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*Full Length Research Paper*

# Allelochemical effect of *Trianthema portulacastrum* L. on *Amaranthus viridis* L. supports the ecological importance of allelopathy

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**The effect of allelopathy on many aspects of plant ecology including occurrence, growth, and structure of plant communities, dominance, and diversity has had a check red history among ecologists. Field studies showed the increase of *Amaranthus viridis* density in an infested area by *Trianthema portulacastrum* more than uninfested area, while no difference in soil physicochemical characters at both areas. Bioassay was done under two different temperatures 25 and 35°C with different types of *T. portulacastrum* leaf and stem extracts. The untreated seeds showed very small germination percent at the high temperature, while the treated seeds showed very high percentage. Different types of extracts had highly significant increase in seed germination, radicle and plumule growth of *A. viridis*. Treatment by *T. portulacastrum* extracts increased soluble protein, amylase and total phenol of *A. viridis*. Stimulation of all the previous parameters was higher in high temperature than in the lower. HPLC analysis for *T. portulacastrum* leaf and stem estimated eight phenolic compounds. The present study supports the ecological importance of allelopathy in Agriculture.**

**Key words:** Allelopathy, *Trianthema*, *Amaranthus*, stimulation, ecology.

## INTRODUCTION

Allelopathy is a chemical interaction between plants that includes stimulatory (Sogaard and Doll, 1992) as well as inhibitory influences (Callaway and Aschehoug, 2000; Huang et al., 2000). The majority of studies on allelopathy are concerned with inhibitory effects ignored in the stimulatory effects. Allelopathy as a mechanism of plant interference in agro ecosystems (Putnam and Duke, 1974) offers an opportunity to manage weeds in a crop sequence (Kobayashi, 2004), but could also adversely affect crop yields (Kimber, 1973)) and influence choice of rotation. It has increasingly received attention by scientists and has played important roles in plant biodiversity and sustainable agriculture.

The ecological significance of allelopathy in plant communities has had a check red history among ecologists (Fitter, 2003). Stowe (1979) stated that species can be shown to have allelopathic properties in bioassays, and that bioassay may, for many communities, have no ecological meaning. Bais et al. (2003) have highlighted a potentially important ecological role for allelopathy in exotic plant invasion. *Trianthema portulacastrum* (horsepurslane) is one of the serious weeds of cotton and maize crops in many countries and could reduce crop yields by 32% (Balyan and Bhan, 1989). Recently Hussain et al. (2011) reported that *T. portulacastrum* possess strong anthelmintic activity. *Amaranthus* species are widely distributed throughout the world's temperate and tropical regions even before man converted some of them into cosmopolitan weeds and domesticated others (Sauer, 1967). The very high number of seeds produced, prolonged seed viability (Weaver and McWilliams, 1980) and seed dormancy (Baskin and Baskin, 1977) result in great accumulations of *Amaranthus* seeds in the soil. They are able to compete with crops for water, nutrients and light, causing severe reductions in yield, quality and harvest efficiency

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(Vangessel and Renner, 1990).

*A. viridis* considered as one of the fast growing annuals of weed is widespread throughout different habitat types in Egypt (Kosinova, 1975; Shaltout et al., 1992). Previous studies proved the inhibition effect of some plants on *Amaranthus* spp. growth, such as *Polygonum sachalinense* (Izhaki, 2002) and coffee plants (Rizvi et al., 1981). On the other side, Niel and Rice (1971) reported the stimulation effect of *Ambrosia psilostachya* on *A. retroflexus*. Horse purslane (*T. portulacastrum*) recently has become one of crop fields at Beni suef and Fayoum regions at Egypt, (author's personal observation). Field observations reveal the increase of *A. viridis* to establish within patches of *T. portulacastrum*. In the present work, field and experimental studies for the role played by the allelopathic potential of *T. portulacastrum* in the increasing of *A. viridis* were done searching its positive association pattern. It is well known that interactions with different environmental factors play an important role in the expression of allelopathy (Kobayashi, 2004; Tang et al., 1995). We designed to determine if temperature, as an important germination factor for the target species (Dixon and Paiva, 1995), would interact with and alter allelochemical effects from *T. portulacastrum* on *A. viridis*.

