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ASSESSMENT OF SAFFLOWER FOR SUSCEPTIBILITY TO *PYTHIUM ULTIMUM*, THE CAUSAL AGENT OF DAMPING-OFF

ABSTRACT

Genetic resistance against *Pythium* species would be an efficient control of this major seed and seedling fungal pathogen in safflower (*Carthamus tinctorius*), but so far no source has been identified. Therefore, identifying and then incorporating genetic resistance into the cultivars would be an ideal method of control for this disease. So in this study the resistance to seed rot and damping-off caused by *Pythium ultimum* among seventeen genotypes of safflower collected from all major production regions of the crop in Iran and some other countries was investigated. *Pythium ultimum*, previously identified as the most prevalent *Pythium* isolates on safflower, were used to infest the sterilized soil, which was seeded with safflower genotypes in greenhouse conditions. The experiment was conducted as a split plot design in which sterile and *Pythium*-infested soils were considered as main plots, and seventeen genotypes of safflower were sub plots. In sterile media, percent of seedling emergence (SES) and in *Pythium*-infested media, percent of seedling emergence (SEI), percent of diseased seedlings (DSI), percent of uninfected seedlings (USI) and percent of non-emerged seeds (NSI) were recorded. Symptoms showed that in safflower, damping-off occurred in both forms of pre and post emergence. Genotypic variation was existed for level of susceptibility to *P. ultimum*. Significant differences in SES and SEI were detected among safflower genotypes ($P < 0.01$), and a significant correlation between SES and seed weight ($P < 0.05$); SES with SEI ($P < 0.05$) were detected. Result showed that between evaluated genotypes there wasn't any genotype with complete resistance (100%) to *P. ultimum*, so the susceptibilities were assayed. Based on the assessed variables, genotypes LRV-55-295, Aceteria, PI-250537 and IL-111 appear to be highly susceptible to the *Pythium* infection, whereas 34040, Arak281, and Isfahan were the least susceptible to the disease. Further research will be conducted to determine whether the resistance detected in these genotypes is heritable.

Key words: disease, resistance, seed, seedling, soil

INTRODUCTION

Safflower, *Carthamus tinctorius* L., is a member of Compositae family that has been cultivated in different countries for many years as a good source of high linoleic oil and plant dye. The crop grows in almost all parts of Iran because of its high adaptation to drought, salinity and hot conditions (Zeinali, 1999). Like other

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crop plants, safflower cultivation suffers from many plant diseases, especially those caused by soil borne pathogens. Seed rot, pre or post emergence damping-off and root and hypocotyls rot caused by *Pythium* and *Fusarium* reported as the most important soil pathogen derived disease in safflower (Huang *et al.*, 1992; Mundel *et al.*, 1995 and Pahlavani *et al.*, 2007). *Pythium ultimum*, as a pathogen usually finds in all soils that the crop cultivates and its population increases by the cultivation of susceptible host (Mundel *et al.*, 1995). Due to its wide host range, long-lived oospores, ubiquitous nature, and large number of pathogenic types, seed and seedling rot or damping-off caused by Pythium is extremely difficult to control and has been reported to decrease seed yields by up to 25% in safflower (Ahmadi *et al.*, 2008). In safflower, many part of damage to be created on seed and seedling at the time of germination till emergence (Ebrinnia, 2001). Germination is one of the most important stage of the plant growth, therefore plants that well established could guarantee yield of the crop. Different fungal pathogens reduce seed germination and seedling emergence in safflower (Mundel *et al.*, 1995 and 1997). The first *Pythium* damage on safflower reported from Alberta in 1949 (Cormack and Harper, 1952). In Iran this disease reported first time at the experimental fields, Karaj College of Agricultural, on Frio safflower cultivar by Alagha in 1970. Ahmadi *et al.* (2008) reported that *P. ultimum* caused damping-off safflower in Golestan state soils. Huang *et al.* (1992) recognized that *Pythium sp. "group G"*, a form of *P. ultimum* causing damping-off in safflower. Between all types of Pythium that reported as the agent of damping-off in safflower, *P. ultimum* is more frequent (Ahmadi *et al.*, 2008). The high incidence of the disease when take place that the crop cultivated in warm and moist soil (Mundel *et al.*, 1995). Damage of *P. ultimum* on the safflower reported from all over the world, including United States of America (Klisiewicz, 1968), Australia (Kochman and Evans, 1969) Canada (Mundel *et al.*, 1995), Afghanistan, Argentine, India, Mexico and Iran (Abdollahi, 1995). Damping-off caused by *Pythium* species and *P. ultimum* reported on other crops including beet (Ebrinnia, 2001), alfalfa (Hancock, 1991), wheat (Higginbotham *et al.*, 2004), sorghum (Forbes *et al.*, 1987), bean (Dickson and Abawi, 1974) pea (Ohh *et al.*, 1978) and cotton (Joohnson and Palmer, 1985).

