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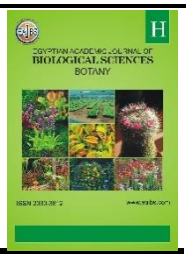

Phytotoxic Activities of Aqueous Leaf Extract of *Datura metel* on Germination and Seedlings of *Zea mays* and *Phaseolus vulgaris*

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ABSTRACT

This study examined the phytotoxicity of the extract of *Datura metel*. It involved the planting of seeds of monocot, yellow maize (*Zea mays*) and a dicot, brown bean (*Phaseolus vulgaris*), growth and analysis of seedlings of the two plants.

Seeds of *Z. mays* were collected at the Institute of Agricultural Research and Training (IAR&T), Ibadan and the seeds of *P. vulgaris* were collected at International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The leaf of *D. metel* was collected. The plant was identified and authenticated (FHI 111862) at Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria. The collected leaves were carefully washed under running tap water and air-dried at room temperature for four weeks. The dry leaves were milled into a fine powder and extracted. The phytotoxic potentials of the extract were evaluated using a standard method. At the end of the experiment, seedlings were harvested, stem and leaf were separately collected, and shoot length of seedlings was measured, and kept frozen for the estimation of total protein, soluble sugar, free amino acids, proline, and plant pigments.

The findings revealed that different concentrations of aqueous extract of the leaf of *D. metel* retarded the rate of germination of seeds, significantly inhibited the shoot length of *P. vulgaris* and *Z. mays* and reduced concentrations of chlorophyll, free amino acids, protein, sugar, and proline.

In conclusion, this study revealed that the extract exhibited a deleterious effect on germination, growth and biochemical parameters in seedlings of *P. vulgaris* and *Z. mays*.

INTRODUCTION

Allelopathy is an occurrence whereby a plant produces compounds or substances which influence the development of other plants, this can be beneficial or detrimental (Mushtaq and Siddiqui, 2017 and Shah *et al.*, 2018). In this context, allelopathy is an essential instrument in selective biological weed management. Allelopathy is considered an attractive method for weed management due to its environmental friendliness (Delcour *et al.*, 2015 and Dallali *et al.*, 2017).

D. metel grows aggressively colonized its immediate environment. It produces highly viable seeds, widely cultivated beyond its native range in tropical regions. It does not allow other plants in its immediate environment to grow well (Jonasson and Afshari, 2016). The shape of the fruit is like an apple covered with sharp thorns. The plant is widely cultivated in heat regions across the globe. The plant also grows in the Southwestern part of Nigeria, where it is known for its narcotic and hallucination properties (Kuganathan and Ganeshalingam, 2011; (Nandini *et al.*, 2015). *D. metel* is invasive, known as a garden thug, noxious weed, and sleeper weed. Therefore, this study investigated the effect of leaf extract (aqueous) of *D. metel* on the germination of seed and biochemical parameters in seedlings of *Z. mays* and *P. vulgaris* with a view of understanding the mode of action of its phytoconstituents.

MATERIALS AND METHODS

Seeds of *Zea mays* (Low Nitrogen Protein Yellow Variety) were collected at the Institute of Agricultural Research and Training (IAR&T), Ibadan and the seeds of *Phaseolus vulgaris* (Ife brown) were collected at International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The viability of the seeds was tested and assayed using the biochemical method.

Chemicals and Reagents:

The chemicals and reagents were acquired from various sources of quality grade. Proline ($C_6H_9NO_2$), Nihydrin ($C_9H_6O_4$), L-glutamic acid, Bovine serum albumin (BSA), Orthophosphoric acid (H_3PO_4), Folin-Ciocalteu's Phenol Reagent, Aluminum chloride ($AlCl_3.6H_2O$), Acetic acid (CH_3COOH), Phenol (C_6H_5OH), Sodium hydroxide (NaOH), Disodium ethylene diamine tetra-acetic acid (Na_2EDTA), Sodium chloride (NaCl), Sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$), Disodium phosphate (Na_2HPO_4), $NaHCO_3$, Na_2CO_3, Sodium/Potassium tartrate ($Na\text{-}K\text{-}C_4O_6$), Copper sulphate pentahydrate ($CuSO_4.5H_2O$), Potassium hydroxide (KOH), Ethanol (C_2H_5OH), Methanol (CH_3OH). All buffers, reagents, and solutions were prepared with distilled water.