## MATERIALS AND METHODS

### Field study: Density of *A. viridis*

The selected study sites were agricultural lands in Fayoum region (29° 18' 28N, 30° 50' 24E), Egypt. 20 stands each of 10 quadrates (1×1 $m^2$) was randomly plotted in an infested and uninfested areas with *T. portulacastrum*. The density of *A. viridis* in each of the stands was recorded. Soil samples were collected from the studied stands and its physicochemical character analyzed according to methods of Jackson (1962).

### Plant and seeds collection

Field grown *T. portulacastrum* plant were harvested at maturity and dried under shade for a few days. Seeds were collected from matured *A. viridis* from fayoum, Egypt, and were stored in paper bags at 20 ± 2°C in the laboratory until germination tests were performed.

### Extraction and bioassay methods

Leaves and stems from well dried *T. portulacastrum* plants were chopped into about 2 cm pieces with fodder cutter. Chopped plant material was dried in an oven at 70°C for 48 h. The oven dried material was ground in a grinder and passed through a 40 mesh screen, then separately extracted by soxhlet with Dichloromethanol (DCM), methanol, and later with ethanol. Extracts to be assayed were dissolved in DMSO (Dimethyl sulfoxide) and diluted with distilled water to reach the test concentration 100 ppm. *Amaranthus* seeds were sown in 9 cm diameter Petri dishes moistened with 5 ml of various extracts, while control received distilled water in equal amount. Petri dishes were divided into two groups, one placed in the dark at 25 ± 1°C and the other group were placed at 35 ± 1°C for 10 days, the previous two temperature degrees were selected from a preliminary experiment. Each treatment was replicated five times.

### Extraction and determination of soluble proteins

Proteins were extracted by placing an aliquot of known fresh weight of plant tissue (approximately 2 g blotted dry) and one gram of sand in a clean mortar at 1 to 5°C. Then 5 ml of 0.3 M cold phosphate buffer (pH 6.8 to 7) was added and grinded until a fine tissue – water slurry is produced, after that 20 ml distilled water was added and grinded again. Pour tissue extract into 15 ml centrifuge tubes with centrifuged at 3.000 g for 15 min or until clear, supernatant was taken as the total protein extract. Total soluble proteins were determined quantitatively according to Bradford method (1976). One ml of the soluble protein extract was diluted to 1:1 with distilled water. 0.1 ml from diluted tissue extract mixed with 5 ml Coomassie Blue reagent. After 30 min, the absorbance was read at 595 nm against water blank. The protein concentration was determined by using bovine serum albumin standard curve, and then calculates the free protein content as mg protein $g^{-1}$ fresh weight.

### Extraction and assay of amylase

The extraction of crude enzyme was carried out by the method cited by Monerri et al. (1986). A known weight of fresh plant tissue was ground with cold 100 mM sodium acetate buffer pH 5.5 (containing 10 mM $CaCl_2$ and 1 mM NaCl) using fine sand in porcelain mortar. The mixture was centrifuged at 10,000 g for 10 min. The clear supernatant was completed up to a known volume with sodium acetate buffer. Amylase activity was assayed by the method adopted by Bilderback (1974). Crude enzyme extract (0.1 ml) was mixed with 0.5 ml starch solution. The reaction was performed at 37°C for 15 min, and then stopped by adding 1 ml of iodine reagent. The volume of the reaction mixture was completed up to 3 ml with distilled water. The control was processed as the samples, but the reaction stopped at zero time. The absorbance of blue colour was recorded at 620 nm. The difference in reading between control and sample was proportional to enzyme activity. The total amylase activity was expressed as the decreases in absorbance per gram per unit time fresh weight.

