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Allelopatic effects of some medicinal plant essential oils on plant seeds germination

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ABSTRACT

The effect of essential oils from some medicinal plants on seed germination was studied with the aim of assessing their potential use as bioherbicides. The experiment was conducted as factorial based on completely randomized design (CRD) with three replications. Seeds of 3 summer crops including lettuce (*Lactuca sativa*), pepper (*Piper longum*) and tomato (*Solanum lycopersicum*) were exposed to essential oils of rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*) and anise (*Pimpinella anisum*) at 3 different concentrations (25 and 50% diluted and undiluted). Treated seeds were grown in a growth chamber at 25°C for 5 days. The number of germinated seeds in each Petri dish was daily counted. After five days seed germination percentage (Ge) was calculated. Biplot analysis was performed using genotype plus genotype environment interaction (GGE) method. Results showed that the allelopathic effect on Ge was varied among studied plants, which was mainly due to i) differences in the composition of the studied essential oils and ii) different allelopathic effects of the studied essential oils on Ge. Accordingly, compared to the individual use, combining several essential oils would have a greater inhibitory effect on Ge of weeds.

Key words: allelopathy, thyme, rosemary, anise, lettuce, tomato, pepper

Introduction

Efficiency of field crops should be increased to meet the rising global demand for agricultural products. In agricultural farms, weeds are responsible for a considerable part of the economic loss via reducing the quality and quantity of the yield. Therefore, weed management plays a key role in the modern agriculture.

Weed management is commonly achieved through mechanical, chemical and biological methods. Although the use of chemical compounds is the dominant method however, application of herbicides is generally accompanied with harmful side effects on the environment. Therefore, replacement of these ingredients with the natural compounds would be of significant advance in the protection of our environment.

Unlike the chemical herbicides, bioherbicides "such as plant extracts and essential oils" never cause environmental poisoning because they neither stay long in soil nor penetrate underground water (Rassaeifar et al., 2013).

Plant essential oils and water extracts can be used as a medium for the expression of allelochemical activity to depress the growth of other organisms (Bonanomi et al., 2006; Macías et al., 2007). Several researchers have suggested the use of allelochemicals for weed suppression in the laboratory and also application under field conditions (Farooq et al., 2011). Jamil et al. (2009) described the utilization of allelopathic water extract as an important and useful way of exploiting the allelopathic potential of crop plants to manage weeds.

Most of the herbs are cultivated for medicinal purposes. However, in this research, we evaluated the *in vitro* possible phytotoxicity of the essential oils from three different medicinal plants *Rosmarinus officinalis*, *Thymus vulgaris* and *Pimpinella anisum* in terms of their use as bioherbicides to control weeds via simulation of their effects on seed germination of three summer crops *Lactuca sativa*, *Piper longum* and *Solanum lycopersicum*.

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Materials and Methods

Experimental site and studied factors

The study was performed in the laboratory of Agronomy and Plant Breeding Department of Shahed University in 2014. The experiment was carried out as factorial based on a completely randomized statistical design with 3 replications. Studied factors comprised: i) three summer crops including lettuce, peppers and tomato, ii) essential oil extracted from three medicinal plants rosemary, thyme and anise and, iii) three different concentrations of each essential oil (25 and 50% diluted and undiluted).

Extraction of the essential oils

To prepare essential oils, fresh rosemary and thyme branches were collected from Medicinal Plants Research Farm of Shahed University and air dried in the shade at room temperature for 5 days. Dried braches were then crushed using scissors. Anise seeds were also collected and milled using a mixer.

Conventional extraction was performed at atmospheric pressure using 100 g of weighted crushed materials by a Clevenger apparatus for 3 hours. Obtained extracts were diluted with distilled water in order to get concentrations of 25%, and 50%. We used ether to facilitate the dilution of essential oils.

Seed treatment and collection of the data

First, seeds were sterilized by using sodium hypochlorite solution for 20 minutes. Subsequently, seeds were washed several times with distilled water. A total of 30 seeds from each plant were placed in a Petri dish. Then, seeds were treated with the different essential oil solutions (Totally, 81 Petri dishes were used). Afterward, petri dishes were transferred to a growth chamber and kept at 25°C. The number of germinated seeds in each Petri dish was daily recorded. After 5 days, the germination percentage was estimated using the following simple equation:

$$\text{Ge\%} = \left(\frac{\text{The number of germinated seeds after five days}}{\text{Total number of seeds}}\right) \times 100$$

In fact, the magnitude of the Ge index shows a greater degree of germination for treated seeds representing less allelopathic effect of essential oils on Ge.