Pythium inoculum levels decrease with stubble burning and soil fumigation. Due to its harmful effects on soil quality and societal concerns for air quality, stubble burning is not a viable option for growers, and soil fumigation is impractical for large-scale crop production. Tillage also decreased *Pythium* inoculum levels in the top 10 cm of soil. For growers using direct-seed cropping systems as a means of soil erosion control, tillage is not an option as a control measure for Pythium root rot. Metalaxyl, a fungicide specific to oomycetes, is often used as a seed treatment for some crops, and it protects the germinating seedling from *Pythium* infection and damping-off. However, Metalaxyl provides little or no protection for the growing roots. Biological control of *Pythium* spp. using bacterial seed treatments also has had limited success, and no bacterial

seed treatments for safflower are commercially available. Producers have no long-term, sustainable option for controlling Pythium damping-off in the commercial fields. Incorporating genetic resistance into safflower cultivars would create an ideal, effective and inexpensive method of control for *Pythium* damping-off.

Overall, safflower is considered as a susceptible plant to diseases (Dajue and Mundel, 1996). Root and stem rot of safflower, caused by *Macrophomina phseolina,* is a serious disease of safflower in commercial fields over the world (Govindappa *et al.*, 2005; Pahlavani *et al.*, 2007). Malaguti (1950) reported that barbed safflower cultivars are more susceptible than no barbed cultivar to damping-off caused by *Phytophthora palmivora*. Thomas (1970) reported that two safflower cultivars have different susceptibility after 3, 5 and 9 week to *P. ultimum*. Availability of resistant to seedling diseases in safflower is limited and available cultivars did not show effective resistance to this disease. Recently research was done about this disease in north Canada and introduced new sources of resistance, including cultivar Saffire, to damping-off caused by *P. ultimum* (Mundel *et al.*, 1995). The objective of this research was to examine, the variation in susceptibility to *Pythium* damping-off among safflower germplasm developed in diverse environmental regions in order to identify donors of potential resistance genes useful for cultivar improvement.

MATERIALS AND METHODS

This study was conducted in research greenhouse at Gorgan University of Agricultural Sciences and Natural Resources (GUASNR) Iran, in 2007.

Plant materials

Seventeen safflower genotypes included released cultivars, accessions and advanced breeding lines were chosen for this evaluation (Table 1). All were provided by Seed Production Research Centers and reproduced at least for two generations in the field research of GUASNR, Iran. The genotypes were chosen in previous years, based on their genetic diversity and breeder recommendations, included winter and spring growth habit types, seed size, oil content and ornamental market classes. Eight genotypes have large seed size (Aceteria, Hartman, Syrian, CW-74, IL-111, PI-250537, LRV-55-295 and 541-5), four genotypes were of medium (Isfahan, Arak2811, Dinger and LRV-51-51) and five genotypes were belonged to small size group (Zarghan-259, IUTM12, 34062, 34074 and 34040) (Table 1). Seven of these genotypes, were originated from Iran, and were chosen based on results of an early screening for resistance to the pathogen.