### Extraction and determination of phenolic compounds of *Amaranthus*

Phenolic compounds were extracted from dried shoot tissues according to the method outlined by Jindal and Singh (1975). A known weight of the powdered tissues was extracted three times with 80 % aqueous ethanol, and each extract centrifuged at 3000 g for 20 min. The clear supernatants were combined, evaporated under reduced pressure and completed up to a known volume with water. This extract contained soluble phenolic aglycones and glycosides. The phenolic glycoside was hydrolyzed with one ml of 2 N HCl in a boiling water-bath for 30 min to cleave the glycosidic bond. After cooling, the extract was neutralized and made up to known volume with distilled water. Folin-Ciocalteau phenol reaction (A.O.A.C., 1990) was used to estimate the phenolic content in extracted tissues. One ml sample was mixed with 1 ml of Folin-Ciocalteau phenol reagent and one ml of 20% anhydrous sodium carbonate, and then completed up to 5 ml with distilled water. The absorbance of the blue colour was measured after 30 min. at wavelength 650 nm against water-reagent blank. The phenolic content was obtained from a standard curve of pyrogallol, and then calculated as mg phenolic $g^{-1}$dry weight. Subtraction of phenolic aglycone content before and after hydrolysis gave the phenolic glycosides content.

**Identification of phenolic composition in methanol extract of both steam and leaf of *T. Portulacastrum* by HPLC**

The standard phenol compounds used for HPLC analysis were (P-Hydroxybenzoic acid, Caffeic acid, Vanillic acid, Ferulic acid, o-coumaric acid, Pyrogallic acid, Protocatechuic acid and trans-Cinnamic acid). The methanol extracts of both stem and leaf were acidifying with dilute phosphoric acid to pH 2.5, and then partitioned two times with an equal volume of diethyl ether. The combined diethyl ether layers were evaporated to dryness and the resulting residue was dissolved in dissolved in HPLC grade MeOH to give 1000 p.p.m. a 20 µl was injected into HPLC. The HPLC analysis were carried out on a Shimadzu class-LC 10 AD chromatograph system (Shimadzu, Japan) supplied with shimadzu SPD-10 AUV-VIS. The used Column is phenomene × C18 (25cm 4.6 mm i.d, with 5 µm particle size). SGE Syringe (50F-LC) with 50 µl fixed loop used for injection.

**Statistical analysis**

All data of germination and seedling growth were subjected to the least significant difference (LSD) test to determine the significant differences among the mean values at the $P < 0.05$ and $P < 0.01$ probability levels, using a 'general linear model' procedure of the Statistical Analysis System (SAS) program.

## RESULTS

### Field studies

Table 1 show that no significantly difference in soil physicochemical character in each of infested and uninfested lands by *T. portulacastrum*. In general, the results revealed that *A. viridis* density in the infested area was significantly higher than that in the uninfested areas by more than five folds (t-test, $P < 0.05$).

### Effect of extracts on seed germination and seedling growth

High temperature significantly decreased the seed germination of *A. viridis* (Figure 1) and all different types of *T. portulacastrum* extracts from both leaf and stem had high statistically significant ($p = 0.01$) on seed germination, by more than 22 folds. Growth of *A. viridis* radicle and plumule at both treatment temperatures showed higher significantly increase (Figure 2). No significantly difference obtained among different extract types on seed germination or radicle and plumule growth (Figures 2 and 3). Leaf methanol extract showed the highest effect on radicle and plumule at 25°C (Figure 2a). On the other hand leaf ethanol extract exhibited the highest effect on radicle and plumule at 35°C (Figure 2). DCM extract from leaf and stem exhibited the lowest effect on seed germination and seedling growth at both temperature treatments (Figures 1 and 2). Methanol stem extract showed the highest effect at both temperature treatments on plumule growth, and the highest effect on radicle at only 25°C while ethanol extract gave the highest effect at 35°C (Figure 3).

### Effect of Extracts on biochemical compounds of the target plant

Both leaf and stem extracts increased soluble protein, amylase, phenol aglycone and total phenol at 25°C (Figures 4 to 8). This increasing was more at 35°C for all above parameters except phenol aglycone which exhibited a decrease by about 15 and 20% when treated with leaf and stem extracts respectively (Figure 6). The highest increase was recorded in phenol glycoside content at 35°C when treated with ethanol and methanol stem extracts. Table 2 shows the HPLC analysis of *T. portulacastrum*. Eight phenolic compounds were identified and quantified in the methanol extract of *T. portulacastrum* using HPLC analysis.

