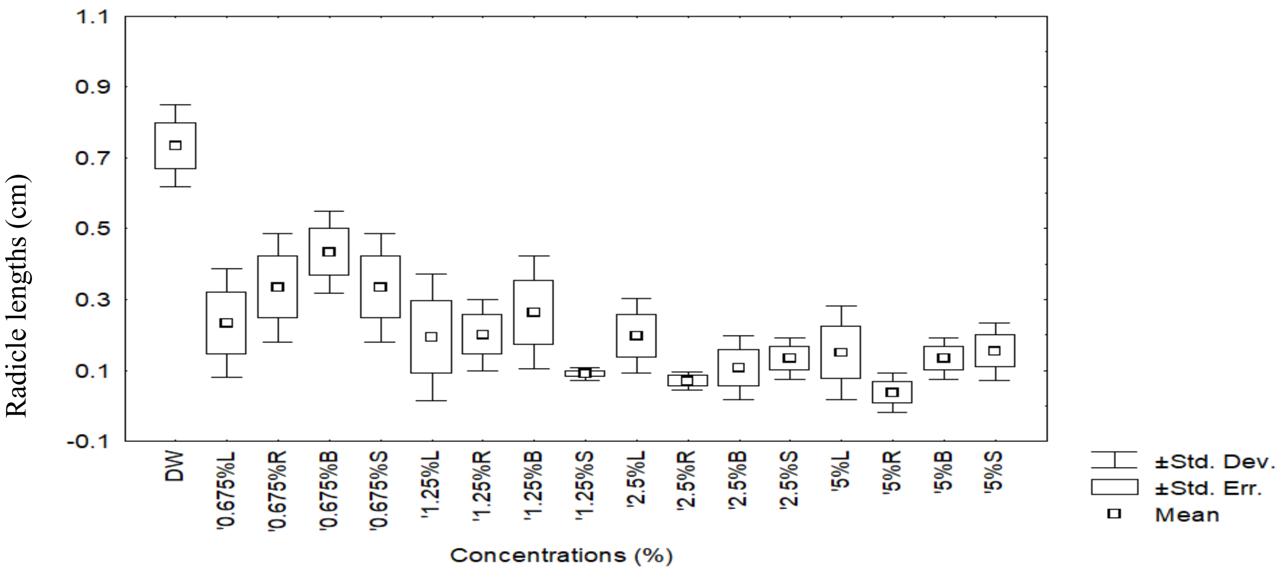

Figure 7: Lengths of *V. subterranea* (L.) Verdc. radicle. Standard error is indicated by a ± standard
deviations above and below the mean.

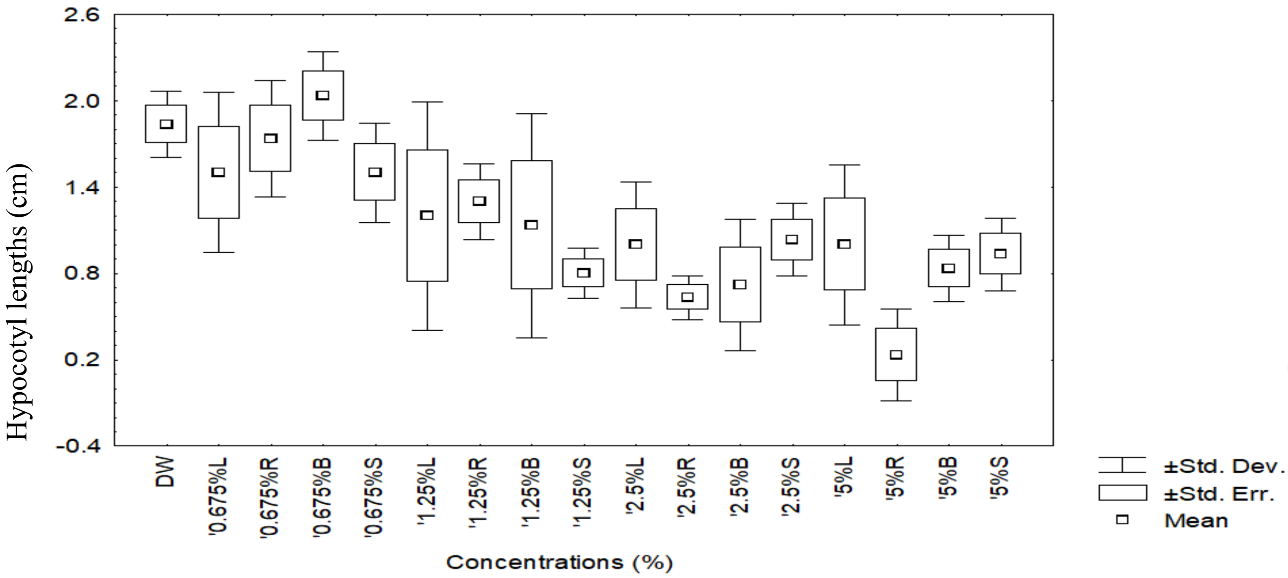

Figure 8: Lengths of *V. subterranea* (L.) Verdc. hypocotyl. Standard error is indicated by a ± standard deviations above and below the mean.

