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## Research Article

# Allelopathic Potentials of *Artrmisia monosperma* and *Thymus vulgaris* on Growth and Physio-Biochemical Characteristics of Pea Seedlings

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## Abstract

**Background and Objective:** Several medicinal plants are reported as having allelochemicals potentials and favorable results have been obtained in this regard. The present investigation aimed to evaluate the effect of different concentrations of leaf aqueous extract of two medicinal plants, *Artrmisia monosperma* and *Thymus vulgaris* on growth criteria, physio-biochemical attributes and antioxidant enzyme activities of pea (*Pisum sativum* L.) seedlings. **Materials and Methods:** Germination of pea was performed in Petri dishes under different concentrations (0, 2, 4, 6, 8 and 10%) of leaf aqueous extracts of the two medicinal plants collected from natural habitats in Saudi Arabia. At the end of experiment (10 days), the growth and physio-biochemical characteristics of pea seedlings were measured. The data were statistically analyzed by one-way ANOVA analysis of variance using SPSS program. **Results:** Leaf aqueous extracts of *A. monosperma* and *T. vulgaris* reduced germination capability, shoot and root length, total free amino acids and proline content. Contrariwise, there was an increase in carbohydrates, proteins, K<sup>+</sup>, Ca<sup>2+</sup> and the activity of antioxidant enzymes. Lipid peroxidation and H<sub>2</sub>O<sub>2</sub> contents were significantly enhanced with increasing concentrations of both extracts. The highest inhibitory or stimulatory effects of leaf aqueous extract were observed at concentrations 8 and 10%. *T. vulgaris* was more effective than *A. monosperma*. **Conclusion:** This investigation indicated that the two medicinal plants had allelopathic compounds with strong potential, which may play important role in weed control and used as an alternative of chemical compounds. The aqueous extract of *T. vulgaris* has allelopathic potential more than *A. monosperma* and could be evaluated as an allelopathic species.

**Key words:** Antioxidant enzymes, allelopathy, carbohydrate, proline, lipid peroxidation

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Many plants including medicinal plants were reported to interact chemically with other plant species<sup>1</sup>. Such chemical interaction is known as allelopathy. The process of allelopathy is any direct or indirect damaging effect by one plant on another plant by production of chemical compounds (Allelochemicals) that leakage into the other plant, depending on their doses leached to plant environment<sup>2</sup>. These chemicals are present in the different parts of plants and released into the environment by root exudate, filtrate from aboveground parts and volatilization or decomposition of plant material<sup>3</sup>. Leaves consider the most consistent producers of allelopathy substances<sup>4</sup>. Allelochemicals can alter the contents of plant growth regulators or induce imbalances in various phytohormones, which inhibit the growth and development of plants<sup>5</sup>. Allelopathic inhibition may be due to the toxic effects of a single compound or the interaction of a group of some chemicals<sup>6,7</sup>. The complexity, interaction and concentration of allelochemicals control the effect on germination and growth of plant<sup>8-11</sup>. The allelochemicals which inhibit the growth of some plants at a certain concentration may stimulate the growth of the same and different plants at different levels<sup>12</sup>. Dongre and Yadav<sup>13</sup> found reduction of lentil dry weights when treated with water extracts of various weeds. Kakati and Baruah<sup>14</sup> showed that germination of seeds and seedling growth of mung bean were inhibited by different concentration of leaf extract of *Azadirachta indica* and *Paederia foetida* Linn. Many investigators have reported the effect of allelochemicals on physio-biochemical processes of the recipient plants<sup>15-17</sup>. Huang *et al.*<sup>18</sup> indicated that the allelochemicals of *Alternanthera philoxeroides* have stimulatory effect on germination, growth and some metabolic activities of *Zoysia matrella* at lower concentrations, while they have inhibitory effects at higher concentrations.

*Artemisia* (*Artemisia monosperma*) and thyme (*Thymus vulgaris*) are important medicinal plants in Saudi Arabia traditional system of medicine and traditionally have been used for several medicinal purposes. *A. monosperma* is a common plant in deserts of Saudi Arabia and one of the most common medicinal species of artemisia<sup>19</sup>. It contains bioactive compounds such as sterols, terpenes, flavonoids, saponins and tannins<sup>20</sup>. The quantitative analysis of aqueous extract of *A. monosperma* contained the phenolic compounds and flavonoids that might be implicated as allelochemicals agents<sup>21</sup>. *T. vulgaris* is used as spice, ornamentals and exporter of essential oil<sup>22</sup>. Soliman and Zatout<sup>23</sup> revealed that the volatile oils of thyme species had toxic effect on germination of seed and growth of some plants.

Pea is one of the most important leguminous crops in Saudi Arabia, but its production is limited by allelochemicals<sup>24,25</sup>. It is utilized for various purposes like fresh peas, dry pulses and edible podded type. It considers an important source of carbohydrate and protein, water-soluble fibers, vitamin B1 and antioxidants<sup>26</sup>.

Using of chemical compounds is reducing due to their negative effects in the products of agriculture. So, the investigators are looking for new methods to use the natural compounds as an alternative of chemical compounds. The use of effective compounds of herbal has considered as natural compounds. Allelopathic effect of medicinal species against temperate crop is well studied<sup>8,23,27</sup>.

