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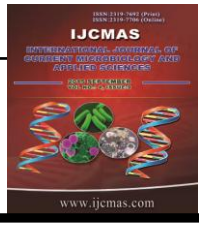

Original Research Article

Allelopathic potential of *Trichodesma africanum* L.: Effects on germination, growth, chemical constituents and enzymes of *Portulaca oleracea* L.

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Keywords

Trichodesma africanum, *Portulaca oleracea*, seed germination, growth, chlorophyll, metabolites.

A B S T R A C T

In this investigation the effect of aqueous leaf extract of *Trichodesma africanum* L. on seed germination, growth, chlorophyll, metabolites, ATP-ase activity, NADH-oxidase activity and hydrogen peroxide content in *Portulaca oleracea*L. leaves was investigated. The results indicate that aqueous leaf extract of *T. africanum* reduced seed germination, root length, shoot length, chlorophyll content, soluble carbohydrate, insoluble carbohydrate and total carbohydrate contents. Also, the leaf extract of *T. africanum* reduced the soluble protein, insoluble protein and total protein contents. In addition, the treatment led to an increase in H_2O_2, and the activities of NADH-oxidase in *P. oleracea leaves*. However, the treatment caused a reduction in ATP-ase activity in a concentration-dependent manner. The results indicate the possible application of *T. africanum* aqueous leaf extract natural bioherbicide of *P. oleracea* and this is an economic procedure.

Introduction

The term allelopathy is used for describing the chemical interaction between two plants. Allelochemicals can be present in leaves, flowers, roots, bark, and fruits of plants (El-Shora and Abd El-Gawad, 2015; Saadaoui et al., 2015).

Vast array of secondary metabolites in plants are known as allelochemicals. They belong to various chemical classes such as alkaloids, phenolics, flavonoids, terpenoids and cyanogenic glucosides (Saleh and Madany, 2013; El-Shora and Abd El – Gawad, 2014; Mishra, 2015).

Moving of allelochemicals into the rhizosphere happen through leaching from leaves as well as other aerial parts of the plants by root exudation, volatile emissions, and the breakdown of leaf litter and bark (Weir et al., 2004; El-Shora and Abd El–Gawad, 2014).

The allelochemicals action on plants is known to be a diverse action and it includes a large number of biochemical reactions resulting into their modifications and finally affecting the growth of target plants (Yu et al., 2003; Elisante et al., 2013).

The influence of allelochemicals usually occurs in the early life cycle of plants causing inhibition of seed germination and/or inhibition of seedling growth (Einhellig, 1995; El-Shora and Abd El–Gawad, 2014).

Among the known influences of allelopathy are, anatomically abnormalities, reduction in uptake, reduction in seed germination, reduction of seedling growth, and chlorosis. The rate of the inhibition by allelochemicals is dependent on the concentration of tested plant extract (El-Shora and Abdel – Gawad, 2015; Mishra, 2015).

Portulaca oleracea

Portulaca olercea L. (purslane) (Family: Portulacaceae) is a general troublesome weed worldwide. It is competitor for crops, can quickly establish, regenerates by the method of vegetative reproduction and affect the growth of crop plants (Mohamed and Hussein, 1994).

Thus, the present work aimed to investigate the allelopathic effect of leaf extract of *T. africanum* on seed germination, growth, metabolites, pigment content and ATP-ase enzyme activity in *P. oleracea*.

Materials and Methods

Plant material

T. africanum was collected from WadiHaqul in the desert of Egypt. The plant leaves were air dried for few days and ground.

Preparation of aqueous extract from *T. africanum*

A sample (10 g) of the air dried powder was mixed with 100 ml distilled water followed by shaking overnight at 4°C. The mixture was filtered using cheesecloth and centrifuged for 30 min at 3000 g. The resulting supernatant represented the stock solution 10 % (w/v). The various tested concentration (2, 4, 6, 8 and 10 %) were prepared from the supernatant by subsequent dilutions with distilled water.

