# Effects of savory essential oil on germination parameters of Fusarium infected-seeds of wheat (*Triticum aestivum* L.)

**Fatemeh Rahimian and Hamid Reza Eisvand***

*Faculty of Agriculture, Department of Agronomy and Plant Breeding, Lorestan University, Iran*

## Abstract

High use of synthetic pesticides has detrimental effects on the environment. An alternative option is application of natural pesticide compounds. The aim of this study was to evaluate the possibility of replacing Vitavax with savory essential oil to inhibit physiological damages to wheat (*Triticum aestivum* L.) seeds caused by *Fusarium oxysprum.* A pot experiment was carried out on wheat seeds as factorial on the basis of completely randomized design with three replications. Factors were fungal infection (*Fusarium oxysprum* infection and non-infection), and fungicide (control, savory essential oil at 10 and 20 ppm and Vitavax 2 g per kg). Fusarium infection decreased seed physiological quality. Using savory essential oil could not inhibit fungal infection in seeds and seedling but Vitavax could. In addition, the savory essential oil decreased somewhat germination percentage and its inhibitory effect on germination increased with concentration. Although there are some reports about anti-fungal effects of savory essential oil under *in vitro* conditions, such effect was not observed under this pot experiment. This may be due to application of low concentration in this experiment; however high concentration will kill the seeds.

*Keywords: fungicide; savory essential oil; seed; organic agriculture*

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## Introduction

Wheat is the most important plant in the world that has an important role for supplying human food. This plant is propagated by seeds; therefore, seed quality is one of the factors that affects the yield (Ghorbani et al., 2008). Germination rate and vigor index are two important characteristics that represent seed physiological quality of wheat.

Root and crown rots are diseases that can damage wheat (Wiese, 1987). A set of fungi (*Fusarium oxysporum, F. eguisstei F. acuminatum*, and *Bipolaris sorokiniana)* are causative pathogens for such diseases (Fedel-Moen and Harris, 2010).

Treatment of the seeds with a suitable and effective fungicide is one of the ways for controlling fungal diseases. Nowadays, many synthetic fungicides are used in agriculture. Application of Vitavax as seed treatment is common in many regions of Iran for controlling root rot in wheat and barley. As use of synthetic pesticides has unpleasant consequences in the environment, there is a strong tendency to replace these ingredients with natural ones.

Savory plant belongs to the mint family, which grows in some parts of Iran. This plant has antimicrobial properties (Azaz et al., 2002). Flavonoids and terpenoids are the important

*Corresponding author
*E-mail address*: eisvand.hr@lu.ac.ir
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chemical compounds in savory. Monoterpenoides such as caravacrol and thymol are two important ingredients in savory essential oil (Samsamshariat, 1995). *In vitro* anti-fungal effects have been reported for savory essential oil (Essawi and Srour, 2000; Sokovic et al., 2002; Yazdanpanah et al., 2010). Yazdanpanah et al., (2010) reported that savory essential oil at 400 ppm or more inhibited growth of *Alternaria citri*; however, low concentrations (below 400 ppm) only slowed the growth and could not inhibit it.

The aim of this study was to evaluate the effects of essential oil of the savory on germination parameters of fusarium-infected seeds of wheat in order to investigate the possibility of replacing Vitavax with savory essential oil.

## Materials and Methods

Wheat (*Triticum aestivum* L. var. Chamran) seeds were prepared from Agriculture and Natural Resources Research Center of Lorestan, Khorram Abad, Iran. In order to prepare inoculums, *Fosarium oxysprum* was isolated from wheat plants in the field based on the method used and reported by Banihashemi (2010) and Saremi (1998).

Before using inoculums (including $5\times10^6$ spore per $cm^3$) in pot experiment, their pathogenicity was tested *in vitro*. In this test, the inoculum was used on sterile seeds as three replicates incubated at 21 °C and RH= 70%. Results of this test showed that the inoculum could infect seeds. The seeds were inoculated by inoculums (distilled water for control and *fusarium inoculums*) before they were treated with fungicides. Inoculation was done through spraying inoculums on the seeds.

## Fungicides (organic and chemical)

Savory essential oil was prepared from Khorraman Pharmaceutical Company, Iran. The essential oil was diluted by toein 80% to 10 and 20 ppm (in a preliminary experiment we found that savory essential oil at more than 30 ppm inhibits seed germination, and kills seeds). Also the Vitavax, a commercial synthetic fungicide, was used at 2000 ppm concentration, a common dose which is used by farmers as seed treatment. After 24 h of Fusarium inoculation, the seeds were treated by fungicides (savory essential oil and Vitavax) and then were planted.