The local communities of Saudi Arabia have been using the medicinal plant species for curing various diseases for a long time. The beneficial medicinal effects of these plants result from their secondary compounds. The effect of these chemicals is not limited to animals and human body alone, but also on plants. However, some studies on evaluation of allelopathic effects of some medicinal plants have been attempted and favorable results have been obtained in this regard, while information about the allelopathic of the tested medicinal plants on pea plant is limited. Therefore, the study aimed to investigate the comparative evaluation of allelopathic activities of *A. monosperma* and *T. vulgaris* on seed germination, seedling growth and some physio-biochemical parameters of pea plant. In addition, recognition of new medicinal plants containing allelopathic compound with strong potential, which may play important role in weed control, is very important to reduce application of synthetic herbicides in the agricultural systems.

## MATERIALS AND METHODS

**Collection of plant materials and preparation of allelopathic aqueous extracts:** Laboratory studies were conducted within the period of March-December, 2016 in Dammam University, Saudi Arabia. Fresh and healthy leaves of two medicinal plants *Artemisia monosperma* and *Thymus vulgaris* were collected at maturity from their natural habitats in Saudi Arabia to evaluate the allelopathic potentials of their aqueous extracts on growth and some metabolic activities of pea seedling. The leaves materials were dried in an oven for 48 h. The dried materials were powderized into fine powder and packaged in paper bags for further use. The allelopathic water extracts were prepared by mixing 100 g leaf powder in 1000 mL of distilled water and allowed for 48 h in dark with occasional shaking and kept in at 25°C. After 24 h, the solutions were filtrated and centrifuged at 12000 rpm, then the clean and pure extracts which were collected completed

to 1000 mL, this give 10% aqueous extract and kept at 4°C for use. The extract was considered as a stock solution with 10% concentration. From stock solution (10%), the other concentrations, 2, 4, 6 and 8% can be made.

**Seed germination:** Germination test was performed in Petri dishes under different concentrations (0 (distilled water), 2, 4, 6, 8 and 10%) of the allelopathic water extract of the two medicinal plants (*A. monosperma* and *T. vulgaris*). Healthy and uniform seeds of pea (*Pisum sativum* L.) were sterilized with 1% sodium hypochlorite and washed with distilled water more than one time. Fifteen seeds were placed in sterilized Petri dishes provided with two layers of filter paper, moistened with suitable amount of different concentrations of allelopathic aqueous extracts of the two medicinal plants. Distilled water (0%) was used as a control. Three replicates were prepared from each concentration. To keep the filter paper moist with aqueous leaf extracts, equal volume of distilled water was added to the Petri dishes when moisture content of the blotting paper declined. The Petri dishes were kept at room temperature (23±2°C). Germination was determined by counting the number of germinated seeds at 24 h intervals for successive 5 days.

**Harvesting:** At the end of experiment (10 days), the seedlings were harvested and washed thoroughly with sterile distilled water three times. Some of these seedlings were frozen in liquid nitrogen and the others were oven dried and powderized into fine powder and stored in sealed glasses at room temperature for later analysis.

**Determination of growth parameters:** Germination percentage, length of root and shoot (cm) and fresh and dry weight (g) of pea seedlings were measured. To determine fresh weight, the harvested freshly seedlings were rinsed with deionized water and blotted on paper towels before being weighed (fresh weight). To determine the dry weight, the freshly tissues are dried in an aerated oven at 70°C to constant weight.

**Physio-biochemical measurements:** Soluble, insoluble and total carbohydrates were extracted and measured according to Thayermanavan and Sadasivan<sup>28</sup> method. Protein fractions were determined according to Bradford<sup>29</sup>. Free amino acids content was determined according to the method of Lee and Takanashi<sup>30</sup>. Proline was estimated by method of Bates *et al.*<sup>31</sup>.

**Determination of mineral elements:** Dried powder samples (0.5 g) were transferred to 50 cm<sup>3</sup> digestion flasks and

supplemented by 2 mL perchloric acid 80% and 10 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and the flasks heated gently over a hot plate until the solution become colorless. Digested material diluted by double distilled water to 100 mL. The mineral elements Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> were determined in the extract solution by atomic absorption spectrophotometer model Perkin Elmer 3110 USA.

#### **Assay of antioxidative enzyme activities**

**Extraction of enzymes:** Pea seedlings (5 g) were powdered in homogenization buffer containing 50 mM potassium phosphate (pH 7.5), 1 mM EDTA, 0.1% Triton x-100 and 1.0% polyvinyl pyrophosphate (PVP). Then the homogenate was filtered throughout 4 layers of muslin fabric and subjected to centrifugation at 15,000×g at 4°C for 10 min and the supernatant was stored at 4°C and used for CAT, POD, APX and SOD assays.

**Catalase activity (CAT) (EC 1. 11. 1. 6):** It was measured as described by Claiborne<sup>32</sup>. One unit of catalase was defined as the amount that decomposes 1 mmol H<sub>2</sub>O<sub>2</sub> per min at 25°C and pH 7.8.

**Peroxidase activity (POD) (EC 1. 11. 1. 7):** The activity of POD was determined according to a modified method based on Reuveni and Reuveni<sup>33</sup>. One unit of peroxidase was defined as 1.0 milligram of purpurogallin formed from pyrogallol in 20 sec at pH 6.0 at 20°C.

