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The Effects of Fungicidal Seed Treatments on Seed Germination, Mean Germination Time and Seedling Growth in Safflower (*Carthamus tinctorius* L.)

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ABSTRACT

Seed-borne pathogens cause incorrect scores determining seed germination rate in safflower. A laboratory experiment was planned to search for an effective fungicide treatment for safflower seeds inhibiting the seed-borne infections during germination and early seedling growth. Two safflower cultivars (Olas and Linas) and five fungicides (Thiram, maneb, mancozeb, metalaxyl, and captan) were tested. Germination, mean germination time, and seedling growth parameters were investigated for determining the effectiveness of the fungicides. Results showed that germination percentage was not adversely affected by the fungicides and a higher germination percentage was obtained from the seeds treated with fungicides. Mean germination time shortened with fungicide treatments and more rapid germination was observed in thiram and metalaxyl. Fungicides increased root and shoot growth, especially in thriam and metalaxyl. The seedling weight of safflower cultivars was not changed by the application of fungicides. The infection rate of two safflower cultivars was different and untreated seeds of cv. Olas were infected. The seeds treated with fungicides successfully prevented the seed-borne infections, the minimum infection rate was obtained from the seeds treated with thiram, maneb and mancozeb. It was concluded that pretreatment of safflower seeds with thriam or mancozeb should be beneficial for avoiding seed-borne pathogens before germination test, and these applications may be tested under field conditions in terms of emergence and seed yield performance.

1. Introduction

Safflower (*Carthamus tinctorius* L.) is one of the most promising oilseed crops to meet the vegetable oil demand because it is adapted to drought and saline conditions under rainfed conditions in Turkey (Kaya et al., 2019). It belongs to the *Asteraceae* family and its seeds are called an achene. Achenes contain a single seed covered by pericarp, which makes achene suitable for placing the pathogens between seed and the pericarp. These pathogens like fungi, bacteria, and viruses adversely affect germinating seeds, seedling development, and healthy plant growth. Seed-borne pathogens prevent directly and indirectly the uniform seedling emergence and stand establishment of safflower due to reduction in germination and viability of seed (Pawar et al., 2013). For these reasons, they must be controlled by seed treatments.

Fungal pathogens are the most hazardous pathogen damaging germinating seeds and early seedling development of safflower. The most common fungi are Alternaria blight (*Alternaria carthami*), Fusarium wilt (*Fusarium oxysporum* Schlecht. f. sp. *carthami*), Verticillium wilt (*Verticillium albo-atrum*), Phytophthora root rot (*Phytophthora drechsleri*), rust (*Puccinia carthami*), brown leaf spot (*Ramularia carthami*), Cercospora leaf spot (*Cercospora carthami*), and Macrophomina root rot (*Macrophomina phaseolina*) (Chattopadhyay et al., 2015). However, several fungicides and their combinations have been extensively used for controlling these pathogens (Pawar et al., 2015). In previous studies, Sudisha et al. (2006) and Pawar et al. (2013) found a significant reduction in disease indices with pre-sowing fungicidal seed treatments. Similarly, Ellis et al. (2011) reported that seed treatments with captan and fludioxonil alleviated fungi damage by 46% and 48%, respectively over control in soybean. Addrah et al. (2020) observed that different rates of pathogen contamination on sunflower varieties and fungicide application decreased in contamination rate from 98% to less than 10%. According to Bardin et al. (2003)

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Pythium species, which damage seedling growth in peas, sugar beet, canola, and sunflower, decreased with Thiram application, as well as increased seedling emergence rates in the field. Inhibited seed-borne fungi in rice associated with seed treatments with mancozeb, metalaxyl, benomyl, carbendazim and thiram was reported by Ibiam et al. (2008). In our study, it was aimed to determine if there are the beneficial effects of some fungicides for decreasing the seed-borne infection during germination and early seedling development of two safflower cultivars.