Statistical analysis

Calculations were done in Microsoft Excel 2013 software environment. As demonstrated by Yan (2001), GGEbiplot is a windows application that performs biplot analysis of two-way data that assume an entry × tester structure. Hence, analysis of the Ge was performed according to the GGEbiplot method using GenStat statistical software version 12.1.0.3338.

The biplot model is defined as follows (Wang & Galletta 1998):

$$\hat{Y}ij - \mu - \beta j = \lambda_1 \cdot \xi i_1 \cdot \eta j_1 + \lambda_2 \cdot \xi i_2 \cdot \eta j_2 + \varepsilon ij$$

where $\hat{Y}ij$ is the genotypic value of the combination between entry i and tester j; μ is the grand mean; βj is the mean of all combinations involving tester j; $\lambda 1$ and $\lambda 2$ are the singular values for PC1 and PC2, respectively; ξi_1 and ξi_2 are the PC1 and PC2 eigenvectors for entry i, respectively; ηj_1 and ηj_2 are the PC1 and PC2 eigenvectors for tester j, respectively; and εij is the residual of the model associated with the combination of entry i and tester j.

In GGEbiplot, combinations of essential oils by concentrations were considered as testers (9 environments), while the summer crops were entries (3 genotypes).

Results

Figure 1-A shows the GGEbiplot output obtained using GenStat software. This diagram has three main elements including: (i) the average tester coordinate (ATC), which is a small red circle, indicating the position of the average tester, (ii) the ATC abscissa, which is a thick line that passes through the biplot origin and the ATC pointing to the average tester from the biplot origin, and (iii) the ATC ordinate, which is a thick line that is perpendicular to the ATC abscissa.

The Ge index rises on the ATC abscissa direction and toward the right side of the biplot. Therefore, Figure 1-A indicates that tomato had the highest Ge index, whereas lettuce had the lowest. The Ges of entries were ranked as tomato > pepper > lettuce. The positive end of the ATC abscissa was at the side of the biplot origin, where the ATC was located. Thus, tomato showed an acceptable Ge in response to the allelochemicals. On the other hand the projection of entries onto the ATC abscissa shows their relative variation. Thus, the Ge of pepper was the most variable (least stable), whereas tomato was highly stable.

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The ATC is defined as a virtual tester whose PC1 and PC2 scores are equal to the average PC1 and PC2 scores, respectively, across all testers (Yan & Hunt 2002). Hence, the allelopathic effects of testers are also approximated by their projection onto the ATC abscissa. Thus, Figure 1-A shows that Th100 and Th50 had the severest allelopathic effects on Ge of entries, whereas R25 followed by A25 had the poorest allelopathic effects. The allelopathic effect of essential oils were ranked as Th100= Th50 > T25 > A100 > R100 > R50 ≈ A50 > R25 > A25. As explained by Yan (2001) an ideal tester should be highly discriminating of the lines and be highly representative of all testers. It is therefore defined as the tester that has the longest vector of all testers (i.e., the most discriminating) and zero projection onto the ATC ordinate (i.e., the most representative of the testers). Therefore, in this study, R25 were the best tester. This means that R25 had the poorest allelopathic effect on Ge because, if R25 is the ideal environment therefore the Ge should be averagely high for all entries.

Since we are looking for allelopathic effect on Ge therefore, the R25 cannot be a worthy tester for our purpose. Instead, a tester positioned farther from R25 should show a more allelopathic effect with regard to the fact that less Ge will happen in it.

Figure 1-B shows the polygon view of the biplot, which visualizes the interaction patterns between entries and testers. This polygon was formed by drawing straight lines that connected the entry markers. In this figure, the biplot was divided into three sectors. All testers except Th25 fell in sector 1, whereas tomato was the vertex of the polygon indicating that tomato had relatively a good Ge across all studied testers. Also, tester Th25 fell in sector 2, whereas pepper was the vertex of the polygon. Thus, Th25 was the best environment for pepper. This means that except Th25, other studied testers had significant allelopathic effect on the pepper. No tester fell in sectors 3, representing no tester was ideal to gain a notable Ge for lettuce. In other word, all essential oils had a significant allelopathic effect on Ge of lettuce. These results suggested the existence of interaction between studied plants and essential oils regarding studied plants showed different responses to testers.