**Ascorbate peroxidase (APX) (EC 1. 11. 1. 11):** Its activity was determined according to the method of Chen and Asada<sup>34</sup>. Ascorbic acid oxidation was recorded by decreasing the absorbance at 290 nm for 1 min.

**Superoxide dismutase (SOD):** It was determined by the method described by Spitz and Oberley<sup>35</sup>. One unit (U) of SOD is the amount of enzyme necessary to cause 50% inhibition of the colour reaction at 25°C and pH 7.8.

Each enzyme activity was expressed as enzyme unit per gram fresh weight of plant.

**Determination of hydrogen peroxide and lipid peroxidation:** The content of H<sub>2</sub>O<sub>2</sub> was assayed according to the method of Jena and Choudhuri<sup>36</sup>. Lipid peroxidation was determined by quantifying the concentration of malondialdehyde (MDA) using the thiobarbituric acid method described by Zhao *et al.*<sup>37</sup>. Amounts were calculated using an extinction coefficient of 155 mM<sup>-1</sup> cm<sup>-1</sup> and are given as nmol MDA g<sup>-1</sup> FW.

**Statistical analysis:** The data were statistically analyzed by one-way ANOVA analysis of variance using SPSS program. Values in the figures indicate the mean values  $\pm$ SD based on three independent determinations ( $n = 3$ ) and the Least Significant Difference (LSD) was used to test the differences between treatments and  $p \leq 0.05$  was considered statistically significant.

**RESULTS AND DISCUSSION**

**Growth parameters:** Allelopathic effects of leaf aqueous extracts of both *A. monosperma* and *T. vulgaris* on seed germination, root and shoot length and fresh and dry weight of pea seedlings had been shown (Fig. 1a-e). The data (Fig. 1a) showed that germination percentage was significantly