Germination and growth of *P. oleracea*

P. oleracea seeds were germinated according to El-Shora (1993).The seed wee surface sterilized by 0.1 % (w/v) $HgCl_2$ followed by washing several times under running tap water and later in distilled water. Seeds were divided into 6 groups, the first group soaked in distilled water to represent the control. The other five remaining groups were soaked in 2, 4, 6, 8 and 10 % (w/v) aqueous leaf extract of *T. africanum*. Ten uniform seeds were placed in each of 6 clean Petri dishes which have been lined with two layers of filter paper and moistened with 10 ml of the aqueous leaf extract used. The Petri dishes were incubated at 25 ℃ for 14 days. At the end of the incubation period, the length of roots and shoots was measured in 5 seedlings picked up randomly.

Determination of total chlorophyll

The total chlorophyll content was determined according to the method adopted by Shankar et al. (2003). A sample of 0.5 g fresh weight of *P. oleracea* leaves was ground in 10 mL of 95% ethanol followed by centrifuging at 5000 rpm for 15 minutes. The resulting supernatant was collected and the absorbance was measured at both 664 nm and 647 nm against reagent blank.

Determination of total and water soluble carbohydrates

A sample of 0.5 g dry weight was hydrolyzed in 4NHCl for 2 hours in a boiling water bath for total carbohydrates. Also, 0.5 g of dry sample was extracted by

distilled water for two hours. Both extracts were filtered and completed to a definite volume and determined by the anthrone sulphuric acid method (Fales, 1951). A calibration curve using pure glucose was made from which the data ware calculated.

Determination of insoluble carbohydrates

The insoluble carbohydrate was calculated as the difference between the total carbohydrate and the water soluble carbohydrate of the same sample.

Determination of water soluble and water insoluble proteins

A sample of (0.5 g) was boiled in 10 ml distilled water for two hours, cooled and the water extract was centrifuged. The resulting supernatant was aspirated and completed to a particular volume by distilled water the soluble proteins were determined according to the method of Bradford (1976).The residue remaining from the previous step after extraction of water soluble was homogenated in a definite volume of 1 N NaOH. One ml of this homogenate was used for estimation of the insoluble proteins by the method of Bradford (1976).

Determination of total proteins

Total protein was calculated as the sum of water soluble and water insoluble proteins.

Estimation of hydrogen peroxide content

Hydrogen peroxide was measured according to Alexieva et al. (2001). A sample of 0.5 g were homogenized with liquid nitrogen and suspended in chilled 5 ml of 0.1 (w/v) TCA. The homogenate was centrifuged at 10,000g for 5 min.

A sample of 0.5 ml of the resulting supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 2 ml of 1 M potassium iodide (KI). The reaction was developed for 1 h in darkness. The absorbance was measured at 390 nm. Hydrogen peroxide concentration was determined using a given H_2O_2 standard curve.

Preparation of enzyme extract

The enzyme extract was prepared according El-Shoraand El-Naqeeb (2014). A sample (0.5 g) of leaf fresh weight were homogenized in 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 1% (w/v) PVP and 0.5% (v/v)Triton X-100 at 4°C. The homogenate was filtered through four layers of cheese cloth and centrifuged at 8,000 g for 20 min at 4°C. The extract was collected for estimation of enzyme activities.

Assay of ATP-ase activity

ATP hydrolysis was measured by releasing of Pi. Standard reaction mixture of 3 ml contained: 50 mM Tricine-KOH buffer (pH 8.0), 10 mM ATP, 10 mM $MgCl_2$, and 0.2ml of ATP-ase extract. The phosphate released from ATP was measured colorimetrically using ammonium molybdate.

Assay of NADH-oxidase activity

NADH oxidase activity was determined according to Morret (1995) as the disappearance of NADH measured at 340 nm in a reaction mixture containing 25 mM Tris-Mes buffer (pH 7.5), 1mM KCN to inhibit any oxidase activity, and 150 µM NADH at 37C with stirring.