Table 1

Name, origin and 1000-seed weight of 17 safflower genotypes used in this study

Genotype	Origin	1000-seed weight [g]
Arak2811	Iran	36.73
Isfahan	Iran	34.87
Zarghan259	Iran	31.00
IL-111	Iran	43.73
LRV-55-295	Iran	39.23
LRV-51-51	Iran	36.73
IUTM12	Iran	30.50
Hartman	U.S.A	38.10
CW-74	U.S.A	44.27
Syrian	Syria	43.73
Aceteria	Canada	43.30
PI-250537	Unknown	45.40
Dinger	Unknown	37.13
541-5	Unknown	45.27
34062	Unknown	32.13
34074	Unknown	31.97
34040	Unknown	24.53

Preparation of Inoculums and artificial inoculation of soil

Isolates of *Pythium* were isolated from rotted and no germinated seeds and also apparently diseased seedlings that was grown in the experimental field in 2006. The rotted seeds and 3 to 5 millimeters pieces of root from diseased plants separated and were thoroughly washed with distilled water, transferred to 0.5% sodium hypochlorite for 1 min and washed again in sterile water for 2 min and then cultured on potato dextrose agar (PDA) and corn meal agar (CMA) mediums. The cultures were incubated in 25°C during four days in darkness for isolation by a hyphal type method (Singelton *et al.*, 1992). Pathogen distinction was done based on zoospore forms, sporangium, zoospore, antridium, oogonium, number and joint between the antridiums to oogonium, according to monographs of Vander Plates-Niternik (1981).

Preparation and sterilization of soil greenhouse

Enough soil (clay loam with pH=7.7 and EC=0.96) was prepared form the field and sterilized in autoclave for three successive times. Each experimental

unit was a 50 cm diameter plastic pot (their surfaces were almost 400 cm^2) with enough depth. Plots were filled with 4 to 5 kg sterilized soil. Hempseed extract was used for making inoculums. First, 80 plastic bags each containing 500 g of sand that mixed with 300 mm hempseed extract inoculated with two 5 mm diameter pieces of 4 days colonies of *P. ultimum*. The bags were kept in a dark incubator at 25°C for 72 hours. Then the inoculated sands were mixed with the sterilized soil of 136 plots in a 1 to 8 ratio. After that, about 500mm distilled water was added to each plot and plots were kept in room temperature to provide more suitable condition for pathogen growth. Finally, the plots with inoculated soils transferred to the greenhouse for running the experiment.

The experiment was carried out in a factorial fashion with four replications in which sterile and infested soil considered as main plot and seventeen safflower genotypes were sub plots. For seeding, first 50 seeds were thoroughly washed with distilled water and transferred to 10% sodium hypochlorite for 1 minute and again washed in sterile water for 2 minutes. Then these seeds were planted in each plot in a 3 cm depth. In sterile plots, seeds were planted in sterilized soil. Finally, plots of sterile and infested media were irrigated with 500 mm distilled water and were kept in normal greenhouse condition form first June to late July, 2007. During this time irrigation with distilled water was done regularly.

To make more insurance about the inoculation, samples of germinated seed, diseased seedlings and ungerminated seeds were randomly chosen and washed in sterile water, dried on paper towel, transferred to 2% water agar in petridishes. The petridishes were incubated at room temperature for 3 days, and examined for the presence of the pathogen, resembling those identified as *Pythium ultimum* which has been used for production of the inoculated soils.

Data collection and Statistical Analyses:

In the infested plots, number of emerged and damped-off seedlings was recorded daily up to 20 days after first emergence. Finally, in sterile plots, percent of seedling emergence (SES) and in Pythium-infested media, percent of seedling emergence (SEI), percent of diseased seedlings (DSI), percent of uninfected seedlings (USI) and percent of non-emerged seeds (NSI) were calculated.

Resistant index (RI) was calculated according to Fischer and Maurer (1978). This index was calculated based on below formula that indicated real percent of emergence in infested media:

$$RI = \left[1 - \left(\frac{SES - SEI}{SES}\right)\right] \times 100$$

which in that, RI was Resistance Index, SES was percent of seedling emergence in sterile media, and SEI was seedling emergence in infested media. With considering RI, genotypes with higher RI were considered as more resistant.