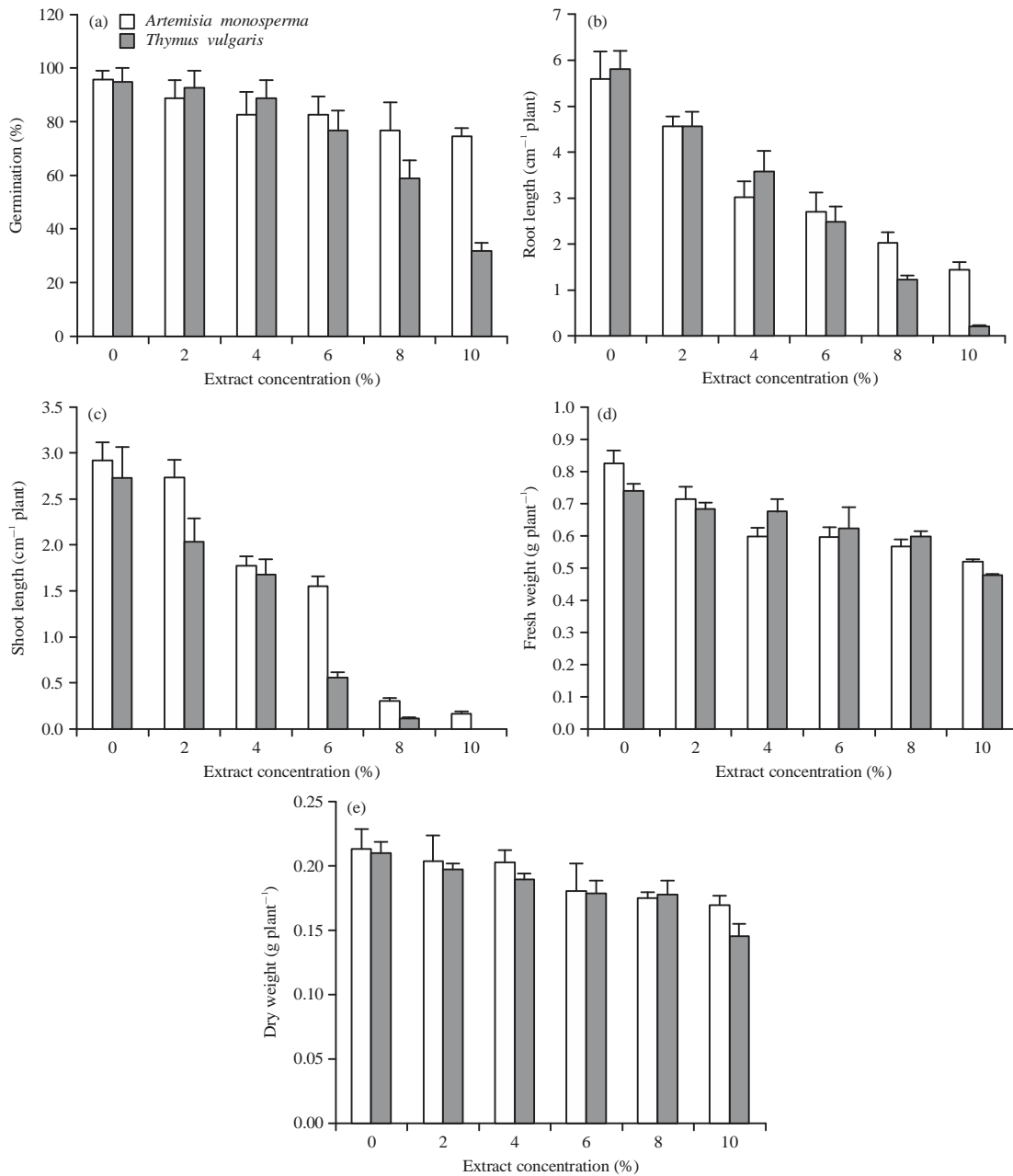


Fig. 1(a-e): Allelopathic effects of *Artrmisia monosperma* and *Thymus vulgaris* Leaf aqueous extract on (a) Germination%, (b) Root and (c) Shoot length (cm), (d) Fresh and (e) Dry weight ( $g^{-1}$  plant) of pea (*Pisum sativum* L.) seedlings. Vertical bars represent  $\pm$ SD

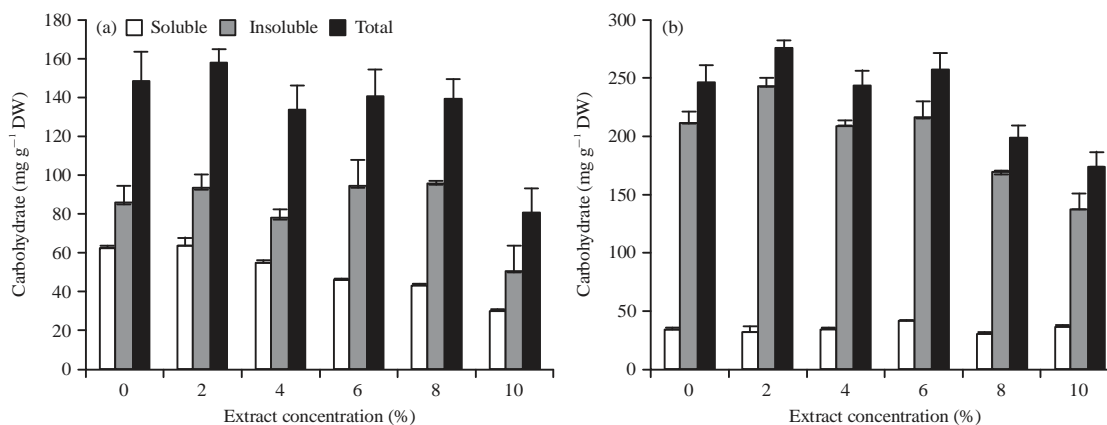


Fig. 2(a-b): Allelopathic effects of (a) *Artrmisia monosperma* and (b) *Thymus vulgaris* Leaf aqueous extract on carbohydrate contents (mg g<sup>-1</sup> dry weight) of pea (*Pisum sativum* L.) seedlings. Vertical bars represent ±SD

decreased with an increasing concentration of both leaf aqueous extracts. Compared to the control, maximum inhibitions were recognized under high concentration (10%) of leaf extracts. The highest reduction in seed germination treated with *T. vulgaris* was more obvious (32%) than *A. monosperma* (75%) as compared with control. This highly allopathic effect of *T. vulgaris* than *A. monosperma* may be due to the presence of different levels of allelochemicals in the leaf extracts of both.

Both extracts from *A. monosperma* or *T. vulgaris* significantly reduced the root and shoot length (Fig. 1b and c) with the increase in concentration of these aqueous extracts. The present results revealed that leaf aqueous extracts were more capable for inhibiting shoot length than root length of the pea seedlings in contrast with some studies, which revealed that root growth is more susceptible to allelochemicals than shoot growth<sup>38,39</sup>. The degree of inhibition varied with leaf extract and treatment. Ashrafi *et al.*<sup>3</sup> and El-Shora *et al.*<sup>16</sup> reported that the inhibition of plant growth depends on the concentrations of the allelochemicals and the inhibition of shoot length may be due to the presence of phenols which affect in cell division, biosynthetic processes and mineral uptake. It was worthy to note that there was no shoot length of pea seedlings under 10% leaf aqueous extract of *T. vulgaris*. This might be due to its lethal allelopathic effects on pea seedlings growth. These results were supported by Nasrine *et al.*<sup>40</sup> who stated that the germination efficiency, plumule and radicle length of *Bromus tectorum* were completely inhibited at the highest concentration of aqueous extracts of the donor species level (10%).

Compared with the control, no significant changes have been detected in fresh and dry weight pea seedlings (Fig. 1d and e) at low concentrations (up to 4%) of both leaf

aqueous extracts, while they were significantly decreased at higher concentrations. The reduction in dry weight at 10% was more pronounced (69.2%) in response to *T. vulgaris* than *A. monosperma* (79.4%) compared to control. These results are in agreement with those reported by other investigators<sup>11,41,42</sup>. The reduced germination and seedlings growth inhibition have been attributed to the presence of water soluble inhibitors (allelochemicals) which are leached out from *A. monosperma* and *T. vulgaris* leaves resulting in changing of some metabolic activities and could inhibit the elongation, expansion and division of cells for seedlings growth<sup>43,44</sup>. Cai and Mu<sup>45</sup> found that the higher concentrations of *Datura stramonium* L. aqueous leaf extracts inhibited root elongation and inhibited cell division in root tips of soybean plant. Gulzar and Siddiqui<sup>46</sup>, concluded that extract of the weed *Calotropis procera* inhibited the germination and seedling growth of brassica due to its phytotoxic effects. Many enzymes like  $\alpha$ -amylases, proteases and lipases play an important role during seed germination and some enzymatic functions are inhibited by the presence of allelochemicals<sup>10,47</sup>. Aqueous extract of *T. vulgaris* leaves induced more inhibitory effects on pea growth than aqueous extract of *A. monosperma*. Consequently, it could be concluded that the aqueous extracts from *T. vulgaris* have allelopathic potential more than *A. monosperma* and could be evaluated as an allelopathic species.