Collection, Identification and Treatment of Plant Material:

The leaf (fresh) of *D. metel* was collected at Ajebamidele Area, Ile-Ife (7˚49 N, 4˚07 E) and Iperindo (7˚29 N, 4˚30 E), Atakunmosa East Local Government Area, Osun State, Nigeria. The whole plant was taken to Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where it was identified and authenticated. The specimen copy was deposited and a voucher number (FHI 111862) was collected.

The collected leaves were carefully washed under running tap water and air-dried on the bench at room temperature for four weeks. The dry leaves were milled into a fine powder in a manual grinder and kept in a tight container until used for the study.

Preparation of Aqueous Leaf Extract of *D. metel*:

The aqueous leaf extract of *D. metel* was prepared according to the procedure earlier reported (Oyedapo and Amos, 1997). Briefly, the powdery form of the leaf (500 g) was exhaustively extracted with distilled water (2 L) for 72 h with irregular shaking. The suspension was filtered through a clean chease cloth and Whatman No. 1 filter paper into a clean container. The residue was washed and re-extracted with the same water until the filtrate became clear and colourless. The combined filtrate was concentrated to a thick slurry *in vacuo* at 40 ˚C before dispensing into clean dried Petri dishes. The Petri dishes were then transferred into a desiccator for the complete removal of moisture. The residue was kept in the air-tight container in the freezer for analysis. The extract was termed aqueous leaf extract of *Datura metel* (ALEDM).

Evaluation of Phytotoxicity Assay:

The evaluation of allelopathic potentials of the extract was done as described in the earlier methods of Rank (2003) and Fasaanu *et al.* (2013) with slight modification.

Seed Germination Assay:

Petri dishes (60) were layered with cotton wool and moistened with different concentrations of the leaf extract (0, 100, 200, 300, 400 and 500 µg/ml) in 5 replicates. Then, 10 viable seeds of *Z. mays* and *P. vulgaris* were selected and placed in separate Petri dishes and kept saturated by regular moistening with the extract. Germinating seeds were counted daily for 7 days. The percentage germination of the seed was estimated using the expression:

$$\text{Percentage Germination} = \frac{\text{Number of seeds germinated}}{\text{Total number seeds planted}} \text{ X } 100$$

Collection of Seedlings of Maize and Bean:

On day 8, seedlings were harvested, stem and leaf were separately collected, shoot length (cm) of seedlings were measured, wrapped in aluminium foil and kept frozen for the estimation of total protein, soluble sugar, free amino acids, proline, and plant pigments.

Biochemical Analyses:

Preparation and Estimation of Soluble Proteins Concentration:

Leaf and stem of maize and bean seedlings were prepared using normal saline of 0.1 M NaOH. Typically, maize seedling leaf (1 g) was homogenized first with normal saline (10 ml) and centrifuged at room temperature using Bench Centrifuge (90-2 Centrifuge Searchtech Instruments) for 10 minutes. The supernatant was collected and the debris reextracted in 10 ml 0.1 M NaOH at room temperature for 1 hr and followed by centrifugation as before. The two supernatants were combined, filtered and the filtrate was used for the quantification of protein content. The process was repeated for the extraction of maize seedling stem, as well as leaf and stem of bean seedlings. The protein extracts were stored frozen in the freezer for protein estimation. The soluble protein concentrations of homogenates (stem and leaf) were determined spectrophotometrically according to Lowry *et al*. (1951) method as modified (Schacterle and Pollack (1973). Bovine albumin was used as a standard protein for the preparation of the standard curve.

Preparation and Estimation of Soluble Sugars Concentration:

The extraction and estimation of the concentration of soluble sugars from the maize and beans seedlings were carried out based on the method of Noseda *et al*. (1999) as reported by Akinwunmi and Oyedapo, (2013) with slight modification. The seedling (1 g) in triplicate was extracted with cold distilled water over a period of 3 hr in order to afford enough soluble sugar, followed by centrifugation for 15 min (3500 rpm). The supernatants were collected and it was kept as cold-water polysaccharides and the residue was subjected to further extraction. Into each residue was added another 10 ml of the solvent (distilled water) and kept in a water bath (85˚C) for another 3 hr, cooled down and then centrifuged. The supernatants were collected and kept as hot water polysaccharides. The two extracts (cold and hot water) were used for the estimation of the concentrations of polysaccharides. The estimation of the concentrations of soluble sugar in cold and hot water extracts was carried out using a Phenol-Sulphuric acid reaction method as described by Dubois *et al.* (1956). D-glucose was used as a standard sugar (250 µg/ml) for the preparation of the calibration curve. Concentrated sulphuric acid was run through the burette by the wall of test tubes to avoid splashing on the body.