## DISCUSSION

The higher density of *A. viridis* in the vicinity of *T. portulacastrum* might be related to the presence of allelochemicals in the soils through leaching from above-ground tissue, root exudation, volatilization, or decomposing dead plant tissue. The recorded similarity in soil texture and no clear difference between the soil nutrients (chemical contents) at both localities could be preliminary evidence that soil texture properties and competition are not the determinant factors in *A. viridis* density.

Various studies documented the influence of temperature on the germination characteristics of *Amaranthus* species, such as *A. retroflexus* seeds, (Guo and Al-Khatib, 2003; Steckel et al., 2004). Germination results obtained from the present work differ from many previous studies. Cristaudo et al. (2007) reported that full germination (90 to 100%) of *A. gracizans* occurred at temperatures ranging between 30 and 40°C. Bavec and Mlakar (2002) recommended for more than 80% emergence in sandy loam a temperature above 21°C for *Amaranthus*. Webb et al. (1987) found that the fastest emergence and the highest percentage emergence occurred between 24 and 34°C. The difference in our results can be explained due to the age of used seeds. The age of the seeds used in the present study was only one month after harvest; this age may be the reason of the narrow temperature range. It was stated that ageing of seeds and the consequent release of dormancy extend the range of temperatures at which seeds germinate in *Amaranthus* and other species (Baskin and Baskin 1998, Baskin et al., 2002). Niel and Rice (1971) reported that rhizosphere soil from *A. psilostachya* stimulated the growth of several plant species, among them *Amaranthus retroflexus*, that occurred in the same field. The results of

**Table 1.** Density of A. *viridis* and soil physicochemical characters in infested and uninfested areas by *T. portulacastrum.*

| Variable | Uninfested | Infested | LSD at 5 % |
|---|---|---|---|
| Density (plants $m^{-2}$) | 3 ± 0.2 | 17 ±2.8 | 2.5 |
| % sand | 58.00±6.5 | 56.8±14.2 | 5.2 |
| % clay | 23.80±4.3 | 22.3±2.2 | 3.6 |
| % loam | 18.20±1.2 | 20.9±6.1 | 3.4 |
| pH | 7.6±0.9 | 7.7±1.1 | 0.9 |
| Organic matter (%) | 1.1±0.2 | 1.3±0.08 | 0.9 |
| Nitrogen (%) | 0.26±0.03 | 0.24±0.04 | 0.2 |
| Potassium (mg 100 g−1) | 1.58±0.2 | 1.47±0.03 | 0.8 |
| Sodium (mg 100 g−1) | 3.2± 0.5 | 3.01±0.14 | 0.6 |
| Phosphorus (mg 100 g−1) | 18.00±3.2 | 16.80±2.3 | 0.4.1 |

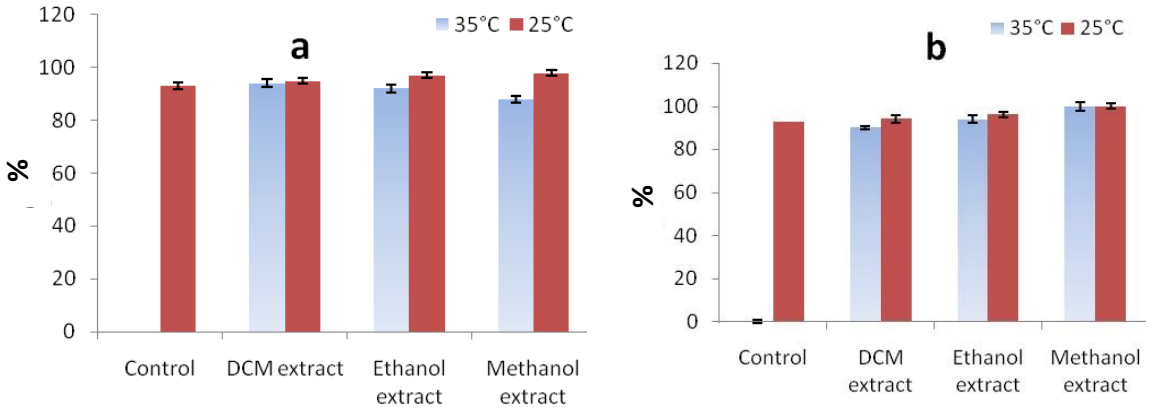


**Figure 1.** Effect of different extracts of *T. portulacastrum*, leaf (a) and stem (b) on seed germination of *A. Viridis*. DCM: Dichloromethanol, Bars indicate the standard deviation.