Activity was measured spectrophoto-metrically over two intervals of 5 min each in presence or absence of hormone or growth factor. Extinction coefficient of 6.22 was used to determine specific activity.

Results and Discussion

The effect of aqueous leaf extract of *T. africanum* on the germination of *P.oleracea* seeds was investigated as shown in Fig.1. The results indicate that treatment with leaf extract resulted in inhibition of seed germination of *P. oleracea* and the inhibition is concentration-dependent.

The influence of leaf extract on seed germination seems to be mediated through a disruption of normal cellular metabolism rather than through damaging of organelles. The process of reserve mobilization usually takes place rapidly during early stage of seed germination seems to be delayed or decreased under allelopathy stress conditions (Gniazowska and Bogatek, 2005; Mishra, 2015).

The leaf extract of *T. africanum* also caused a remarkable reduction in seedling growth of *P. oleracea*). The inhibition of root length was concentration-dependent (Fig. 2). This inhibition may be due to the inhibitors which are leached out from plant leaves resulting in changing of macromolecules such as proteins, lipids as well as nucleic acids (Hussain and Reigosa, 2011)..

The inhibition of shoot length (Fig.3) may be due to the presence of phenols. It is possible that these phenolic compounds interfered with the phosphorylation pathway and inhibiting the activation of Mg^{2+} and ATP–ase activity. Another possibility is that the inhibition of shoot length may be attributed to reduction in the synthesis of carbohydrates, protein, and nucleic acids (RNA and DNA). A third possibility is that the inhibition of shoot length may be due to the interference of phenols in cell division, biosynthetic processes as well as mineral uptake. It has been found that the influences of allelochemicals on seed germination, and the growth of shoot and root may be due to reduction in cell division (Gholami et al., 2011; Singh and Chaudhary, 2011) which resulted from damage of cell membrane caused by allelochemicals.

In the present investigation the content of total chlorophyll was reduced (Fig. 4). The reduction in chlorophyll contents observed in all the concentrations might be due to degradation of chlorophyll pigments or reduction in their synthesis and the action of flavonoids or other phytochemicals present in leaf extract.

Allelochemicals can reduce the chlorophyll and porphyrin content and in turn affecting photosynthesis and the total plant growth (Siddiqui and Zaman, 2005). The photosynthesis potential in plants is directly proportional with the chlorophyll content present in leaf tissues which play an important role in photochemical reactions (Schlemmer et al., 2005).

The soluble carbohydrate (Fig. 5), insoluble carbohydrate (Fig. 6) and the total carbohydrate (Fig. 7) were reduced in *P. oleracea* leaves by *T. africanum* leaf extract. The reduction in carbohydrate fractions and the total carbohydrate could be due to reduction of photosynthesis because depletion of chlorophyll content under the treatment.

Also, it is possible that allelochemicals in *T. africanum* leaf extract might have interfered with the pathways of photosynthesis and consequently decreased all the metabolites including carbohydrates (Sinyl and Ranjana, 2003). Allelochemicals can inhibit PSII components and ATP synthesis (Inderjit and Duke, 2003).

Treatment of *P. oleracea* seedlings with leaf *T. africanum* extract resulted in reduction of soluble protein (Fig. 8) insoluble protein (Fig. 9) and total protein (Fig. 10). It is

possible that the phenolic compounds in leaf extract *T. africanum* may reduce the incorporation of certain amino acids into proteins and thus reduced the rate of protein synthesis.