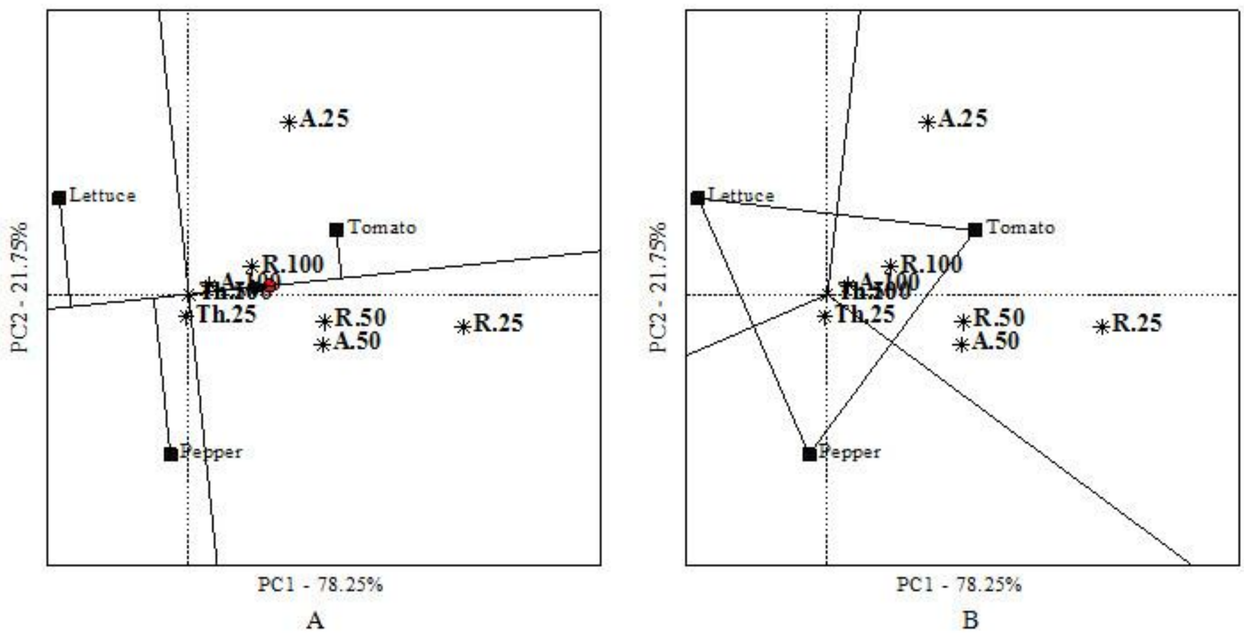

Figure 1. *Convex-hull view (A) and Polygon view (B) of the GGEbiplot derived from study of seed germination of 3 summer crops (tomato, lettuce and pepper) subjected to different concentration (25 and 50% diluted and undiluted) of essential oils of 3 medicinal plants thyme (Th), rosemary (R) and anise (A).Crops are in lower case, and essential oils are in upper case. PC1 and PC2 are first and second principal components, respectively.*

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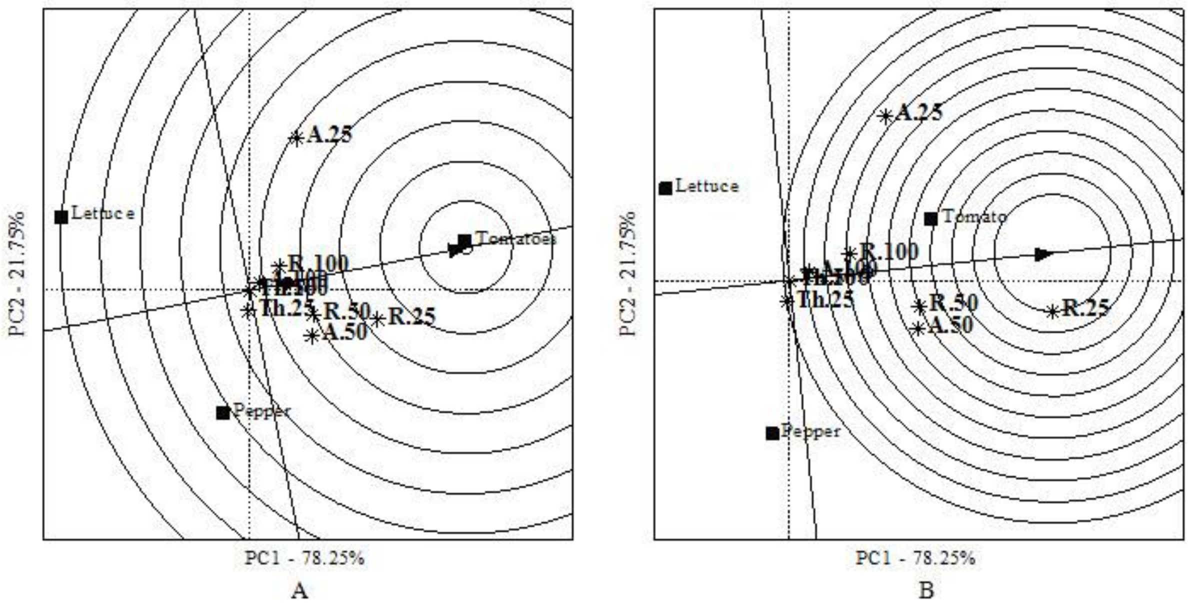