Data were analyzed using SAS (2005). Studied variables, SES, SEI, DSI, USI and NSI were used in statistical analyses as split plot design. Before analysis the normality test of Kolmogorov-Smirnov (KS) was done for all the studied variables. By the results of KS test a square root data transformation was performed on data (Zar, 1984). Analysis of variance was conducted to test significance of genotype and soil infection main effects, as well as to identify any significant interactions among them. For each media, comparisons were made between genotypes using LSD test at the 0.05 probability level. Also the coefficient of correlation was calculated between all studied variables and RI.

RESULTS AND DISCUSSION

A range from 24.53 to 45.40 gr was existed for 1000-seed weight of the evaluated genotypes (Table 1). The highest seed weights were belonged to genotypes PI-250537, 541-5 and CW-74, and also genotypes 34040, IUTM12 and Zarghan259 had the lowest seed weight. Among the local genotypes, IL-111 and IUTM12 were the smallest and largest seed bearing genotypes, respectively (Table 1). Some *Pythium*-infected seeds were rotted and had small gray or black spot on their seed coat, so were inducing for emergence (Fig. 1). The pathogen, *Pythium ultimum*, invades the hypocotyls, cotyledons or some parts of emerging seedlings and caused rotting and collapse of infected tissues and so showed damped-off seedlings (Fig. 1). Symptoms were distinguished in this study were similar to those mentioned by Howard and David (2005) in the study of *Pythium* on safflower.

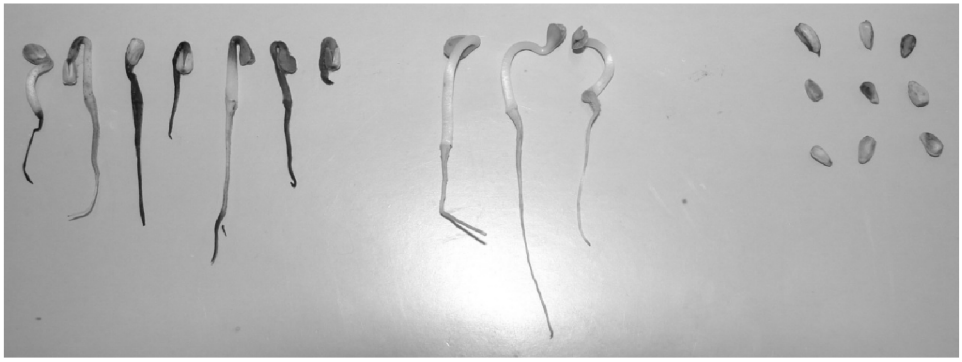

Fig 1. Occurrences of seed rot (right) and seedling death (left) in safflower due to *Pythium ultimum* infection. Normal seedlings are in the middle of the picture. The small gray or black spot on seeds, and dark-brown to black collapsed tissues on seedlings are obviously visible

The analysis of variance showed a significant effect of infested-media on percent of seedling emergence ($P < 0.01$; Table 2). As shown in Table 3, the pathogen considerably decreased emergence of seedlings from 60.1 % at the sterile media to 10.1 % at the infested media. This observation indicated that seeds and seedlings in safflower are greatly susceptible to *Pythium ultimum*, the causal agent of damping-

off. Damage of pathogens like *Pythium* and Fusarium that cause damping–off in safflower and other crops were numerously reported by other researchers (Thomas, 1970; Mundel *et al.*, 1995 and 1997; Ohh *et al.*, 1978). Huang *et al.* (1992) indicated that in naturally *P. ultimum* infested soil, the incidence of damping-off was high in safflower and dry pea, with less than 16 % of seedling surviving, moderately high in sugar beet, and low in canola, which had more than 70 % of seedling surviving. The significant effect of media also qualified that the infection method used in this study had enough potential for creating effective disease inducing media. The percent of seedling mergence in infested media was much less than the sterile media, with the infested media has 46 %, on average, that those of the sterile media. Effects of infesting soil with Pythium were reported by others in former studies (Dhingra and Sinclair, 1978; Mundel *et al.*, 1995). However, the level of infection in the glasshouse grown bioassay material was higher than those reported by Palooj *et al.* (2009) in the field condition, and also was lower than the infection level caused in the paper towels media that reported by Huang *et al.* (1992) and Pahlavani *et al.* (2009). This could have been predicted because of the optimum infection conditions that provide in controlled environments like glasshouse, in where high moisture is available for plant and pathogen, increasing infection.