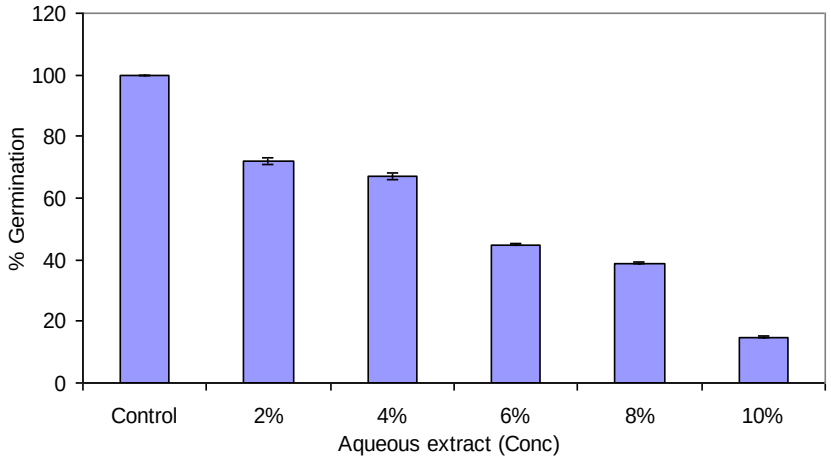

Fig.1 Effect of aqueous leaf extract of *T. africanum* on seed germination of *P. oleracea*

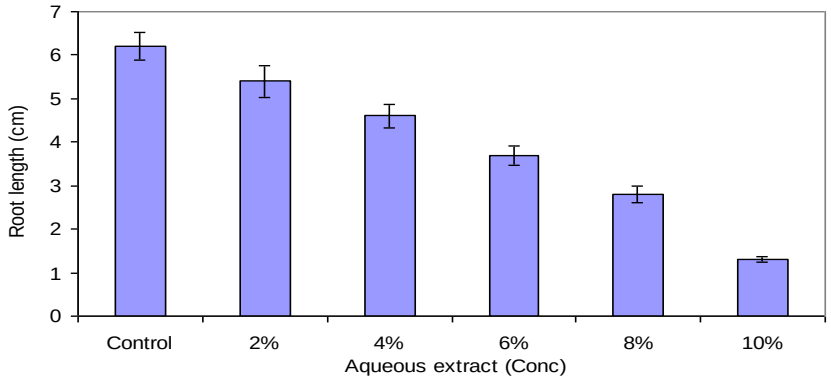

Fig.2 Effect of aqueous leaf extract of *T. africanum* on root length of *P. oleracea*

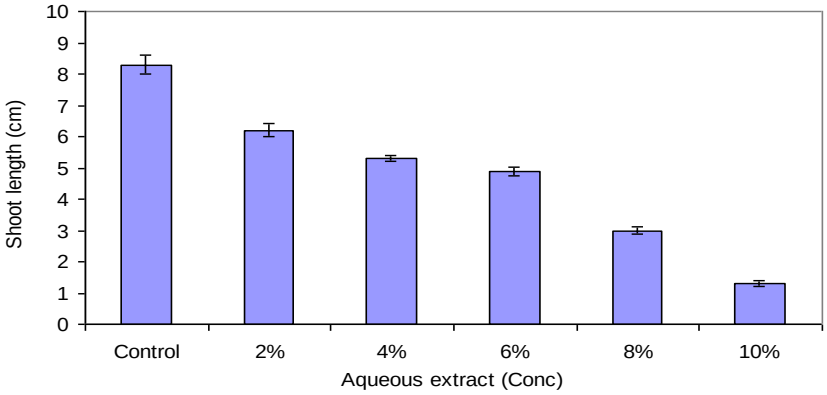

Fig.3 Effect of aqueous leaf extract of *T. africanum* on shoot length of *P. oleracea*