It was clearly indicated in the figure 7 that elongation of *V. subterranea* (L.) Verdc. radicle was drastically affected by almost all concentrations (i.e. from 0.675% - 5%) of root, leaf, stem bark and soil extracts. Even though seeds of *V. subterranea* (L.) Verdc. germinated, however, extracts of the different parts of *Androstachys johnsonii* Prain might have possessed allelochemicals which affected the enzymes responsible for plant hormone synthesis[29] ,thus resulting in hindering of roots and shoots development. However, of the four extract concentrations, 2.5% and 5% extracts of root, stem bark and soil significantly inhibited the elongation of radicle of *V. subterranea* (L.) Verdc. Nevertheless, elongation of *V. subterranea* (L.) Verdc. hypocotyl behaved in the same manner as the hypocotyl of *Z. mays* L. At higher aqueous extract concentrations, elongation was inhibited, but as the concentrations were ameliorated, elongation was promoted. At concentration of 0.675% of stem bark, elongation of hypocotyl was even more than that of the control. This suggests that elongation of *V. subterranea* (L.) Verdc. hypocotyl was strongly promoted at 0.675% of stem bark aqueous extract.

These results suggest that elongation of both *Z. mays* and *V. subterranea* (L.) Verdc. radicles were more sensitive to the allelopathic effects of *A. johnsonii* than germination. Moreover, degrees of growth promotion and inhibition produced by aqueous extracts of *A. johnsonii* differed with plant parts differences, suggesting that activation of allelochemicals in *A. johnsonii's* parts varied greatly, to produce targeted effects. Even though seeds of *V. subterranea* (L.) Verdc. managed to germinate in all concentrations of root, stem bark, leaf and soil leachates, but elongation of radicles was significantly ($p<0.05$) inhibited (Table 1). Essentially, these results are comparable to findings of other researches[30] in a sense that some concentrations of extracts significantly inhibited early growth of some treated plant species.

Statistical results on comparisons between responses of crops treated with distilled water and various concentrations of root, stem bark, soil and leaf leachates of *A. johnsonii* Prain are outlined in Table 1. There is no significant difference between the germination rate of *Z. mays* L. treated with distilled water versus all concentrations of leaf, root, stem and soil leachates,

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except the germination rate of *Z. mays* L. treated with 2.5% to 5% root leachates, 5% bark leachates and 5% soil leachates (Table 1 group A). Table 1 (group B) shows significant differences between germination rate of *V. subterranea* (L.) Verdc. treated with distilled water and various treatments of leachates of other different parts of *A. johnsonii*. There is no significant difference between

V. subterranea (L.) Verdc. treated with distilled water versus 0.675% root leachates, bark leachates, soil leachates, 1.25% - 2.5% leaf leachates, bark leachates, soil leachates and leaf leachates. Significant differences were found between *V. subterranea* (L.) Verdc. treated with distilled water versus 0.675% and 5% leaf leachates, 1.25%, 2.5% and 5% root leachates, 2.5% and 5% bark leachates, 2.5% and 5% soil leachates.

Table 1: One-way analysis of variance of seed germination, lengths of radicles and hypocotyls of *Z. mays* L. and *V. subterranea* (L.) Verdc. P < 0.05. SS refers to sum of squares, df to degree of freedom and MS refers to mean of squares.

Treatments	SS	df	MS	F-VALUES	P
No. of germinated seeds of *Z. mays* L.	107.25	16	6.70	5.34	0.00
No. of germinated seeds of *V. subterranea* (L.) Verdc.	137.41	16	8.59	3.77	0.00
Radicle lengths of *Z. mays* L. seedlings	12.99	16	0.81	6.24	0.00
Radicle lengths of *V. subterranea* (L.) Verdc. seedlings	1.34	16	0.84	6.56	0.00
Hypocotyl lengths of *Z. mays* L. seedlings	75.12	16	4.69	11.63	0.00
Hypocotyl lengths of *V. subterranea* (L.) Verdc. seedlings	10.43	16	0.65	3.59	0.00

Elongation of radicle of *Z. mays* L. treated with distilled water differed significantly with elongation of radicle of *Z. mays* L. treated with all leachate treatments except of those treated with 0.675% leaf and bark leachates (Table 2). Radicles of *V. subterranea* (L.) Verdc. were significantly affected by allelochemicals found in leaf, root, bark and soil from *A. johnsonii* canopy. This is supported by the fact that there are significant differences between the radicle length of *V. subterranea* (L.) Verdc. treated with distilled water versus all treatments (Table 2).