Soil was sterilized twice during 24 h for 15 min at 120 °C and 1 atmosphere air pressure. The pots were filled with soil and 25 seeds were planted in each. Six pots were considered for each treatment (i.e., two pots per each replicate). The pots were irrigated with distilled water and transferred to a greenhouse with the temperature set at 27 °C. Arrangement of the pots in the greenhouse was done according to a factorial experiment on the basis of completely randomized design with three replications. The number of emerged seedling was recorded daily. Percentage and rate of emergence, mean emergence time, and vigor index were determined using the following equations (1, 2, 3, 4), respectively:

$$\text{Emergence percentage} = \frac{b*100}{a} \quad (1)$$

where, a is the number of planted seeds, and b is number of emerged seedlings;

$$\text{Speed of emergence} = \sum_{1}^{j} \frac{n_i}{Di} \quad (2)$$

where $n_i$ is the number of emerged seedlings on day *i* and $D_i$ is the number of days after beginning the experiment (Agrawal, 2004);

$$\text{Mean emergence time} = \frac{\sum D \times n}{\sum n} \quad (3)$$

where *n* is the number of emerged seedlings and *D* is the number of days after beginning the experiment (Cezabator, 1962);

Vigor index = mean length of seedling × percentage of emergence/100 (Abdul-baki and Anderson, 1973) (4)

## Data analysis

MSTATC software was used for data normality test, analysis of variance, and means comparisons.

Table 1
One-way ANOVA for the effects of Fusarium infection and fungicides on some traits of wheat

| | | | Mean | Squares | | | |
|---|---|---|---|---|---|---|---|
| Sources of variance | df | Emergence percentage | Speed of emergence | Shoot height | Root length | R/S Ratio | Vigor Index |
| Fungal infection (A) | 1 | 9440.66** | 13.25** | 87.78** | 61.76** | 0.24** | 804.23** |
| Fungicide (B) | 3 | 1092.22** | 7.04** | 8.82** | 0.65 ns | 0.21** | 43.62* |
| A×B | 3 | 1200.66* | 0.12ns | 8.84** | 24.18** | 0.07ns | 96.79** |
| Error | 16 | 274 | 0.28 | 0.68 | 1.37 | 0.03 | 10.80 |
| CV (%) | | 22.82 | 13.67 | 8.92 | 10.21 | 12.63 | 16.8 |

Ns,*, and **: non-significant, significant at 5% , and 1% , respectively

## Results

Emergence percentage was affected by Fusarium infection (Table 1, P≤0.01) and decreased significantly (Fig. I). In addition, there was an interaction between Fusarium infection and fungicide treatments for emergence percentage (Table 1, P≤0.05).

Emergence percentage continuously increased in Fusarium-infected seeds by applying and increasing savory essential oil as well as applying Vitavax. However, in non-infected seeds, using savory essential oil at 20 ppm concentration decreased seedling emergence (Fig. I, A). Among the fungicide treatments, Vitavax was most effective for mitigation of Fusarium damages and improved seedling emergence noticeably although it could not remove Fusarium adverse effects completely.

Effects of Fusarium infection and fungicide treatments were significant on seedling shoot height. In addition, there was an interaction between Fusarium infection and fungicide treatments for this trait (Table 1). Shoot height decreased when the seeds were infected by Fusarium (Fig. II, A). Savory essential oil and Vitavax had an inhibitory effect on seedling height; this effect was more obvious in the infected seeds. However, low concentrations of savory essential oil (10 ppm) did not have any significant effect on reduction of shoot height in the non-infected seeds (Fig. II, A).

Root length decreased by Fusarium infection (Fig. II. B). All fungicide treatments especially savory essential oil at 20 ppm and Vitavax had negative effects on root length in non-infected seeds. In Fusarium-infected seeds, savory essential oil at 10 ppm did not have any effect, but savory essential oil at 20 ppm and Vitavax reduced root length (Fig. II, B).

The R/S ratio was affected by fungal infection as well as fungicide treatments (Table 1, P≤0.05). Infection of the seeds with Fusarium increased R/S (Fig. III, A). Vitavax application decreased R/S significantly (Fig. III, B). Among the fungicides, savory essential oil had the highest R/S ratio (Fig. III, B).