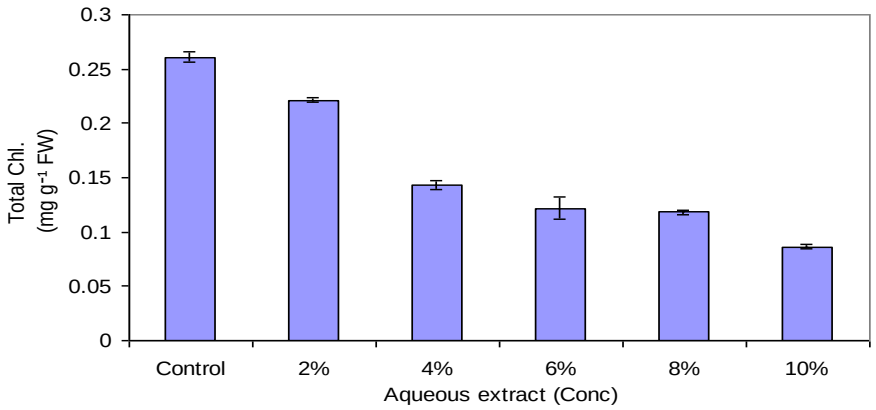

Fig.4 Effect of aqueous leaf extract of *T. africanum* on total Chl. of *P. oleracea.*

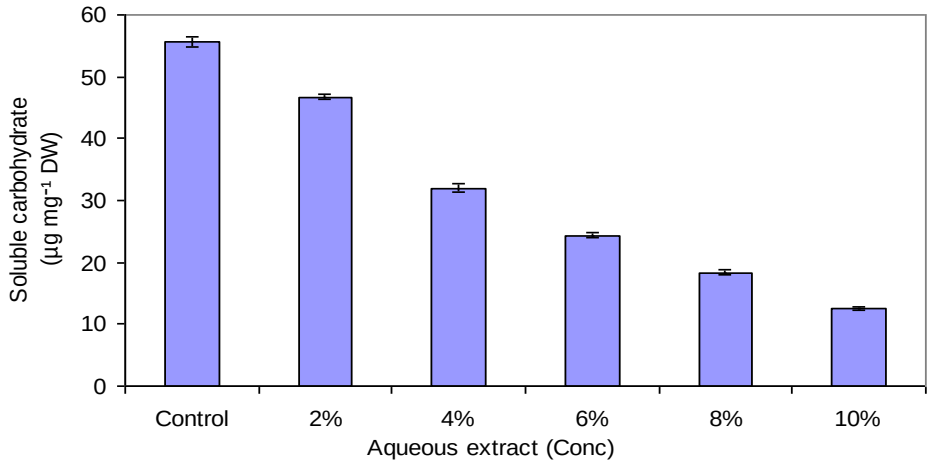

Fig.5 Effect of aqueous leaf extract of *T. africanum*on soluble carbohydrate of *P. oleracea.*

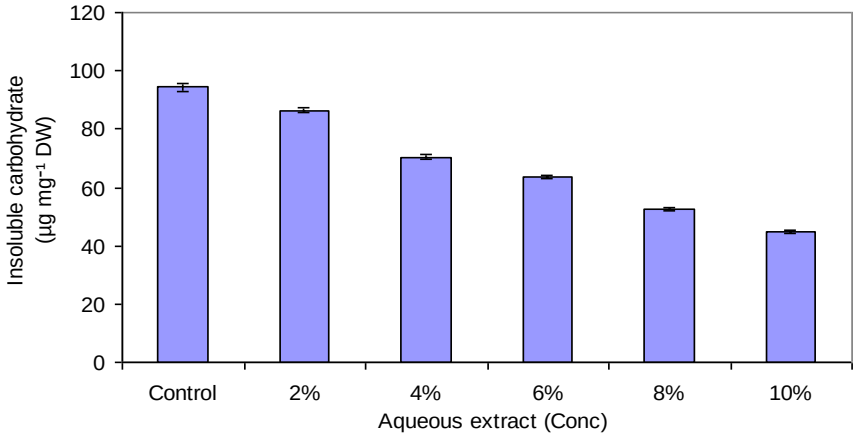

Fig.6 Effect of aqueous leaf extract of *T. africanum* on insoluble carbohydrate of *P. oleracea*