Extraction and Estimation of Plant Pigments Concentration:

The extraction and estimation of chlorophyll contents of the leaves of seedlings (maize and bean) cultivated in varying concentrations of the extract were carried out according to the method of Gross (1991). Chlorophyll extraction involved homogenizing leaf (0.5 g) in 5 ml of 70% (v/v) acetone using pestle and mortar with washed sand as

abrasive. The homogenates were first filtered and then centrifuged at 3500 rpm, and then supernatants were collected and kept for chlorophyll assays.

The extracts were diluted appropriately (10-fold dilution) and followed by reading absorbance at 645 nm and 663 nm respectively. Acetone (70% (v/v) was used as blank. The expressions below (Arnon, 1949), was used to calculate the amounts of plant pigments in the leaf of seedlings.

Chlorophyll a (g/l) = (0.0127 x OD 663 nm) + (0.00269 x OD 645 nm)
Chlorophyll b (g/l) = (0.0229 x OD 645 nm) + (0.00468 x OD 663 nm)
Total chlorophyll (g/l) = (0.0202 x OD 645 nm) + (0.00820 x OD663 nm).

Extraction and Estimation of Proline Concentrations:

The extraction and quantification of proline were carried out according to Bates *et al.* (1973) as described by Fallah *et al.* (2018) with slight modification. Fresh seedling (0.5 g) was homogenized (triplicate) with 3% (w/v) sulfosalicylic acid (10 ml) in mortar and pestle on ice, followed by centrifugation (at 3500 rpm for 15 min). The upper layer of the homogenate was collected after homogenization for estimation of proline. The upper layer (proline extract), 2 ml was pipetted into a clean tube, followed by the addition of 2 ml of glacial acetic acid, followed by the addition of ninhydrin reagent (2 ml). Ninhydrin reagent is a mixture of ninhydrin (1.25 g), glacial acetic acid (30 ml), and 6 M orthophosphoric acid (20 ml). The reaction mixture was vortexed, boiled in a hot water bath (1 h at 100 ˚C). Tubes were removed, cooled down, and each tube was vigorously mixed with 4 ml of toluene. Toluene was used as blank. Absorbance was read using a spectrophotometer (at 520 nm). Standard proline (20 µg/ml) was used to prepare a standard calibration curve. The proline concentrations were extrapolated from the calibration curve which was represented as mg/g fresh weight.

Estimation of Free Amino Acid Concentrations:

Total free amino acid contents of leaf and stem of *Z. mays* and *P. vulgarius* treated with varying concentrations of the extract of *D. metel* were estimated following a method of Laitonjam *et al*. (2013) with slight modification using anthrone reagent reaction.

Fresh leaf and stem (0.5 g) of *Z. mays* and *P. vulgarius* were cut into a bit, homogenized with 80% (v/v) ethanol (5 ml) in mortal and pestle on ice. Then followed by centrifugation at 4000 rpm in 20 min, the upper layer (supernatant) of the mixture was separated and kept for analysis. Into each of the clean test tubes arranged in triplicate, supernatant (0.5 ml) was added; this was followed by the addition of distilled water (0.5 ml). The mixture was vigorously shaken and 1 ml of ninhydrin reagent [2% (w/v) Ninhydrin in acetone] was added. The tubes were covered with foil paper, shaken vigorously and boiled for 10 min. The water bath was switched off and the test tubes were removed and allowed to cool down. The absorbance of the samples was undertaken at 570 nm. Glutamic acid (150 µg/ml) was used as a standard amino acid to prepare a calibration curve from which the amount of free amino acid was extrapolated. Into clean test tubes (in triplicate), 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of glutamic acid (150 µg/ml) were added, distilled water was then used to adjust the volumes to 1.0 ml and treated as above. The number of free amino acids present in plant tissue was extrapolated from the calibration curve and represented in mg/g fresh weight of plant tissue.

Statistical Analysis: GraphPad 5 (prism) was used for the analyses. The data were expressed as Mean ± SEM, n = 5 and a One-way Analysis of Variance was used to determine the differences in the data. The level of confidence was set at $p < 0.05$.

RESULTS

Seed Germination of *Z. mays* and *P. vulgaris*:

The phytotoxic effects of varying concentrations of the plant leaf extract on the germination of *Z. mays* are shown in Table 1. The extract caused a decrease in the rate of seed germination. The inhibitory activity of varying concentrations of the extract on seed germination of *P. vulgaris* is given in Table 2. The treatment of the seeds of *P. vulgaris* with the extract at different concentrations slightly decreased the total number of seeds germinated. The decrease in the rate of seed germination was concentration-dependent.