Hypocotyl elongation of *Z. mays* L. was favored by 0.675% and 1.25% leaf leachates only and all other treatments were found to be significantly inhibitory. Unlike in *Z. mays* L., hypocotyls of *V. subterranea* (L.) Verdc. were favored by 0.675% leaf, root, bark, soil leachates and 1.25% root leachates. All other treatments were significantly inhibitory.

Table 2: Duncan multiple range test analysis of seed germination, lengths of radicles and hypocotyls of *Z. mays* L. and *V. subterranea*. A, B, C, D, E and F refer to germination of *Z. mays* L., germination of *V. subterranea* (L.) Verdc. radicle of *Z. mays* L. radicle of *V.*

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subterranea (L.) Verdc., hypocotyl of *Z. mays* L. and hypocotyl of *V. subterranea* (L.) Verdc. respectively. DW refers to distilled water, LL to leaf leachates, RL to root leachates, BL to bark leachates and SL refers to soil leachates.

CONTROL VS TREATMENTS	**GROUPS**						**REPLICATES**
	A	**B**	**C**	**D**	**E**	**F**	**(n)**
DW VS 0.675% LL	ns	*	ns	*	ns	ns	3
DW VS 0.675% RL	ns	ns	*	*	*	ns	3
DW VS 0.675% BL	ns	ns	ns	*	*	ns	3
DW VS 0.675% SL	ns	ns	*	*	*	ns	3
DW VS 1.25% LL	ns	ns	*	*	ns	*	3
DW VS 1.25% RL	ns	*	*	*	*	ns	3
DW VS 1.25% BL	ns	ns	*	*	*	*	3
DW VS 1.25% SL	ns	ns	*	*	*	*	3
DW VS 2.5% LL	ns	ns	*	*	*	*	3
DW VS 2.5% RL	*	*	*	*	*	*	3
DW VS 2.5% BL	ns	*	*	*	*	*	3
DW VS 2.5% SL	ns	*	*	*	*	*	3
DW VS 5% LL	ns	*	*	*	*	*	3
DW VS 5% RL	*	*	*	*	*	*	3
DW VS 5% BL	*	*	*	*	*	*	3
DW VS 5% SL	*	*	*	*	*	*	3

***** = significant difference between DW and treatments, ns = not significant

Germination and growth of *Z. mays* L. treated with leaf leachates of *A. johnsonii* were promoted as compared to leachates of other parts of *A. johnsonii*. The germination rate, elongation of radicle and hypocotyl of *Z. mays* L. treated with leaf leachates were not inhibited compared to those of *Z. mays* L. treated with other leachates. Germination of seeds and early growth of studied crops, more specially *V. subterranea* (L.) Verdc. varied from lowest to highest extract concentrations. According to Ghebrenhiwot et al.[30], germination of plant species treated with the highest concentrations can be hindered whereas lower extracts concentrations brings up promotion in germination and early growth of certain plant species. The fact that *Z. mays* L. may be tolerant to the allelochemical materials should not be ignored because responses of plants to allelochemical materials differ from species to the other due to their genetic make-up.[1] On the other hand, roots of *V. subterranea* (L.) Verdc. are non-tolerant to the allelochemicals contained in *Androstachys johnsonii* Prain, hence the inability of *V. subterranea* (L.) Verdc. to thrive under canopies of *A. johnsonii* Prain in crop farming fields. Consumption and utilization of Bambara groundnut in Sub-Saharan countries for its taste, satiating and nutritional benefits[31] must not be ignored, therefore awareness campaign to farmers from the study areas is necessary.

Conclusion

It is known that seeds radicles and seeds hypocotyls develop into roots and shoots of plants. From observations made in this study, it is apparent that *A. johnsonii* Prain leaf leachates do not inhibit germination and growth of *Z. mays* L. This is supported by the fact that there was no significant difference between germination, the elongation of radicle and hypocotyl of *Z. mays* L. treated with 0.675% leaf leachates and those treated with distilled water. These findings then afford notion of sureness to maize plant farmers to continue using *Androstachys johnsonii* Prain for shade on conditions that watering of their crops becomes intense to reduce toxicity of the leachates.

Roots growth of *V. subterranea* (L.) Verdc. was negatively affected by leachates of *A. johnsonii* Prain, however, shoots development was promoted at low concentrations of stem bark leachates. This evidence suggests that low concentrations of stem bark leachates of *A. johnsonii* can probably serve as *V. subterranea* (L.) Verdc. natural shoots promoters. The inhibitory allelochemicals present in different parts of *A. johnsonii* could be used as potential natural herbicides, pesticides and fertilizers, however, further research to identify and study their mode of action is necessary.

Author Contribution

Authors contributed to the structuring of the manuscript to completion.

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All contributed in data collection and analysis are appreciated.

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