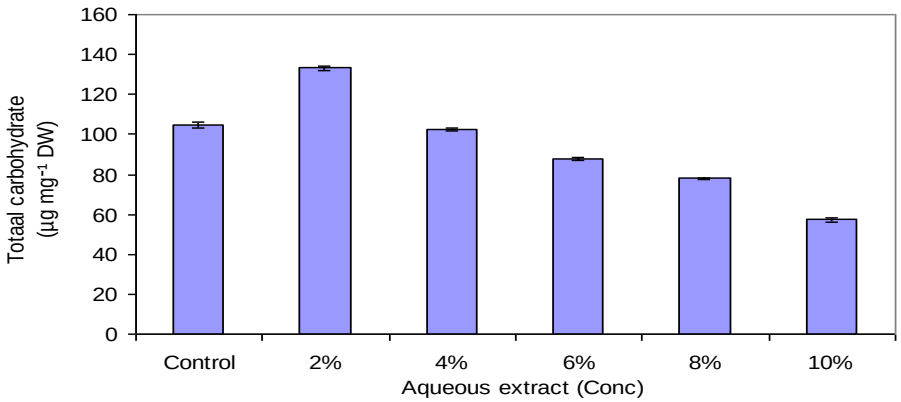

Fig.7 Effect of aqueous leaf extract of *T. africanum* on total carbohydrate of *P. oleracea*

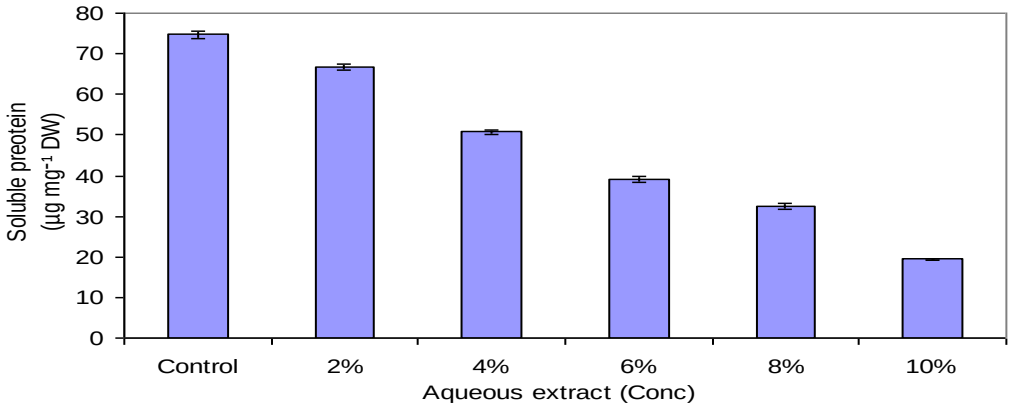

Fig.8 Effect of aqueous leaf extract of *T. africanum* on soluble protein of *P. oleracea*

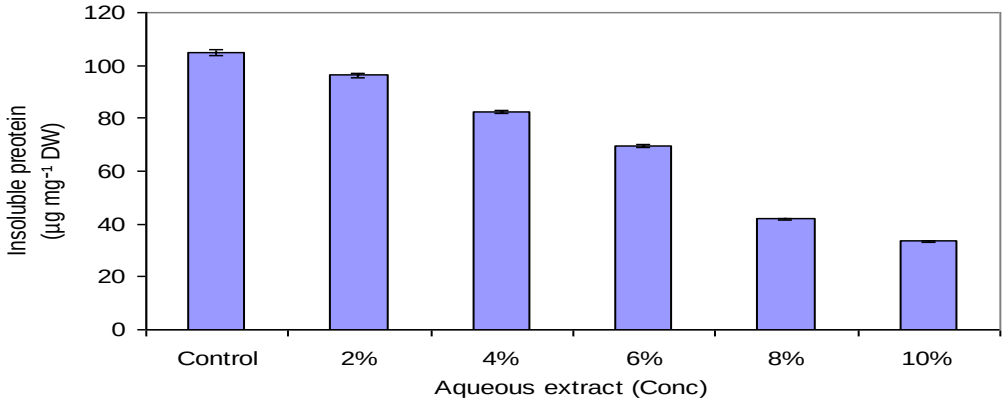

Fig.9 Effect of aqueous leaf extract of *T. africanum* on insoluble protein of *P. oleracea*