Table 1: Percentage Seed Germination of *Z. mays*

Concentration (µg/ml)	Days of Planting		
	2	3	4
0	9 ± 0.00 (90%)	10 ± 0.00 (100%)	10 ± 0.00 (100%)
100	9 ± 0.44 (90%)	10 ±0.22 (100%)	10 ±0.00 (100%)
200	6 ± 0.00 (60%)	7 ± 0.22 (70%)	8 ± 0.22 (80%)
300	7 ± 0.78 (70%)	9 ± 0.22 (90%)	9 ± 0.22 (90%)
400	8 ± 0.22 (80%)	8 ± 0.22 (80%)	8 ± 0.22 (80%)
500	9 ± 0.66 (90%)	10 ± 0.22 (100%)	10 ± 0.00 (100%)

Each value represented Mean ± SEM, n=5 readings. $p < 0.05$.

Table 2: Percentage Seed Germination of *P. vulgaris*

Concentration (µg/ml)	Days of Planting			
	2	3	4	5
0	6 ± 0.22 (60%)	9 ± 0.00 (90%)	10 ± 0.00 (100%)	10 ± 0.00 (100%)
100	7 ± 0.22 (70%)	9 ± 0.44 (90%)	10 ± 0.22 (100%)	10 ± 0.00 (100%)
200	4 ± 0.22 (40%)	6 ± 0.00 (60%)	7 ± 0.22 (70%)	8 ± 0.22 (80%)
300	5 ± 0.22 (50%)	7 ± 0.78 (60%)	9 ± 0.22 (90%)	10 ± 0.22 (100%)
400	6 ± 0.22 (60%)	8 ± 0.22 (80%)	8 ± 0.22 (80%)	8 ± 0.22 (80%)
500	7 ± 0.22 (70%)	8 ± 0.66 (80%)	9 ± 0.22 (90%)	10 ± 0.00 (100%)

Each value represented Mean ± SEM, n=5 readings. $p < 0.05$.

Shoot Length of the Seedlings of *Z. mays* and *P. vulgaris:*

Table 3, was shown the inhibitory potentials of the extract on shoot growth of seedlings of *P. vulgaris* and *Z. mays*. Shoot lengths of *P. vulgaris* and *Z. mays* were decreased with varying concentrations of the extract.

Table 3: Shoot Lengths of *P. vulgaris* and *Z. mays* Seedlings

Concentration (µg/ml)	Shoot Length (cm)	
	Z. mays	*P. vulgaris*
0	16.64 ± 0.80	21.26 ± 1.01
100	16.56 ± 1.10	19.12 ± 0.80
200	15.26 ± 1.60	19.06 ± 0.90
300	14.64 ± 1.70	17.82 ± 0.41
400	13.26 ± 1.10	17.8 ± 0.51
500	13.78 ± 1.40	16.84 ± 0.60

Each value represented Mean ± SEM, n=5 readings. $p < 0.05$.

Concentrations of Soluble Protein in Leaf and Stem of Seedlings of *P. vulgaris* and *Z. mays:*

The effect of the extract on soluble protein concentrations in the leaf and stem of *Z. mays* and *P. vulgaris* seedling was presented in Figure 1**.** The extract significantly caused an

increase in protein concentration of both *Z. mays* and *P. vulgaris* seedling except slight reduction observed in a few groups when compared with the control.

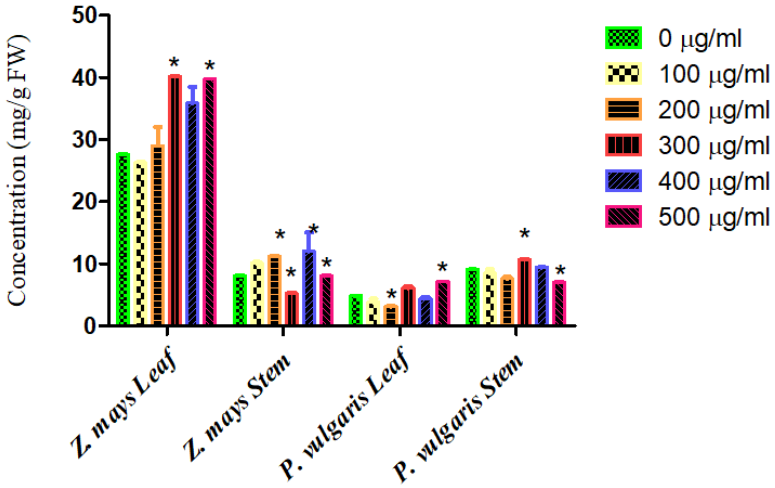

Fig. 1: Concentrations of Soluble Protein in Leaf and Stem of *Z. mays* and *P. vulgaris* Seedlings

Each value represented Mean ± SEM, n=3 readings. $p < 0.05$.
Values with * were significant when compared with control (0 µg/ml).