2. Materials and Methods

A laboratory experiment was conducted at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University in 2020. Five fungicides, thiram (1 g kg^{-1} seed), maneb (1.5 g kg^{-1} seed), mancozeb (2 g kg^{-1} seed), metalaxyl (5 g kg^{-1} seed), and captan (2.5 g kg^{-1} seed) were applied to the seeds of safflower cultivars Olas and Linas. Seeds without fungicidal treatment were used as control. Two hundred seeds from each cultivar were firstly weighed, put into a falcon tube and a required amount of individual fungicide was poured over the seeds. The tube was tightly closed with a cover and shaken with vortex for 5 minutes for uniform coating of seeds with the fungicides (Islam et al., 2007). Untreated seeds were used as control.

Germination test was performed by the procedures of ISTA (2003) rules with two hundred (4×50) seeds of each safflower cultivar for each fungicide. Fifty seeds were inserted into two-layer filter papers wetted with 7 ml of the distilled water for each paper. After filter papers with seeds were rolled, they were placed into a sealed plastic bag to avoid water loss. The packages were incubated at 25°C in the dark and seed with 2 mm radicle was counted every 24 h for 10 d as germinated. To evaluate the speed of germination, mean germination time (MGT) was calculated according to ISTA (2003) rules. MGT= $\Sigma(Dn)/\Sigma n$, where, n is the seed number germinated on day D, and D is the number of days from the beginning of the germination test. On the 10th day, ten seedlings from each treatment were randomly selected to determine the seedling growth traits such as root length (RL), shoot length (SL), seedling fresh weight (SFW), and seedling dry weight (SDW). After the seedling fresh weight was directly weighed, the seedlings were transferred into an oven at 80°C for 24 hours for determination of dry weight (Ergin et al., 2021). Also, infection rate (IR) was determined by applying the following formula:

IR= (Number of seeds on which infection appears / Total number of seeds) × 100 (Arshad Javaid et al., 2006).

The experiment was established at two factors Completely Randomized Design (CRD) with 4 replicates. All the collected data were statically analyzed by using the MSTAT-C computer program. Means were compared with the LSD test to evaluate the differences among them (Düzgüneş et al., 1983).

3. Results and Discussion

The efficacy of fungicides on germination and seedling development is shown in Table 1. The difference between cultivars ($p<0.01$) for mean germination time, seedling length, seedling fresh weight, seedling dry weight and infection rate were statistically significant. Differences between safflower cultivars in terms of germination percentage and root length were not significant. There were significant differences among fungicides in germination percentage, mean germination time, root length, shoot length, and infection rate. Seedling fresh and dry weights were not affected by fungicides. Two-way interaction between cultivar and fungicide was significant for mean germination time, root length, shoot length and infection rate.

Table 1
Analysis of variance on the investigated traits in safflower cultivars and fungicide treatments

VS	DF	Mean Square						
		GP	MGT	RL	SL	SFW	SDW	IR
Total	47	1126	2.44	32.2	7.80	4.21	0.09	22350
Cultivar (A)	1	30	0.08**	0.2	0.80**	0.64**	0.06**	2749**
Fungicide (B)	5	250*	0.35**	16.6**	2.77**	0.50	0.01	11233**
A × B	5	186	1.72**	8.3**	1.34**	0.54	0.01	5616**
Error	36	659	0.28	7.1	2.89	2.53	0.03	2751

*, **: significance level at $p<0.05$ and $p<0.01$, respectively. VS: Variation source, DF: Degrees of freedom, GP: Germination Percentage, MGT: Mean Germination Time, RL: Root Length, SL: Shoot Length, SFW: Seedling Fresh Weight, SDW: Seedling Dry Weight, IP: Infection Rate

Table 2
Germination percentage, mean germination time, and seedling growth parameters of two safflower cultivars treated with five fungicides