Table 2

Analysis of variance for seed and seedling traits of safflower genotypes in sterile and infested soil with *Pythium ultimum* under greenhouse conditions

Mean square			Mean square				
Sv	df	Percent of emergence	Sv	df	DSI	USI	NSI
Block	3	190.62	Block	3	123.50	117.9	357.4*
Media	1	85100.03**	Genotype (G)	16	23.30	71.1	137.7
Error of media	3	169.91	Error	48	18.16	44.6	97.7
Genotypes	16	957.94**					
Genotypes × Media	16	576.4**					
Error of genotypes	96	107.20					

* and **: significant at 5 and 1 %, respectively. DSI, USI and NSI: percent of diseased seedlings, percent of uninfected seedlings, and percent of non-germinated seeds at Phytium ultimum infested media, respectively

There were significant differences among the genotypes for percent of seedling emergence ($P < 0.01$; Table 2). Enough genotypic variability could help researchers to find genotypes carrying resistance genes for decreasing damage of the pathogen in the early growth stages. Respectively, CW-74 and IL-111 at sterile soil, and IL-111 and Syrian at infested soil were the genotypes with the highest and lowest percent of seedling emergence (Table 3). Existence of genetic variation for response to pathogen causing seed rot or damping-off reported by Davia *et al.*, (1982) and Mundel *et al.*, (1995). Sharifnabi and Saidi (2004) studied some safflower genotypes for their resistance to damping-off caused by

Fusarium and concluded that genetic variation are enough for breeding programs that aimed selection genotypes or creating cultivars with resistance to the pathogen.

Table 3

Comparison of means in seed and seedling in 17 safflower genotypes under sterile and infested soil to the *Pythium ultimum*

Genotypes	SES	SEI	DSI	USI	NSI	RI
Arak2811	54.5^{bcd}	17.5^{ab}	6.5^{a}	11.0^{ab}	82.5^{bc}	31.7
Isfahan	66.5^{abc}	17.5^{ab}	7.0^{a}	10.5^{abc}	82.5^{bc}	26.4
Zarghan259	52.5^{cd}	10.0^{abc}	5.0^{ab}	5.0^{bc}	90.0^{abc}	22.4
Hartman	72.5^{a}	17.5^{ab}	7.0^{a}	10.5^{abc}	82.5^{bc}	24.2
Dinger	76.5^{a}	15.5^{abc}	6.5^{a}	9.0^{abc}	84.5^{abc}	19.8
Syrian	76.5^{a}	20.5^{a}	2.0^{ab}	15.0^{a}	79.5^{c}	23.5
Aceteria	74.0^{a}	5.5^{bc}	2.0^{ab}	3.5^{bc}	94.5^{ab}	7.0
PI-250537	73.0^{a}	5.0^{bc}	1.0^{ab}	4.0^{bc}	95.0^{ab}	7.2
CW-74	77.5^{a}	8.0^{abc}	5.0^{ab}	3.0^{bc}	92.0^{abc}	9.7
IL-111	25.0^{f}	1.5^{c}	0.0^{b}	1.5^{c}	98.5^{a}	8.2
LRV-55-295	76.5^{a}	4.5^{bc}	2.0^{ab}	2.5^{bc}	95.5^{ab}	5.4
LRV-51-51	69.0^{ab}	9.5^{abc}	6.5^{a}	3.0^{bc}	90.5^{abc}	14.3
541-5	73.5^{a}	10.0^{abc}	4.5^{ab}	5.5^{bc}	90.5^{abc}	14.1
34062	26.5^{ef}	3.5^{bc}	2.0^{ab}	1.5^{c}	96.5^{ab}	13.8
34074	55.5^{bcd}	13.0^{abc}	2.0^{ab}	11.0^{ab}	87.0^{abc}	25.2
34040	31.0^{ef}	8.5^{abc}	1.0^{ab}	7.5^{abc}	91.5^{abc}	31.9
IUTM12	41.5^{de}	4.0^{bc}	2.5^{ab}	1.5^{c}	96.5^{ab}	11.3
Mean	61.1	10.1	3.7	6.2	99.0	17.4