Concentrations of Soluble Sugar in Leaf and Stem of *Z. mays* and *P. vulgaris* Seedling:

Figures 2 and 3 presented the level of total sugar extracted with cold and hot water from the leaf and stem of *Z. mays* and that of *P. vulgaris* exposed to varying concentrations of the extract of *D. metel.* The extract at varying concentrations caused a significant elevation of concentrations of sugar extracted with cold and hot water from the seedling of *P. vulgaris* as compared to the control. The result showed an increase in the level of soluble sugar extracted with hot water from the seedling of *Z. mays* and a reduction in soluble sugar extracted with cold water when compared with the control.

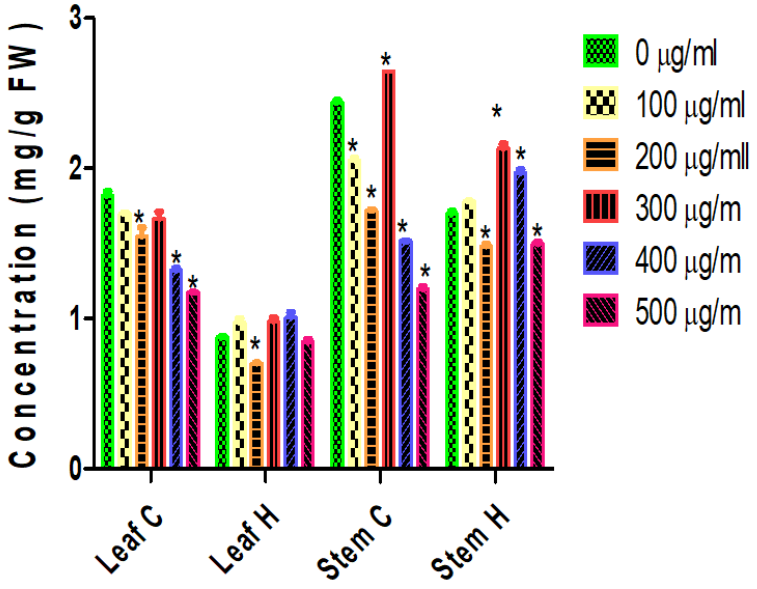

Fig. 2: Concentrations of Soluble Sugar in Leaf and Stem of *Z. mays* Seedling.

Each value represented Mean ± SEM, n=3 readings, $p < 0.05$.
Values with * were significant when compared with control (0 µg/ml).
C = Cold water extract, H = Hot water extract, FW = Fresh Weight.

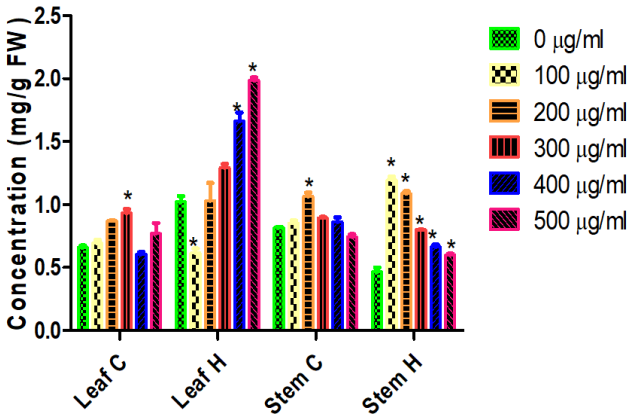

Fig. 3: Concentrations of Soluble Sugar in Leaf and Stem of *P. vulgaris* Seedling
Each value represented Mean ± SEM, n=3 readings, $p < 0.05$.
Values with * were significant when compared with the control (0 µg/ml).
C = Cold water extract, H = Hot water extract, FW = Fresh Weight

Levels of Chlorophyll Contents in Leaf of *Z. mays* and *P. vulgaris* Seedlings:

The effects of the extract on chlorophyll contents in the leaf of the seedlings were shown in Figures 4 and 5. There was a decrease in chlorophyll content of the leaf *Z. mays* seedling but an increase in the group treated with 400 µg/ml of the extract when compared with the control. Significantly, there was a reduction in chlorophyll contents of the leaf of *P. vulgaris* seedling when compared with the control.