**Carbohydrates content:** The soluble, insoluble and total carbohydrate contents of pea seedling in response to allelochemicals effect of *A. monosperma* and *T. vulgaris* (Fig. 2a and b, respectively) were increased at 2% concentrations of both extracts, thereafter there were a gradually decreased in all treatments than control. The

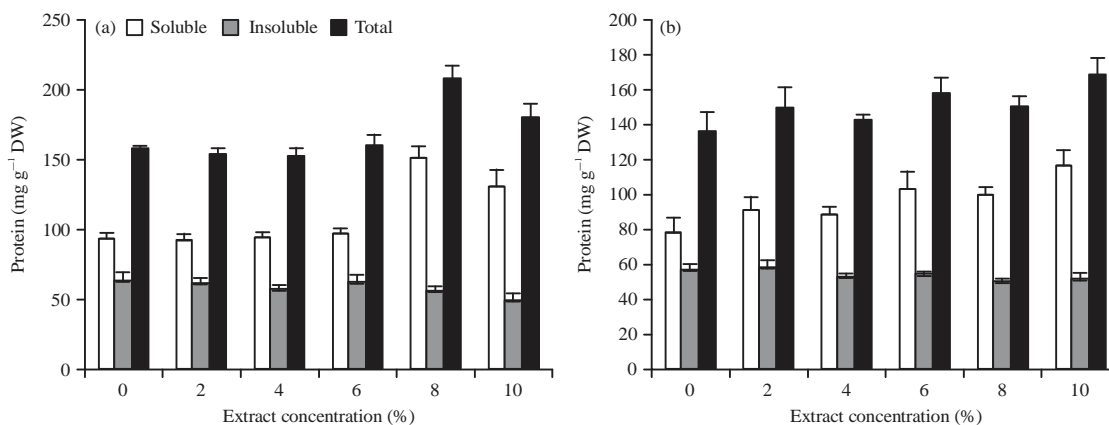


Fig. 3(a-b): Allelopathic effects of (a) *Artemisia monosperma* and (b) *Thymus vulgaris* leaf aqueous extract on protein contents ( $\text{mg g}^{-1}$  dry weight) of pea (*Pisum sativum* L.) seedlings. Vertical bars represent  $\pm$ SD

reduction values in total carbohydrate at high concentration (10%), was higher in case of *A. monosperma* than *T. vulgaris* compared to control. On the other hand, soluble carbohydrate was unaffected in response to *T. vulgaris*. These results are in agreement with those obtained by El-Khawas and Shehata<sup>48</sup> and Mohamadi and Rajaie<sup>49</sup>. They found that sugar contents were significantly reduced in both common bean and maize, as a result of allelopathic effect. El-Shora *et al.*<sup>16</sup> and Hemada and El-Darier<sup>41</sup>, reported that the decrease of total carbohydrate may be attributed to the inhibitory effect of the released allelochemical substances on the synthesis of photosynthetic pigments and therefore, on photosynthesis. This explanation was consistent with the previous results obtained by other investigators for allelochemicals stress that could limit carbohydrate synthesis in plant<sup>16,50</sup>. So, it could be concluded that the allelochemicals of *A. monosperma* and *T. vulgaris* might have interfered with the pathways of photosynthesis and consequently decreased all the metabolites including carbohydrates<sup>51</sup>.

**Proteins content:** Leaf aqueous extracts of *A. monosperma* (Fig. 3a) and *T. vulgaris* (Fig. 3b) were increased the content of soluble and total protein in pea seedlings. On the other hand, insoluble protein was found to be reduced compared to the control. Similar results were observed by a number of investigators<sup>17,21,48,52</sup>. They suggested that allelochemicals can inhibit the ability of plant to absorb water and cause a certain degree of water stress. This explains the accumulation of soluble protein and consequently the total protein in pea seedling in the present study. Therefore, it may be suggested that, the accumulation of soluble protein was at the expense of insoluble fractions and could be regard as an indicator of allelopathy tolerance of pea seedlings. Tripathi *et al.*<sup>53</sup> showed an increase in protein content of soybean compared to the

control by application of *Albizia procera* and *Acacia nilotica* leaf extract. Al-Watban and Salama<sup>21</sup>, reported that aqueous extract of *A. monosperma* decreased the content of soluble sugars at low and moderate concentrations, while increased protein content in common bean seedlings. These results supported present results in pea seedlings. Mersie and Singh<sup>50</sup> and Abu-Romman<sup>39</sup> concluded that allelochemicals resulted in enhanced protein degradation in recipient plant. This clarifies the reduction of insoluble protein of pea seedlings in response to leaf aqueous extract of both extracts.