SES: percent of seedling emergence at sterile media; SEI, DSI, USI, NSI and RI: percent of seedling emergence, percent of diseased seedlings, percent of uninfected seedlings, percent of non-germinated seeds and resistance index at Phytium ultimum infested media, respectively

Genotype × media interaction had significant effect on percent of seedling emergence of the safflower genotypes (Table 2). The source of the interaction mainly involved genotypes Aceteria, PI-250537, LRV-55-295 and 34040 which their ranks were significantly differed over sterile to infested media (Table 3). The dissimilarity of emergence over sterile and infested media shows that, for these genotypes there are different genetic control mechanisms in each environment. It also means that breeding for improving emergence in pathogen free soils don't have significant association with breeding for infested soils. Higginbotham *et al.*, (2004) reported that comparison between percent of emergence in both sterile and infected media is the best way for identification susceptible wheat genotypes to *Pythium* fungus. Because of existence changing

in rank of the genotypes over two levels of soil infestation, the comparison of the genotypes was separately done at the sterile or infested media. Percent of seedling emergence at sterile media (SES) was ranged from 25.0 to 77.5 with a mean 60.1 %, and percent of seedling emergence at infested media (SEI) was varied from 1.5 to 20.5 with a mean 10.1 % (Table 3). The smallest decreases in seedling emergence caused by *P. ultimum* were occurred in genotypes IL-111, 34062 and 34040 with 25.0, 26.5 and 31.0 % at the no infested soils (control), respectively (Table 3). The largest decreases in seedling emergence caused by *P. ultimum* occurred in genotypes CW-74, Acetaria and PI-250537 that had 77.5, 74.0 and 7.03 % emergence at sterile soil (Table 3). NSI, percent of nonemerged seeds at infested soil, was ranged from 79.5 to 98.5 %. NSI shows the amount of seeds that could not emerged on the soil surface or directly means amount of seed rot. DSI, percent of infested seedlings, was relatively little in relation to NSI (Table 3).

In this study, both pre and post emergence damage was observed. Pahlavani *et al.* (2009) reported that symptoms of *Pythium* in safflower express as both pre and post emergence. The amount of post emergence damping-off appeared in table 3 as DSI, percent of infected seedlings. So the smallest pre emergence damping-off (NSI) was observed in genotypes Syrian, Hartman, Isfahan and Arak2811, and the smallest post emergence damping-off (DSI) was occurred in genotypes IL-111, 34040 and PI-250537 (Table 3).

The choice of superior genotypes for their ability to emergence at both sterile and *Pythium* infested soil was made difficult by occurrence of variability in value of SES, SEI, DSI and NSI (Table 3). So, resistance index (RI) was calculated by using the amount of seedling emergence at both sterile and infested soils. Genotypes with a large RI have a small reduction in seedling emergence caused at infested soil with *P. ultimum* in relation to sterile media. Based on RI, the genotypes could classify into resistant and susceptible groups. In this regards, the studied genotypes with RI from 5.4 to 31.9 % characterized from susceptible to resistant (Table 3). The most resistant genotypes were 34040, Arak2811, Isfahan and 34074 with RI of 31.8, 31.7, 26.4 and 25.2 %, respectively (Table 3). Genotypes 34040 with unknown origin with a poor emergence at sterile media had a low SES and a moderate NSI among the studied genotypes (Table 3). Arak2811, an Iranian cultivar, with a good SES and USI had a relatively low NSI (Table 3). Genotype Isfahan that is a breeding line from indigenous material was high in both SES and NSI. The results also revealed that the most susceptible genotypes against *P. ultimum* were LRV-55-295, Aceteria, PI-250537, IL-111 and CW-74, since their RI were 5.4, 7.0, 7.2, 8.2 and 9.7 %, respectively (Table 3). Genotypes LRV-55-295, Aceteria, PI-250537 and CW-74 had a high SES and low USI, but IL-111 had the lowest SES and USI (Table 3). Others including Zarghan259, Hartman, Dinger, Syrian, LRV-51-51, 541-5, 34062 and IUTM12 had a RI in range of 11.3 to 24.2 % and