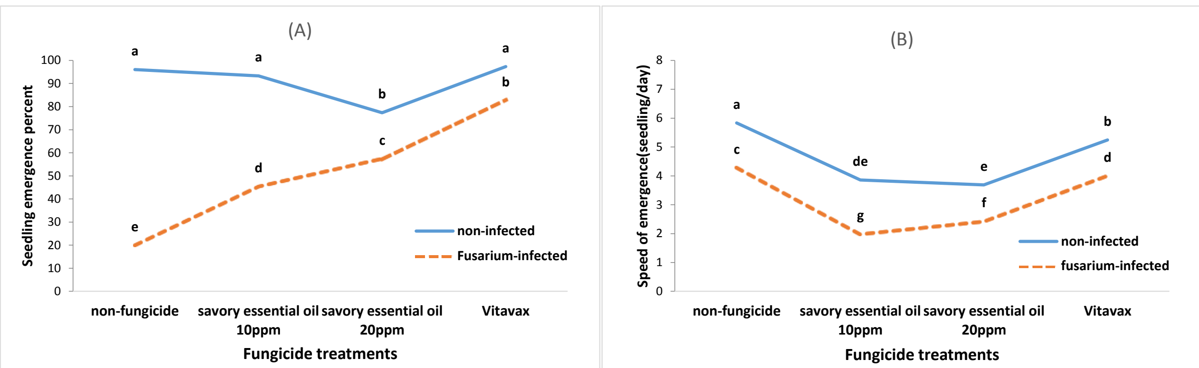


Fig. I. Effects of different fungicides treatments on seedling emergence percentage (A) and speed of seedling emergence (B) of non-infected and fusarium-infected wheat seeds; comparisons of means is done by Duncan multiple range test (P≤0.05).

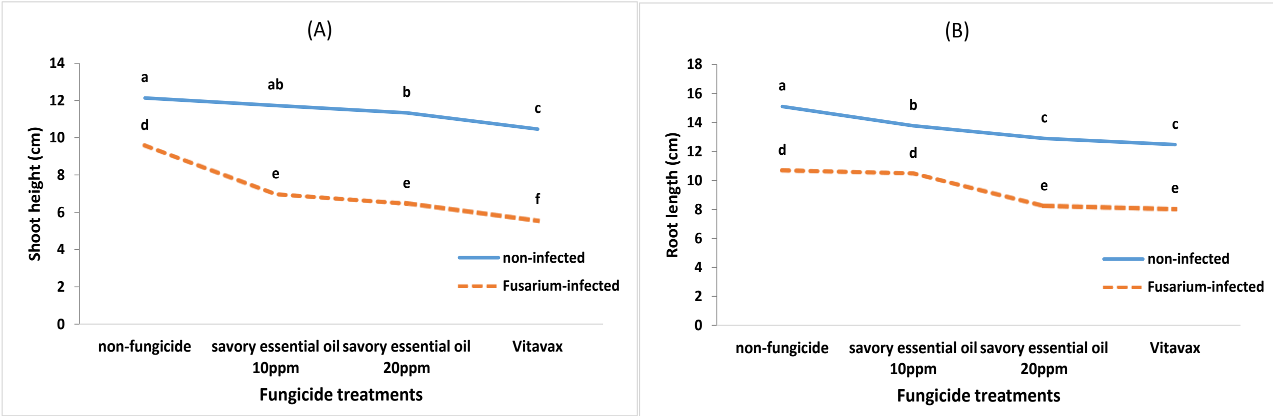


Fig. II. Effects of different fungicides treatments on seedling shoot height (A) and root length (B) of non-infected and Fusarium-infected wheat seeds; comparisons of means are done by Duncan multiple range test (P≤0.05).