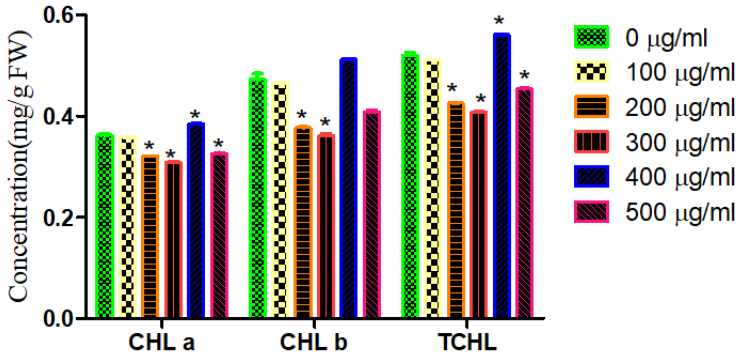

Fig. 4: Levels Chlorophyll Contents in Leaf of *Z. mays* Seedling
Each value represented Mean ± SEM, n=3 readings. $p < 0.05$.
Values with * were significant when compared with the control (0 µg/ml).
CHL = Chlorophyll

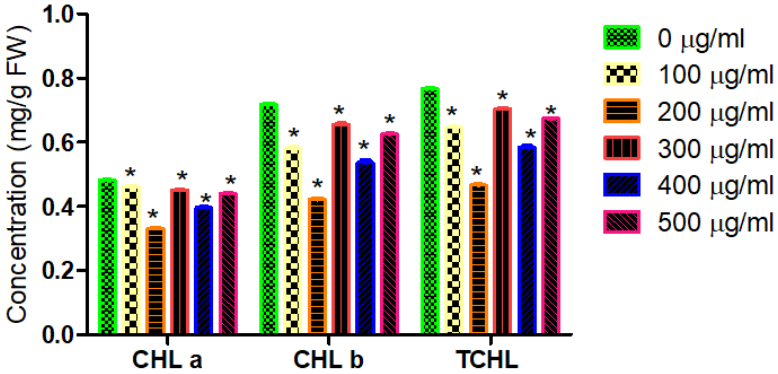

Fig. 5: Levels of Chlorophyll Contents in Leaf of *P. vulgaris* Seedling
Each value represented Mean ± SEM, n=3 readings. $p < 0.05$.
Values with * were significant when compared with the control (0 µg/ml).
CHL = Chlorophyll, FW = Fresh Weight.

Concentrations of Total Free Amino Acid in Leaf and Stem of *Z. mays* and *P. vulgaris* Seedlings:

Effects of the extract on the concentration of free amino acid (total) in the leaf and stem of *Z. mays* and that of *P. vulgaris* seedling are shown in Figure 6. The exposure of *P. vulgaris* to different concentrations of the extract resulted in a significant increase in free amino acids concentration when compared with the control. The increase was concentration-dependent.

There was a decrease in total free amino acids concentration of *Z. mays* seedlings except for an increase observed in the stem of the group treated with 400 µg/ml of extract of *D. metel* when compared with control.

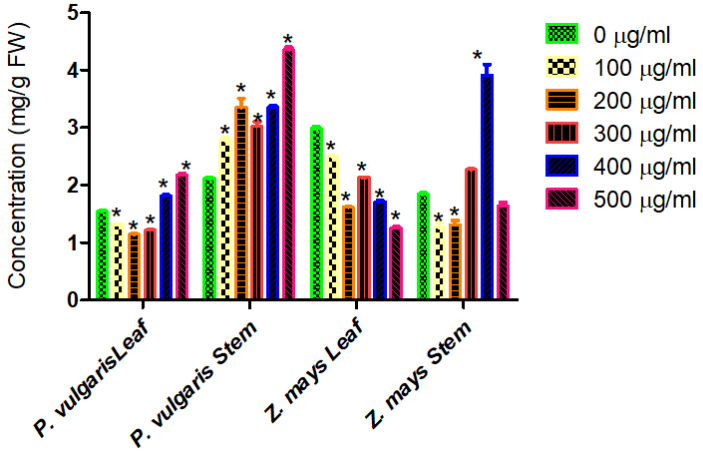

Fig. 6: Concentrations of Total Free Amino Acid in Leaf and Stem of *Z. mays* and *P. vulgaris* Seedlings

Each value represented Mean ± SEM, n=3 readings. $p < 0.05$.
Values with * were significant when compared with the control (0 µg/ml).
FW = Fresh Weight.