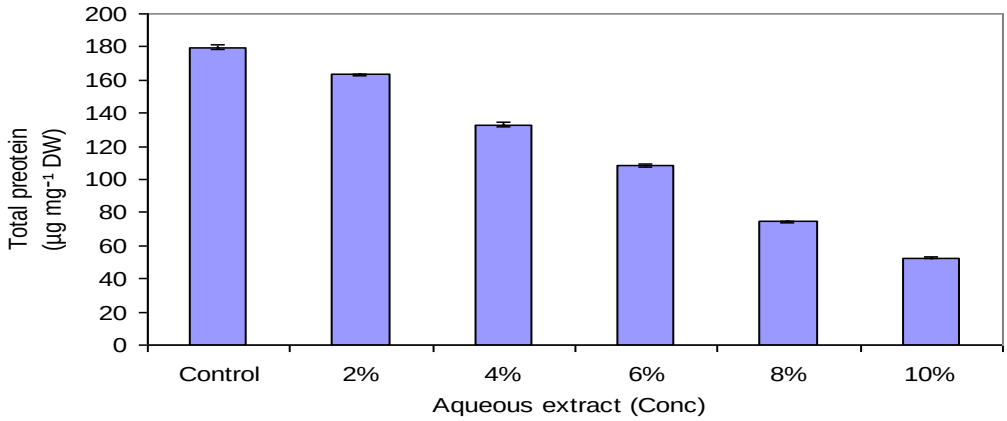

Fig.10 Effect of aqueous leaf extract of *T. africanum* on total protein of *P. oleracea*