**Total free amino acids and proline:** Total free amino acids (Fig. 4a) and proline (Fig. 4b), were negatively affected by increasing the concentration of both *A. monosperma* and *T. vulgaris* leaf aqueous extract. This indicates that the leaf aqueous extract of both *A. monosperma* and *T. vulgaris* may have a significant effect on the enzyme system involved in the incorporation of amino acids into protein and *T. vulgaris* was more effective than *A. monosperma*. Kavitha *et al.*<sup>54</sup>, reported a decrease in amino acids content of green gram and black gram at the higher concentrations leaf extracts of *Vitex negundo*. There was surprising situation in the criteria of free proline, where stress conditions accumulate proline, while the opposite occurred in pea seedlings in response to allelochemicals stress of the two medicinal plants. The reduction in proline content was similar to the result obtained by Azooz<sup>55</sup> who revealed that, free proline decreased with increasing salt stress conditions in sorghum cultivars.

**Mineral contents:** The effect of different concentrations of leaf extracts of *A. monosperma* (Fig. 5a) and *T. vulgaris* (Fig. 5b) on  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  content in pea seedling was presented. Generally all the treatments increased  $\text{K}^+$  and  $\text{Ca}^{2+}$  content. On the other hand, a marked decrease in  $\text{Mg}^{2+}$  was

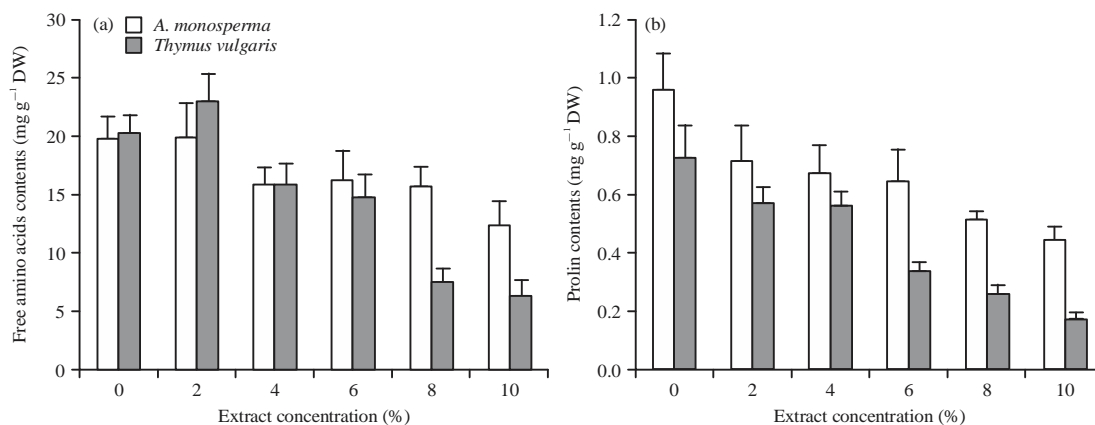


Fig.4(a-b): Allelopathic effects of *Artrmisia monosperma* and *Thymus vulgaris* leaf aqueous extract on (a) Free amino acids and (b) Proline contents (mg g<sup>-1</sup> dry weight) of pea (*Pisum sativum* L.) seedlings. Vertical bars represent ±SD

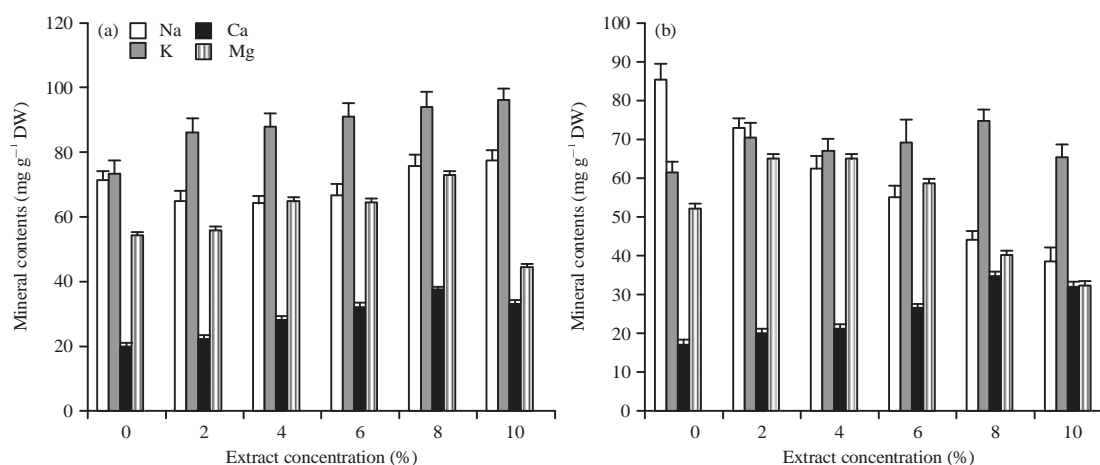


Fig. 5(a-b): Allelopathic effects of (a) *Artrmisia monosperma* and (b) *Thymus vulgaris* leaf aqueous extract on mineral contents of pea (*Pisum sativum* L.) seedlings. Vertical bars represent ±SD