Factors	GP (%)	MGT (day)	RL (cm)	SL (cm)	SFW (mg plant^{-1})	SDW (mg plant^{-1})
Cultivar						
Olas	89.8	1.22^{b}	3.11^{a}	2.77^{a}	214^{b}	25.5^{b}*
Linas	91.3	1.30^{a}	2.98^{b}	2.51^{b}	237^{a}	32.6^{a}
Fungicide						
Control	86.8^{c}	1.25^{bc}	2.25^{d}	2.73^{ab}	228	29.8
Thiram	91.3^{ab}	1.17^{c}	3.51^{ab}	2.84^{a}	227	29.6
Maneb	89.3^{bc}	1.42^{a}	2.73^{c}	2.85^{a}	225	28.5
Mancozeb	93.8^{a}	1.24^{bc}	3.42^{b}	2.78^{a}	232	28.7
Metalaxyl	89.8^{abc}	1.17^{c}	3.88^{a}	2.19^{c}	204	28.3
Captan	92.5^{ab}	1.31^{b}	2.49^{cd}	2.45^{c}	235	29.7
Cutlivar×Fungicide						
Olas × Control	83.5	1.10^{bc}	1.79^{e}	2.88^{bc}	230	26.7
Olas × Thiram	92.0	1.04^{c}	3.79^{ab}	3.30^{a}	227	26.2
Olas × Maneb	88.0	1.79^{a}	2.78^{d}	2.89^{b}	212	25.1
Olas × Mancozeb	93.0	1.16^{bc}	4.10^{a}	2.95^{ab}	224	25.1
Olas × Metalaxyl	87.0	1.09^{bc}	4.19^{a}	2.18^{f}	183	24.7
Olas × Captan	95.0	1.13^{bc}	2.04^{e}	2.42^{def}	207	25.3
Linas × Control	90.0	1.40^{abc}	2.71^{d}	2.58^{b-f}	227	32.9
Linas × Thiram	90.5	1.30^{bc}	3.23^{bcd}	2.38^{ef}	227	32.9
Linas × Maneb	90.5	1.04^{c}	2.69^{d}	2.81^{bcd}	238	31.9
Linas × Mancozeb	94.5	1.33^{bc}	2.74^{d}	2.60^{b-e}	241	32.2
Linas × Metalaxyl	92.5	1.24^{bc}	3.56^{abc}	2.21^{ef}	225	31.8
Linas × Captan	90.0	1.49^{ab}	2.95^{cd}	2.48^{c-f}	263	34.0

*: Means followed by the same superscript letter(s) are not significant at $p<0.05$. GP: Germination percentage, MGT: Mean germination time, RL: Root length, SL: Shoot length, SFW: Seedling fresh weight, SDW: Seedling dry weight.

Fungicide treatments for seed-borne pathogens increased the germination percentage of safflower seeds. The germination percentage ranged from 86.8% in control to 93.8% in mancozeb, shown in Table 2. Similar results were reported by Khairmar et al. (2013) and Choudhary et al. (2013) who found that germination rates in control seeds were 76-84%, while it reached up to 91-95% in the seeds with fungicide applications. Mean germination time was shortened by fungicide treatments especially thiram and metalaxyl with 1.17 days. Among the fungicides, maneb and captan gave a longer time to germinate. Sundaresh et al. (1973) and Gawade et al. (2016) determined retardation in soybean germination in untreated seeds with fungicide and inhibition due to fungal infection (46%); however, seeds treated with thriam and mancozeb were completely free from fungal growth. The seeds treated with fungicides produced longer root length compared to untreated seeds of two cultivars. metalaxyl and thiram treatments gave the longest roots with 3.88 cm and 3.51 cm, respectively. Root length of cv. Olas was more prominently increased by treatment of metalaxyl and mancozeb. Shoot length was significantly enhanced when the seeds were treated with thiram and mancozeb in cv. Olas, maneb and mancozeb in cv. Linas. In general, shoot length in thiram, maneb and mancozeb showed slightly higher or similar to control seeds. Similarly, the longest seedling length in soybean was reached in metalaxyl-M + fludioxonil application (Costa et al., 2019). Choudhary et al. (2013) reported that thiram treatment gave longer roots than captan. The results are in line with the findings of Sultana and Ghaffar (2010) who reported that fungi adversely affect seedling growth, and that healthy seedling growth can be achieved with fungicidal applications. Seedling fresh weight was not changed by fungicide treatments and similar results were observed in all treatments except for cv. Olas were treated with metalaxyl, which produced the lowest fresh weight with 183 mg plant^{-1}. Heavier fresh and dry weight was determined in cv. Linas. No significant changes were observed in seedling dry weight among the fungicides. However, Solorzano and Malvick (2011) stated that the dry weight of corn seedlings had higher values when the seeds treated with fungicide mixtures were used.