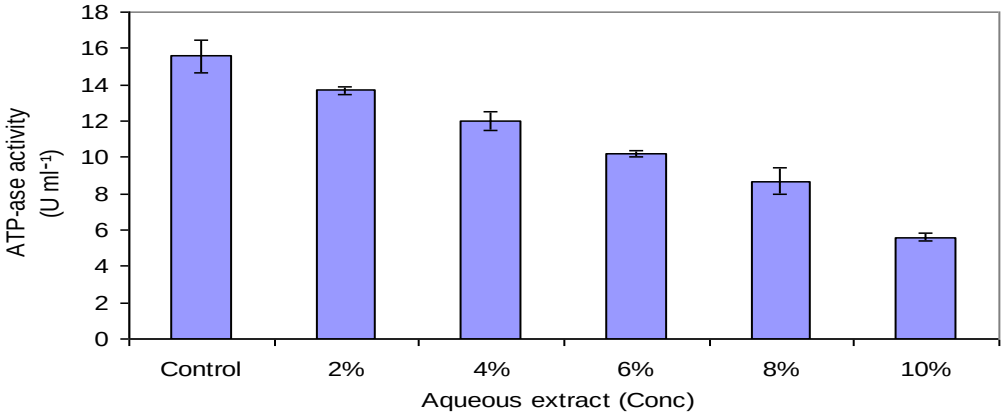

Fig.11 Effect of aqueous leaf extract of *T. africanum* on ATP-ase activity

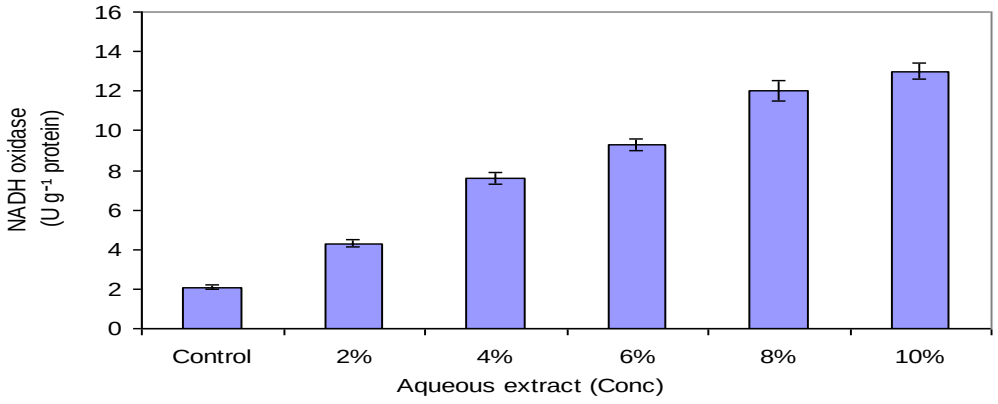

Fig.12 Effect of aqueous leaf extract of *T. africanum* on NADH oxidase in leaves of *P. oleracea*

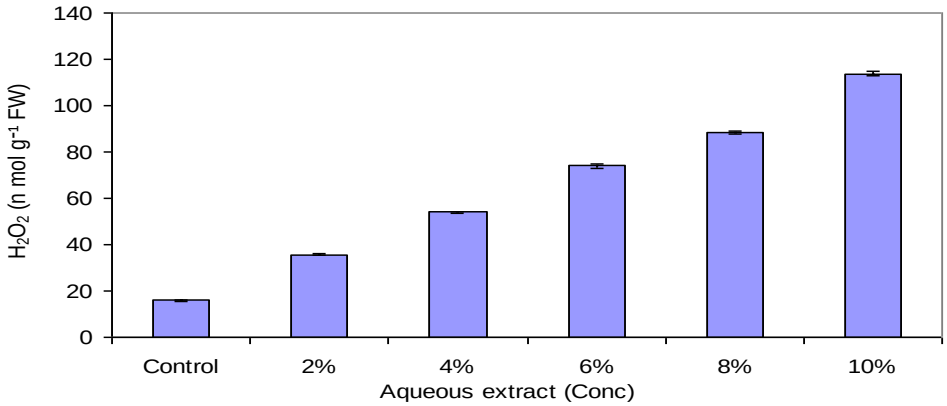

Fig.13 Effect of aqueous leaf extract of *T. africanum* on H_2O_2 concentrations of leaves of *P. oleracea*

The results indicate a reduction in ATP–ase activity in root of *P. oleracea* (Fig. 11) is concentration – dependent. ATP–ase is responsible for generation of proton electrochemical gradient (Michelet and Bountry, 1995). Thus, it indicates the driving force for the uptake, efflux of ions and metabolites across the plasma membrane (Palmgren, 2001).

ATP-ase inhibition by leaf extract possibly leads to reduction of mineral and water uptake by roots. This affect the essential plant functions such as photosynthesis and consequently reduction of growth.

The present results show an increase in H_2O_2 content (Fig 12) in *P. oleracea* leaves under the present treatment. This increase in NADH-oxidase activity is associated with the increase in H_2O_2 content activity (Fig. 13).

The increase in H_2O_2 may be attributed to the resulting reactive oxygen species (ROS) by allelochemicals (El-Shora et al., 2004). The increase in NADH-oxidase activity may cause production of superoxide radical which is harmful kind of ROS (Ding et al., 2007). H_2O_2 causes substantial damage to plants by destruction of proteins, lipids and nucleic acids (Collen and Pedersen, 1996). The production of H_2O_2 as a result of using oxygen as electron acceptor in respiration and photosynthesis is therefore potentially detrimental to the plant. H_2O_2 disturbs photosynthesis by inhibiting a number of enzymes in the Calvin cycle such as fructose bisphosphatase, ribulose phosphatekinase, and ribulose bisphosphate carboxylase/ oxygenase (Rubisco) (Badger et al., 1980; Tanaka et al., 1982; El-Shora, 2003).

In conclusion, treatment of *P. oleracea* seedlings with leaf extract of *T. africanum* led to reduction in germination percentage. Also, the other criteria of growth were reduced. Therefore, the results suggest using the aqueous leaf extract of *T. africanum* as bio-herbicide to control weeds such as *P. oleracea*.

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