observed at the higher levels of leaf extracts. Interestingly, *T. vulgaris* leaf extract significantly reduced Na<sup>+</sup> at all concentrations, while *A. monosperma* caused a decrease at low concentration and then rise under the higher concentrations. Both increases and decreases in minerals uptake have been reported for plants that are subjected to the allelopathic conditions<sup>56-58</sup>. El-Shabasy<sup>58</sup> found that Na<sup>+</sup> content is increased in combined *Prosopis* leaf extract but decreased in combined *Acacia*. Allelopathic inhibition of mineral uptake results from alteration of cellular membrane functions in plant roots. Flavonoids and phenolic acids prevent minerals uptake through disrupting the normal actions of membrane in the root cells<sup>57</sup>. El-Refai and Moustafa<sup>59</sup> noted that the increase in K<sup>+</sup> and Ca<sup>2+</sup> might be a defense mechanism for resistance of Na<sup>+</sup>, which leads to a metabolic damage in plant. Calcium plays an important role

in membrane integrity and selectivity<sup>60</sup>. The reduction of Mg<sup>2+</sup> indicates to its important to plant which regarded as a major signal for controlling plant growth and considers a key elemental constituent of chlorophyll, which responsible for photosynthetic process. K<sup>+</sup> is considered as a key regulatory element in plant metabolic process by promoting Na<sup>+2</sup> exclusion and osmotic adjustment<sup>61</sup>. In present experiment, pea seedlings behaved similarly, at the higher concentration of allelochemicals the level of K<sup>+</sup> was increased, while Na<sup>+</sup> was reduced as compared to control.

**Antioxidant enzyme activities:** Plants form an endogenous antioxidant enzyme defence system to resist adverse conditions, including allelopathic stress. So, the activity of antioxidant enzymes (CAT, POD, APX and SOD) in response to aqueous extract of the two medicinal plants were studied in



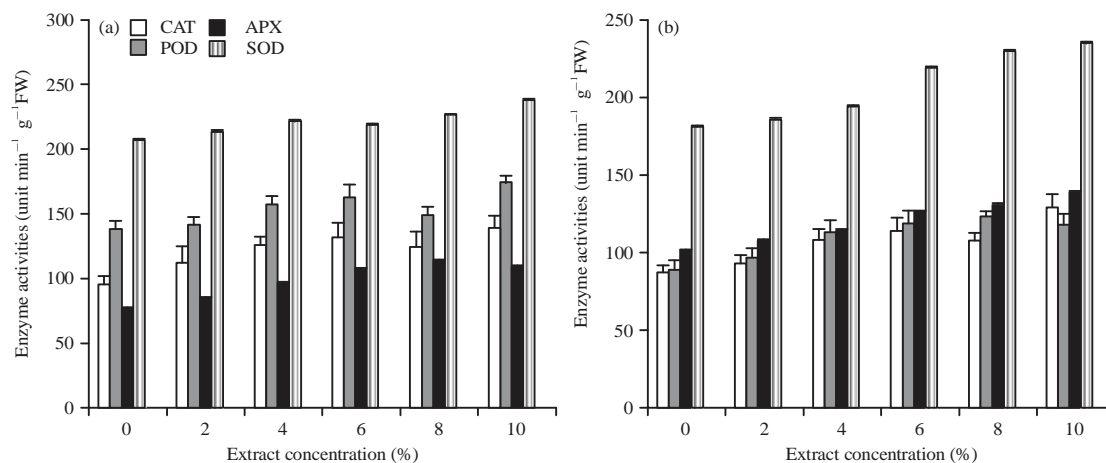


Fig. 6(a-b): Allelopathic effects of (a) *Artemisia monosperma* and (b) *Thymus vulgaris* leaf aqueous extract on enzymes activity (CAT, POD, APX and SOD) ( $\text{Unit min}^{-1} \text{g}^{-1} \text{FW}$ ) of pea (*Pisum sativum* L.) seedlings. Vertical bars represent  $\pm$ SD

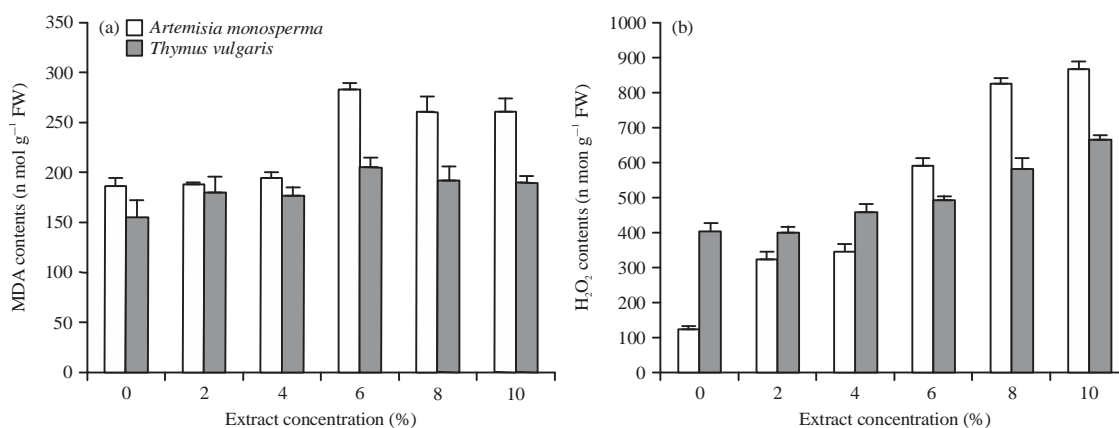


Fig. 7(a-b): Allelopathic effects of *Artemisia monosperma* and *Thymus vulgaris* leaf aqueous extract on (a) Malondialdehyde (MDA) and (b)  $\text{H}_2\text{O}_2$  ( $\text{nmol g}^{-1} \text{FW}$ ) of pea (*Pisum sativum* L.) seedlings. Vertical bars represent  $\pm$ SD