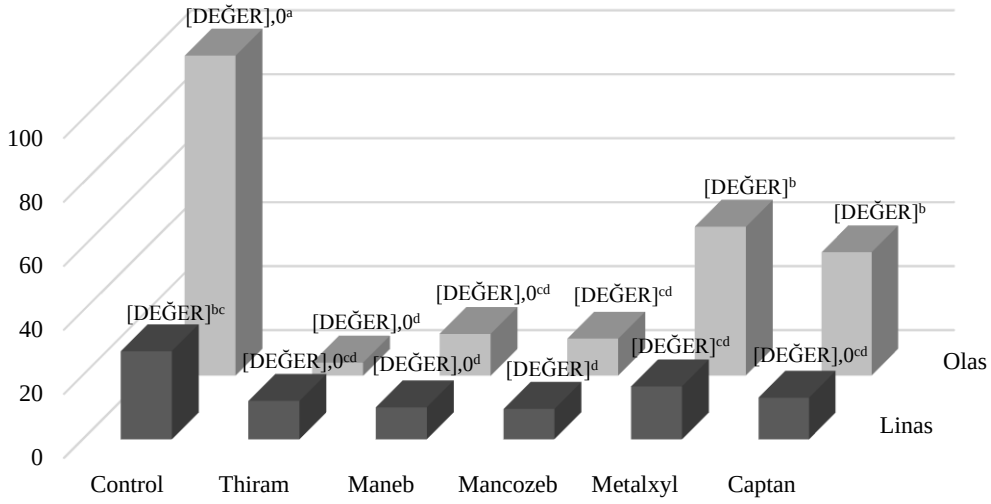

Figure 1
Infected seed rate of safflower cultivars treated with five fungicides during germination test.
*: Values on the each column followed by same letter(s) are not significant at p<0.05

Infected seed rate is considered as the effectiveness of seed treatments by fungicides. Untreated control seeds showed that infection rate could be reached up to 100%, as in cv. Olas (Figure 1). However, fungicides inhibited the seed infection resulting from seed pathogens. The least infection rate was achieved in the application of thiram to seeds of cv. Olas. Our results confirmed the findings of Singh and Jha (2003) and Saroja (2012) who found thiram and carbendazim were the most effective agents inhibiting the growth of *Fusarium oxysporum* f.sp. *ciceri* at 1% concentration among seven fungicides. Also, in the study of Khairmar et al. (2013) was observed that the fungi in the seed mycoflora of different safflower cultivars decreased with thiram and thiram+carbendazim applications. Suresha et al. (2012) reported that the application of carbendazim+thiram significantly reduced the seed infection compared to captan, thiram, carbendazim and carbendazim+captan applications. Moreover, the beneficial effects of fungicidal seed treatments were informed by Munkvold and O'mara (2002) in maize, Habib et al. (2007) in eggplant, Akgül et al. (2011) in peanut, Ellis et al. (2011) in soybean, Dhanamanjuri et al. (2013) in chickpea and maize, Islam et al. (2015) in wheat and Addrah et al. (2020) in sunflower.

In conclusion, germination test is routinely performed for seed certification processes and its results are commonly used for the required amount of seed per unit area; consequently, germination rate must be accurately determined. The main obstacle in germination tests in safflower is seed-borne pathogens which are more rapidly grown than germinating seeds. They multiply more rapidly in optimum germination conditions such as high humidity and temperature than seeds, and germination was restricted by infections. In our study, there were significant differences between safflower cultivars in terms of seed-borne pathogens. This difference results from genotypic variations because the seeds of two safflower cultivars were produced under Eskişehir conditions in 2020. But, detailed researches should be conducted by using several cultivars to make a precise decision. In addition, fungicides were effective to reduce seed-borne pathogens during the germination experiment. Pathogen-free healthy seeds are needed for desired plant populations and high yield. It is argued that seeds treatment with fungicides may favorably affect the emergence, stand establishment and consequently seed yield of safflower under field conditions. In conclusion, all the fungicides inhibited the seed-borne infections without adverse effects on germination rate and allowed to grow the healthy seedlings, and thiram, maneb and mancozeb should be advised for the seed treatments before germination test in safflower.

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