Figure 2. *Comparison of the summer crops with the ideal one (A) and comparison of the essential oils with the ideal essential oil (B). Essential oils are in lower case, and crops are in upper case. PC1 and PC2 are first and second principal components, respectively*

Figure 2 shows the comparison plots. An ideal entry is one that has both high mean Ge and high stability. The center of the concentric circles (Figure 2A) shows the position of an ideal entry. A plant has more Ge if it is closer to the ideal point. Therefore, tomato which was very close to the ideal point of the biplot had the highest mean Ge and stability. So, this result suggests that studied essential oils had minimal allelopathic effect on the tomato (Figure 2A). On the other hand, lettuce is located at the farthest point from the center of the concentric circles (poorest Ge and low stability) suggesting high allelopathic effect. The ideal tester represented by the small circle with an arrow pointing to it (Figure 2B) is the most discriminating of entries and yet representative of the other test environments. Therefore, a tester is more allelopathic if located far from ideal point compared to other testers. Hence, R25 was a desirable essential oil (low allelopathic effect) while, Th50 and Th100 were undesirable (high allelopatic effect).

Discussion

Results of the present work showed that the allelopathic effect on seed germination was varied among studied plants. This outcome can be interpreted from two aspects:

1. Differences in the composition of the studied essential oils

Studies have shown that the main constituent of *P. anisum* essential oil is *cis*-anethole, which represented 97.1% of the whole oil (De Almeida et al., 2010; Tabanca et al. 2006). In *T. vulgaris* oil, thymol, *o*-cymene and carvacrol were the main constituents (De Almeida et al., 2010). Also Boutekedjiret et al. (2003) reported that *α*-pinene (5.2%) *β*-pinene (5.7%) 1,8-cineole (52.4%) and camphor (12.6%) were the main constituents of rosemary essential oil.

De Almeida et al. (2010) divided the above chemical groups into 5 groups including alcohols, aldehydes, alkenes, ketones and phenols. Based on their work, thyme oil belongs to phenols that represent about 33.1% of the total oil composition, respectively. They suggested that monoterpenes were the most abundant components of thyme and rosemary oil while the oil of anise was mainly constituted of non-terpenes.

Vokou et al. (2003) studied the allelopathic activities of 47 monoterpenoids belonging to different chemical groups, to estimate their effects on seed germination and subsequent growth of *Lactuca sativa* seedlings. They found that the most active compounds belonged to the groups of ketones and

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alcohols, followed by the group of aldehydes and phenols. Also, Kotan et al. (2010) suggested that in general, a high presence of oxygenated monoterpenes is linked to a potent phytotoxic activity of plant essential oils. These reports were entirely consistent with our findings. Our study also revealed that essential oils extracted from thyme and anise had the strongest and poorest allelopathic effects on Ge, respectively.

In this study, the observed inhibition of rosemary essential oil was mainly due to its Camphor. Since the monoterpenes in thyme oil are more diverse with higher percentages, therefore the inhibitory effect of thyme oil was more than rosemary oil.

2. Different allelopathic effects of the studied essential oils on the seed germination

Although seed germination is an internally regulated process influenced by genotype, external factors such as light, temperature, moisture, and the presence of certain chemical compounds (phytohormones or organic acids) also strongly influence this process (Kucera et al., 2005; Finkelstein, 2010).

In this experiment, tomato and lettuce had the highest and lowest Ge when the seeds were exposed to same essential oils suggesting the activity of studied essential oils were selective. In this regard, Fischer et al. (1988) reported that cineol was highly toxic against *Schizachyrium scoparium,* but not on *Leptochloa dubia*.

Therefore, the inhibitory effect of a certain compound on certain plant species may not necessarily be maintained on another species. Vokou et al. (2003) believe that such feature of the essential oils is very important, particularly for application as bio-herbicides, where specificity of activity is required. Therefore, researches are needed to identify the most drastic compounds for the different target species.

Accordingly, compared to the individual use, combining several essential oils may have a greater inhibitory effect on seed germination of weeds.

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