this study (Fig. 6a and b). The activities of these enzymes were increased with increasing aqueous extract concentrations of *A. monosperma* (Fig. 6a) and *T. vulgaris* (Fig. 6b). *T. vulgaris* was more effective in most treatments than *A. monosperma*. Highest increase in activities of these enzymes were recorded at 10%. Concordantly with our results, increases of antioxidant enzyme activities have been observed in response to allelochemicals stress<sup>18,62</sup>. They concluded that the oxidative stress defenses occur through enzymatic antioxidant mechanism including catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX). Candan and Tarhan<sup>63</sup> revealed that the antioxidative enzymes might scavenge or suppress the active oxygen radicals and consequently protect the cell membranes from peroxidation under stress conditions. Exposure of pea seedlings to

allelopathic stress of *A. monosperma* and *T. vulgaris*, may produce ROS and alter the activity of antioxidant enzymes such as CAT, POD, APX and SOD to resist oxidative stress<sup>64</sup>. SODs were considered the first line of defence against ROS in the cell. If ROS detoxification cannot keep pace with ROS generation, ROS accumulation leads to progressive oxidative damage and ultimately induces death of plant cells<sup>65</sup>. This explains the lethal effect of 10% *T. vulgaris* aqueous leaf extract on shoot length of pea seedlings.

**$\text{H}_2\text{O}_2$  accumulation and lipid peroxidation (MDA):** The contents of MDA (as an indicator of lipid peroxidation) and  $\text{H}_2\text{O}_2$  (Fig.7a and b, respectively) of pea seedlings were increased continuously in response to both leaf aqueous extract. MDA contents were increased approximately by

39 and 21%, while H<sub>2</sub>O<sub>2</sub> increased by 187 and 63%, in response to *A. monosperma* and *T. vulgaris*, respectively over the control. Several researchers have reported that allelochemicals can induce MDA and H<sub>2</sub>O<sub>2</sub> contents<sup>18,66</sup>. The accumulation of MDA and H<sub>2</sub>O<sub>2</sub> could reflect the oxidative stress and the changes of antioxidant enzymes in pea seedlings. El-Shora *et al.*<sup>16</sup> showed an increase in H<sub>2</sub>O<sub>2</sub> content in *Portulaca oleracea* L. under leaf aqueous extract of *Trichodesma africanum* L. Likewise Lara-Nunez *et al.*<sup>67</sup> and Hatata and El-Darier<sup>68</sup> found that there are a significant increase in H<sub>2</sub>O<sub>2</sub> and MDA of *Lycopersicon esculentum* and *Triticum aestivum* L. plants treated with *Sicyos deppei* and *Achillea santolina* L., respectively. This increase in H<sub>2</sub>O<sub>2</sub> may be related to the resulting reactive oxygen species (ROS) by allelochemicals<sup>16</sup>. H<sub>2</sub>O<sub>2</sub> causes substantial damage to plants by desolation of proteins, lipids and nucleic acids<sup>69</sup>. It disturbs photosynthesis by inhibiting a number of enzymes in the Calvin cycle<sup>70</sup>. Farhoudi and Lee<sup>71</sup> found that the growth of some seedlings was inhibited by an aqueous extract of barley aerial parts through increasing lipid peroxidation. Present results support the idea that inhibition of seed germination may be associated with membrane lipid peroxidation<sup>72</sup>. Apel and Hirt<sup>73</sup> concluded that the adverse stresses induce the production of ROS, such as H<sub>2</sub>O<sub>2</sub> in plants. These ROS may cause oxidative damage to the cell membranes leading to the production of membrane lipid peroxidation and increasing MDA content.

### CONCLUSION

*Artrmisia monosperma* and *Thymus vulgaris* leaf aqueous extracts showed allelopathic potential on germination, growth, mineral uptake and some metabolic activities of pea seedlings due to their phytotoxic effects. Further, allelochemicals stress caused by *A. monosperma* and *T. vulgaris* extracts are producing an oxidative imbalance as supported by generation of ROS and alteration of activity of antioxidant enzymes and high level of lipid peroxidation and H<sub>2</sub>O<sub>2</sub>. The results of the present study revealed that the aqueous extract of the two medicinal plants showed a wide range of activities from partial and complete inhibition to stimulation which may indicate the presence of certain allelochemicals causing both inhibition and stimulation of the tested parameters. *T. vulgaris* is characterized by the higher allelopathic potential than *A. monosperma* and could be evaluated as an allelopathic species. This may indicate that possibilities of using these medicinal plant extracts as herbicides to control the weed growth.

### SIGNIFICANCE STATEMENT

This study elucidate that the aqueous extracts of the two medicinal plants have a wide range of activities and containing allelopathic compounds with strong potential, which may play important role in weed control and could be used as an alternative of chemical compounds.

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