Concentrations of Proline in Leaf and Stem of Seedlings of *Z. mays* and *P. vulgaris*

Phytotoxic effects elicited by the extract of the leaf of *D. metel* on proline concentrations in the seedlings of *Z. mays* and that of *P. vulgaris* (leaf and stem) were reported in Figure 7. The proline concentrations of the leaf of *P. vulgaris* and *Z. mays* seedlings were significantly reduced which was concentration-dependent and there was also an increase in proline concentrations of the stem of *P. vulgaris* and *Z. mays* seedlings.

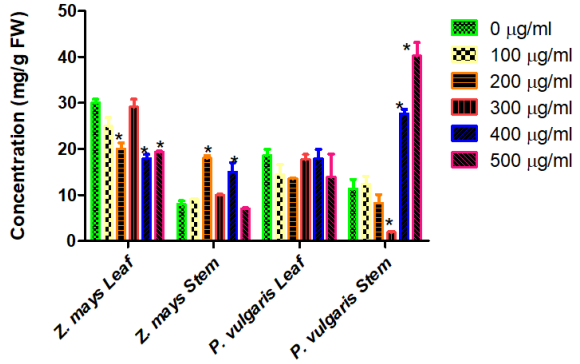

Fig. 7: Concentrations of Proline in Leaf and Stem of *Z. mays* and *P. vulgaris* Seedlings

Each value represented Mean ± SEM, n=3 readings. $p < 0.05$.
Values with * were significant when compared with the control (0 µg/ml).
FW = Fresh Weight.

DISCUSSION

This study was designed to investigate the phytotoxicity of the extract (aqueous) of the leaf of *Datura metel* (a species of Datura) with a view to investigating the mechanism through which *D. metel* affects other plants. This was due to the earlier observation that the plant, *D. metel* does not allow other plants around it to thrive well. The plant tends to be invasive and grow along roadside and dumbing areas.

Germination is a complex and vital stage in plant development which includes imbibition, enzymes activation, respiration and mobilization of food reserves (Salam and Kato-Noguchi, 2010). The seed dormancy undergoes by the plant is very important and paramount for plant regeneration and food preservation for both animals and humans. Biochemical changes that occur during germination provided the basic background for subsequent plant development. The growth and development of plants start after seed germination (Dragoeva *et al*., 2015). The phytotoxic potentials of *Datura metel* extract on the germination of the seed and the growth of the seedling of *Phaseolus vulgaris and Zea mays* revealed that the different concentrations of the extract slowed down the rate of seed germination of *P. vulgaris and Z. mays* when compared with control. This could be due to the deleterious effect of the extract on the stages involved in seed germination such as imbibition, enzymes activation, respiration and mobilization of food reserves as well as the synthesis and activities of enzymes involved in seed germination. The effect of the extract on the germination observed in this study is in agreement with the observation of the study of Nwokeocha and Ezumah, (2015). The study reported that the inhibitory effect of extracts of leaf and stem bark of cashew on the germination and growth of maize seed.

The results of this study revealed that varying concentrations of the extract significantly caused a reduction in shoot length of *P. vulgaris and Z. mays* seedlings and the reductions were concentration-dependent. This could be due to the toxic potentials of the extract to cause disruption of normal cellular metabolism, inhibition of cell division, decrease in levels of the chemical that regulate plant growth and development like gibberellins and indoleacetic acid (Nwokeocha and Ezumah, 2015).

Proteins are essential biomolecules that take part in a critical aspect of plant development and growth. It is one of the plant's primary metabolites. It serves various functions like catalysis of biochemical reactions, facilitation of transportation of materials across the cell membrane, participating in biochemical reactions involved in the generation of energy and cellular structure (Zagorchev *et al.,* 2014). The present findings showed that the extract significantly ($p<0.05$) caused an increase in protein content of Z. *mays* seedling leaf except for a slight decrease in the group treated with100 µg/ml when compared with the control.

The elevated level of concentrations in both Z. *mays* and *P. vulgaris* seedlings could be due to the enhancement of protein biosynthesis or stimulation of the enzymes involved in protein synthesis by the phytoconstituents present in the extract (Verma *et al.,* 2011).