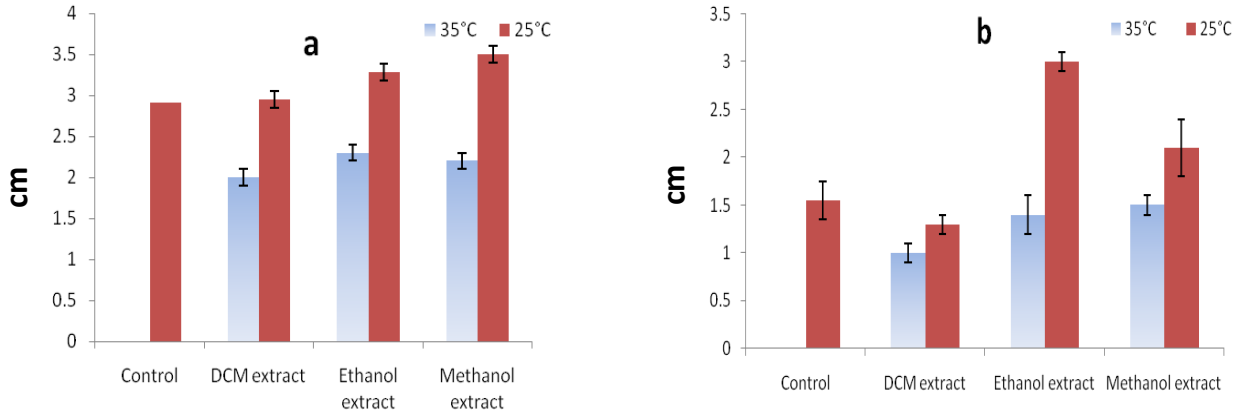


**Figure 2.** Effect of *T. portulacastrum* leaf extracts on radicle growth (a) and plumule growth (b) of *A. Viridis*. DCM: Dichloromethanol, Bars indicate the standard deviation.

this study illustrate that temperature stress enhances allelochemical stimulation and a combination of allelopathic and environmental conditions (as temperature in the present study), may alter growth of species. This

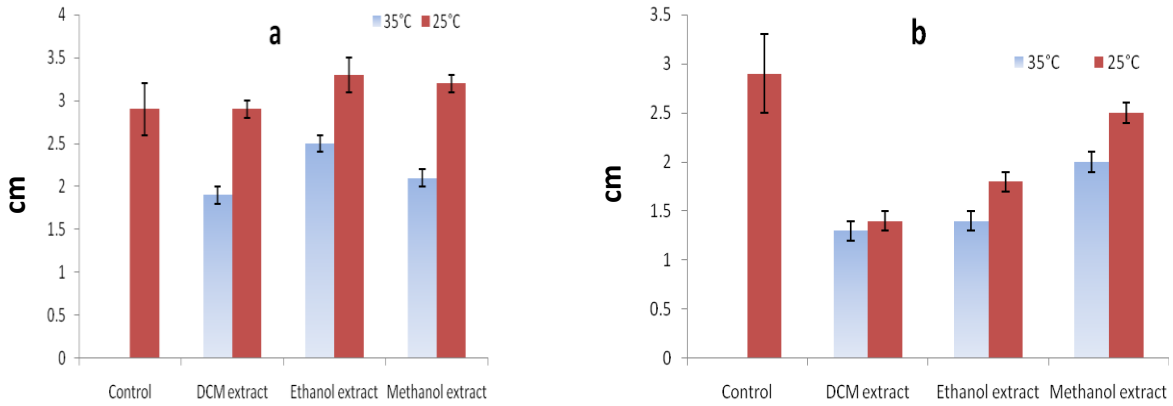


**Figure 3.** Effect of *T. portulacastrum* stem extracts on radicle growth (a) and plumule growth (b) of *A. Viridis*. DCM: Dichloromethanol, Bars indicate the standard deviation.