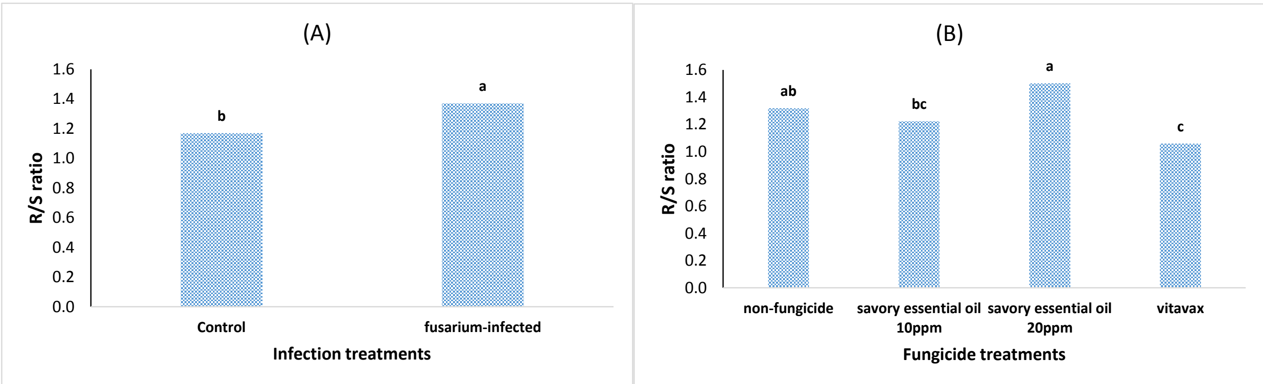


Fig. III. Effects of Fusarium infection (A) and fungicides (B) on R/S ratio of wheat seedling; comparisons of means are done by Duncan multiple range test (P≤0.05).

Fusarium and fungicides affected vigor index. In addition, there was an interaction between Fusarium and fungicide factor for this trait (Table 1). Fusarium infection decreased vigor index (Fig. IV). In non-infected seeds, savory essential oil at 20 ppm and Vitavax decreased vigor index. However, in the Fusarium-infected seeds, all fungicides decreased the vigor index and the adverse effect was exacerbated by savory essential oil at 20 ppm and Vitavax (Fig. IV).

## Discussion

Seed borne diseases can challenge seed germination and seedling establishment. However, their damages depend on the nature and presence of all microorganisms in the soil and seed surfaces. Percentage and speed of germination generally are among the factors which are negatively affected by seed disease. There are many reports that Fusarium spp. decreases the seed germination of crops such as wheat (Alizadeh, 1997), tall wheat grass, and bromegrass (Alizadeh, 2004), and maize (Galli et al., 2005).

In this research, Fusarium infection decreased seedling emergence and speed. Storage fungi reduce germination and seed vigor via weakening embryo and producing necrotic tissue on embryo which may result in its death (Gupta et al., 1993). The savory essential oil and Vitavax

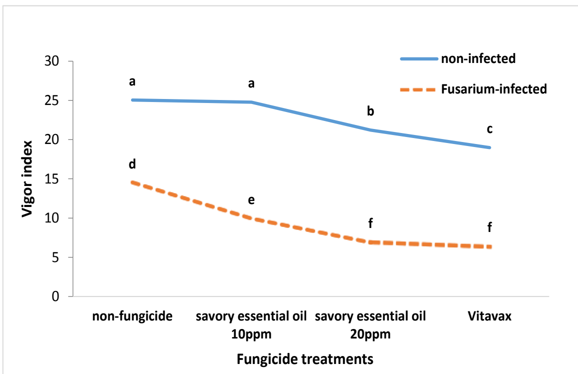


Fig. IV. Effects of different fungicide treatments on vigor index of non-infected and Fusarium-infected wheat seeds; comparisons of means are done by Duncan multiple range test (P≤0.05).

decreased adverse effects of Fusarium. This effect may imply anti-fungal activity of savory essential oil. However, Vitavax was more efficient. Meanwhile, Vitavax could not improve seedling emergence and speed, completely. Maskouki and Mortazavi reported thyme and ajowan essential oils could inhibit fungi growth in food, horticulture, and crop products. They also suggested that these oils could be used instead of chemical fungicides (Maskouki and Mortazavi, 2004). Moreover, there is a positive effect of savory essential oil in increasing seedling emergence of Fusarium-infected seeds while it has an inhibitory effect on speed of seedling emergence. In other word, it has a double sword role.

Savory essential oil and Vitavax decreased shoot height and root length. In comparison to Vitavax, savory essential oil had less negative effects on seedling shoot height. Fusarium increased R/S ratio that mainly occurs because of inhibition of shoot growth more than that of roots. Therefore, it seems that shoot growth is more sensitive to savory essential oil and Vitavax.

## Conclusion

Undesirable and adverse effects of the chemical pesticides are more obvious with time. Therefore, people search natural compounds instead of synthetic ones to have safer foods. Biologic materials and organisms are alternatives for the chemicals. More studies seem to be necessary to modify savory essential oil formulation and find appropriate doses that keep its favorable fungicide effects while its negative effects on germination are eliminated or at least mitigated.

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