Sugars possess and exhibit central regulatory functions in the growth and development of any plant (Korkina *et al.,* 2017). In the growth and development of a plant, the specific function of sugars is diverse, including the molecular networks that drive cell division and expansion and provision of energy and biomass (Lastdrager *et al.,* 2014). Mobilization of sugar is essential to maintain the cellular integrity of plants, especially under stressful conditions. The result revealed that the different concentrations of the extract significantly caused a decrease in the sugar concentrations of *Z. mays* seedling while it caused an increase in the *P. vulgaris* seedling but was not concentration-dependent. The reduction in the levels of concentrations of soluble sugar could be due to its overutilization by the seedling to combat the stress posed by the allelochemicals present in the extractor

mobilization for energy production. It could also be as a result of disruption in the process of photosynthesis or reduction in chlorophyll content which is not unconnected with the reduction in chlorophyll content observed in the leaf of *Z. mays* seedling. The increase in soluble sugar concentration might be due to the increase in biosynthesis of sugar and this agreed with the study of Korkina *et al.* (2017) that showed an increase in sugar levels of sugarcane exposed to stress. Sugar is also a plant stress biomarker.

Chlorophyll is the plant pigment that resides in the chloroplast of a plant. Chlorophyll a and b are the primary leaf pigments responsible for the capturing of light energy. During photosynthesis, chlorophyll captures sunlight energy to generate ATP and NADH that are involved in the production of carbohydrates (Kalaji *et al.,* 2018). In our findings, the chlorophyll contents of the leaves of Z. *mays* and that of *P. vulgaris* seedlings were remarkably affected. The varying concentrations of the extract caused a significant reduction of chlorophyll a, and chlorophyll b and total chlorophyll content of Z. *mays* and *P. vulgaris* seedling leaves. This might be due to the interaction of alleochemicals in the extract with the leave pigments biosynthesis precursors, porphyrin (Li *et al.,* 2010). This reduction in chlorophyll contents could have also resulted from the phytotoxic effect of the extract which could lead to a slow rate or inhibition of photosynthesis, depreciation in oxygen, sugars and energy production (Li *et al.,* 2010). This could lead to growth retardation which is connected with the growth inhibition and decrease in total sugar concentration observed in the study. The reduction in chlorophyll content observed in the study agreed with Kohila and Gomathi, (2018) observed a decrease in chlorophyll content of sugar cane exposed to heat stress.

Amino acids play a crucial role in the biosynthesis of a polymer, protein. They are the backbone or building blocks of all proteins, including antioxidant enzymes. Some amino acids are present in the organisms as free amino acids and they do not take part in protein synthesis, they are referred to as non-proteinous amino acids. These free amino acids are crucial to the growth and development of plant life (Jyothi *et al.,* 2018). The level of free amino acids (total) in the leaves, and stems of Z. *mays* and *P. vulgaris* seedlings were affected by the extract. They also play an important role as building blocks of some biosynthetic pathways, cell signaling and the response of the plant to stress (Hildebrandt *et al.,* 2015). The increase in the concentration of free amino acids could be due to the high rate of their synthesis in plant tissue.

Proline is an osmoprotectant and serves as carbon, nitrogen and energy source for tissues recovering from water or heat stress and heat denaturation of enzymes (Rafaela *et al.,* 2016). The findings of the study revealed a marked increase of proline content in Z. *mays* and *P. vulgaris* seedling stems. An increase in proline concentration could be due to the increase in its biosynthesis. The determination of proline content may be so important in the assessment of the physiological state, more generally to understand stress tolerance in plants (Zhang *et al.,* 2013). Reduction observed in the levels of proline in the study may be due to the mobilization of proline in order to combat the threat posed by the extract (El-Shora *et al.,* 2018). Proline mobilization under stress may perform the role of low molecular weight chaperones. The role of proline in plants under stress includes stabilization, protection of the structure of enzymes. It also protects the structure of proteins, maintenance membrane integrity, protects against reactive oxygen species, also serves as a pool of carbon and nitrogen source for the plant after the plant has undergone stress (Hameed *et al.* 2012). It could also be due to the induction of enzymes responsible for protein synthesis, enhancement of incorporation of proline into protein, or enhancement of proline catabolism. This observation is in accordance with the increase in leaf protein concentration in this study. The accumulation in proline concentration is in harmony with the work of Gharsallah *et al.* (2016) that recorded an increase in proline content of tomato cultivars exposed to salt stress.

Conclusions

In conclusion, the study revealed that the leaf extract of *D. metel* exhibited a deleterious effect on germination, growth and biochemical parameters in seedlings of *P. vulgaris* and *Z. mays*.

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