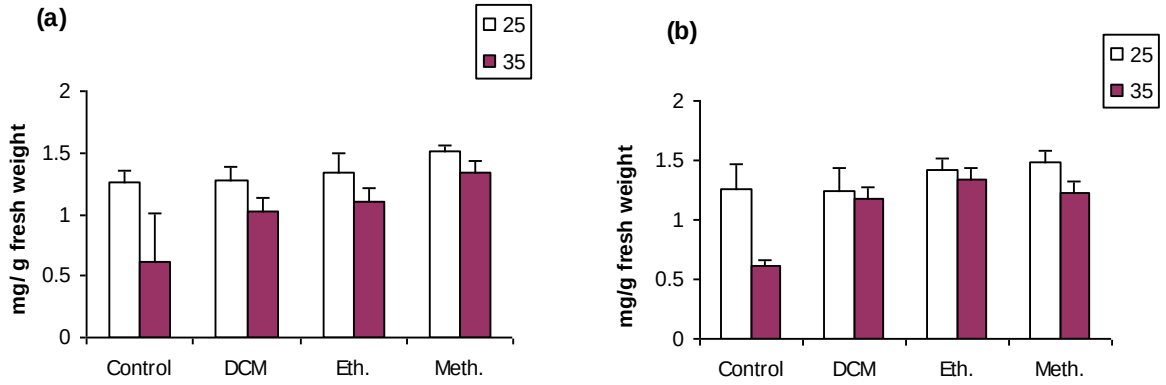


**Figure 4.** Effect of Leaf (a) and stem (b) extract on protein content of *A. viridis*. Bars indicate the standard deviation.

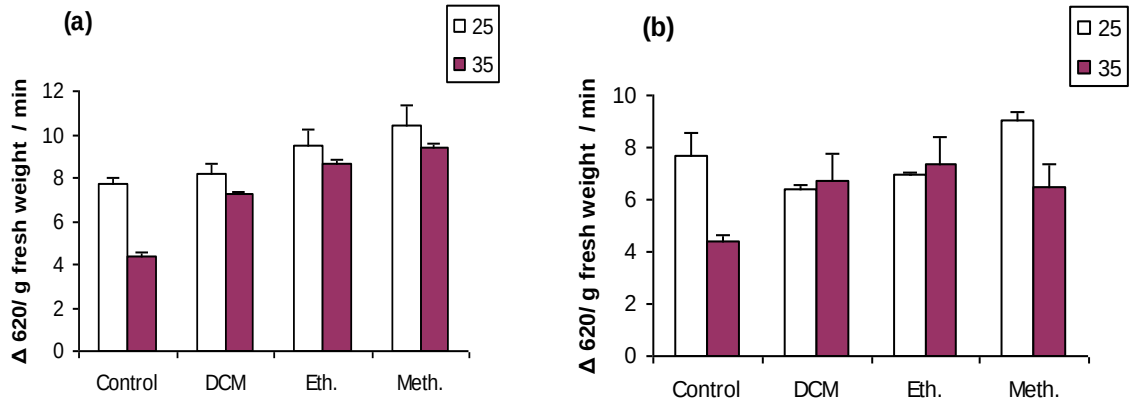


**Figure 5.** Effect of Leaf (a) and stem (b) extract on amylase activity *A. viridis*. Bars indicate the standard deviation.

observation supports the discovery of an ecologically important allelopathic interaction with environmental factors (Bais et al. 2003). Environmental stresses both abiotic and biotic are often implicated as very important

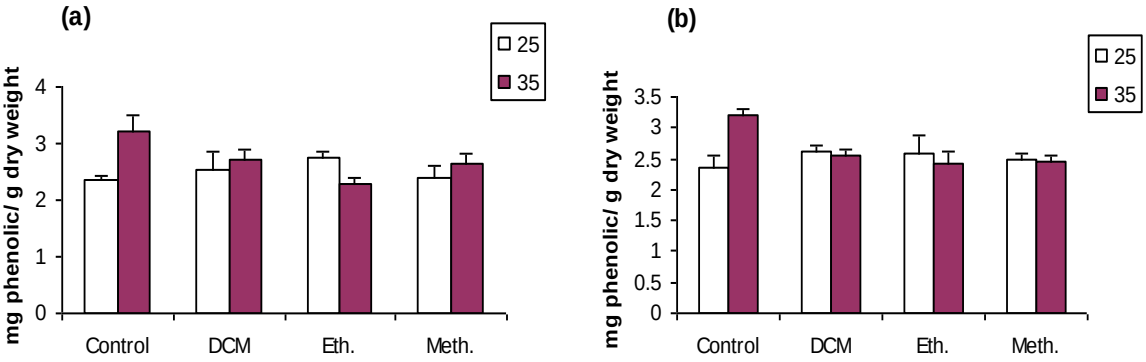


**Figure 6.** Effect of Leaf (a) and stem (b) extract on aglycone content of *A. viridis*. Bars indicate the standard deviation.

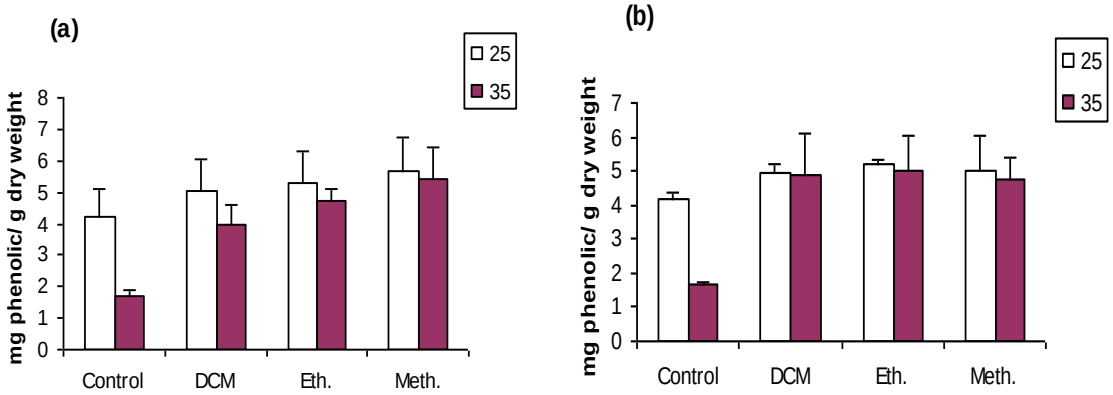


**Figure 7.** Effect of Leaf (a) and stem (b) extract on Glycoside content of *A.viridis*. Bars indicate the standard deviation.

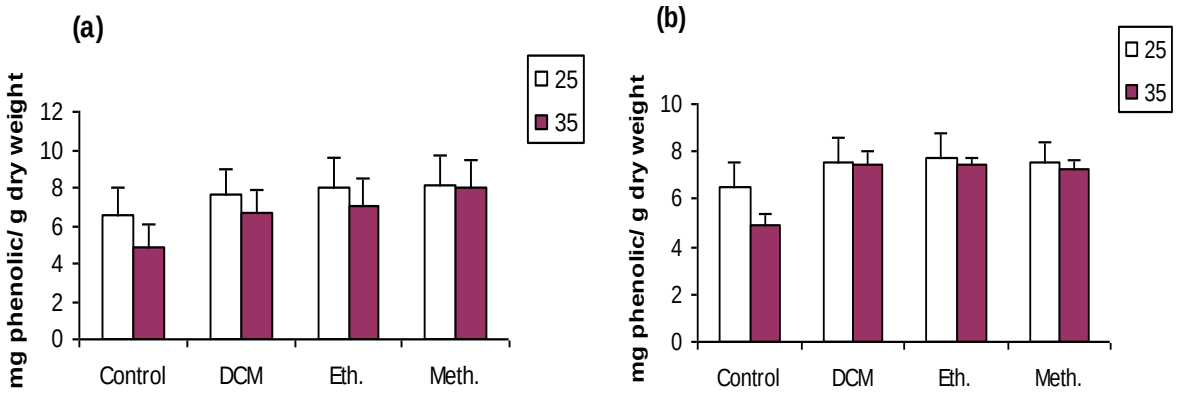


**Figure 8.** Effect of Leaf (a) and stem (b) extract on total phenol of *A. viridi* bars indicate the standard deviation.

factors changing allelopathy manifestation in nature (Anaya, 1999, Einhellig, 2004). Moreover, Nayyar (2002) and Reigosa et al. (2002) stated that allelopathy is more pronounced and could be important when acceptors are affected by environmental stresses, especially when they are severe. Previous reports have suggested an interaction of allelochemical inhibition with temperature. Steinsiek et al. (1982) reported that the inhibitory action

**Table 2.** Quantitative determination of HPLC analysis on some phenolic compounds (± S.E.) present in leaf and stem of *T. Portulacastrum*.

| Plant part | Compound | Total concentration (µg $g^{-1}$ Dry weight) |
|---|---|---|
| Leaf | P-Hydroxybenzoic acid | 3.21± 0.3 |
| | Caffeic acid | 1.24± 0.1 |
| | Vanillic acid | 5.21± 0,6 |
| | Ferulic acid | 21.03± 1.2 |
| | o- coumaric acid | 17.1± 1.1 |
| | Pyrogallic acid | 1.55± 0.3 |
| | Protocatechuic acid | 11.23± 0.9 |
| | trans-Cinnamic acid | 4.46± 0.1 |
| Stem | P-Hydroxybenzoic acid | 3.04± 0.1 |
| | Caffeic acid | 0.00 |
| | Vanillic acid | 3.15±0.3 |
| | Ferulic acid | 7.01± 0.8 |
| | o- coumaric acid | 14.36±1.2 |
| | Pyrogallic acid | 2.09±0.1 |
| | Protocatechuic acid | 9.21±0.9 |
| | trans-Cinnamic acid | 6.17±0.4 |

of extracts from wheat on weed-seed germination and growth was temperature-dependent, and several of the weeds they tested had the greatest inhibition at 35°C. There is common opinion that environmental stress usually, though not always, increase allelopathic effectiveness of the target plants and this is achieved by increased concentrations of allelochemicals and/or by lowering of the threshold of allelocompound concentrations, at which the effects take place (Einhellig, 2004).

The increase of phenolic glycoside content over the aglycone indicates the ability of plants to protect themselves against the external conditions. It is widely accepted that the production of secondary metabolite, particularly phenolic compounds, can play direct role in self-defense and plant protection to cope the stress created by external conditions (Bennett and Wallsgrove, 1994; Dixon and Paiva, 1995). Phenolic compounds are widely spread in different plant tissues and usually conjugated with sugars as glycosides which is a non-toxic, more water soluble and easily transport (Hrazdina and Wanger, 1985; Gutierrez et al., 1996). This result supports the evidence that phenolic glycosides act as defensive and protective compounds in plants (Kobayashi, 2004). Shvets et al. (1996) reported that steroidal glycoside is known to posses' wide range of biological activity including plant growth promotion. The enhancement of amylase activity could accelerate starch hydrolysis and hence the utilization of soluble sugar during seed germination. The adverse changes in amylase activity could be attributed to interference of allelochemicals with enzymes action (Einhellig, 1996). The increase in solouble protein when treated with different types of extarcts in agreement with results obtained by of Baziramakenga et al. (1995). It was found that some allelochemicals stimulate protein synthesis by increasing the incorporation of amino acid into protein in seedlings (Inderjit and Nayyar, 2002). The high effect of *Trianthema* extracts on *A. graecizan* may be due to of stimulatory compounds contained. Several compounds from secondary metabolism have been described as having germination-stimulatory activity (Bouwmeester et al., 2003; Keyes et al., 2001; Tang et al., 1995). Some of the phenolic acids similar to that identified in *T. portulacastrum*, (Table 2), were reported to play an important role in allelopathic interactions, and their biological activities on growth of some crop plants and weeds were studied using different bioassay tests (Chung et al., 2002).

## Conclusion

The current results can lead to a better understanding of the positive association between *T. portulacastrum* and *A. viridis* in the field and support the ecological importance of allelopathy. We recommend to effectively controlling such weeds (*A. viridis*) depleting the soil seed banks by stimulating germination of dormant seeds by adding *Trianthema* extracts and then eliminating the seedlings with herbicides.

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