considered as moderately resistant genotypes. Heritage and Harrigan (1984) screened eleven safflower breeding lines along with Gila, a susceptible cultivar, in the disease nursery for resistance to Phytophtora root rot. They found that accession A1110 from Iran, had a satisfactory resistance to field infection indicating both stem and root rot resistance. They also showed that selections from accession A1110 have proved consistently resistant in both field and greenhouse screening with only 26 to 28 % loss of plants under field conditions. In their study another accession from Iran, A1108, was also studied. A1108 along with others showed excellent resistance to *Phytophtora* (Heritage and Harrigan, 1984).

Table 4

Coefficient of correlation among seed and seedling parameters of safflower in *Phytium ultimum* infested soil

Seed weight		SES	SEI	DSI	USI	NSI	RI
Seed weight	1						
SES	0.58*	1.00					
SEI	0.04	0.44	1.00				
DSI	0.07	0.53*	0.79**	1.00			
USI	0.02	0.30	0.93**	0.53*	1.00		
NSI	-0.04	-0.44	-1.00**	-0.79**	-0.94**	1.00	
RI	-0.51*	-0.16	0.75**	0.46	0.79**	-0.75**	1.00

SES: percent of seedling emergence at sterile media; SEI, DSI, USI, NSI and RI: percent of seedling emergence, percent of diseased seedlings, percent of uninfected seedlings, percent of non-germinated seeds and resistance index at Phytium ultimum infested media, respectively

Coefficients of correlation between emergence and seed parameters are showed in Table 4. There was a significant and positive correlation between seed weight and SES, percent of emergence in sterile media ($r=0.58^{*}$), although there was no association between seed weight and emergence at infected media ($r=0.04$). This means that seed weight had a significant role in safflower emergence just at pathogen free soils. Alinejad (2005) studied the effects of seed size in safflower, and took result that seed size had direct effect on seed germination and seedling emergence. SES, percent of emergence in sterile media, correlated significantly with DSI, percent of infected plant in infected media ($r= 0.53^{*}$). This result showed that number of infected seedlings was increased with increasing of seedling emergence, and it seems that pathogen mainly caused post emergence damping-off. This association could be used in indirect selection to decrease number of seedling infection under infested media to *P. ultimum*. Seed weight also was significantly correlated with RI (r=-0.51*, Table 4). This emphasis again that seed weight could help safflower breeders to find resistant genotype to *P. ultimum*, early in breeding projects.

A significant and positive correlation was detected between DSI and SEI ($r=0.79^{**}$). It seems that increasing of seedlings could prepare more plant tissues for occurring incidence of the disease. This result was in according to report of

Hancock (1991) in study of *P. ultimum* on alfalfa, that with increase of seedling emergence, the percent of diseased seedlings have been increased.

RI, resistance index, had a good relationship with most of the seedling parameters, because its correlation with SEI, USI and NSI was 0.75**, 0.79** and -0.75**, respectively (Table 4). So selection for genotypes with higher RI could be result in increase SEI and USI, but would decrease NSI. This is in agreement with Ahmadi *et al.* (2008) that concluded RI is a good measure for selection of resistant genotypes against *P. ultimum*. In Table 4, there were a significant and negative correlation between DSI and NSI ($r=-0.79^{**}$), and between USI and NSI ($r=-0.94^{**}$). It could be concluded that DSI and NSI are not suitable traits for decision about resistance to *P. ultimum* damping-off in safflower. These results are in agreement with reports demonstrated by Higginbotham *et al.*, (2004) on wheat, indicated that contamination seed by *P. ultimum* is guidance for damping-off.

CONCLUSION

In general the results showed that damping-off in safflower causing by *P. ultimum* occur as pre and post emergence cases. Also genotypes with large seed size (mostly foreign genotypes) had high emergence in both sterile and infested media and genotypes with small seed size (mostly Iranian genotypes) had high resistance to damping-off. Performance of genotypes in both sterile and infected media showed that resistance index is better index for selection resistant genotypes. In this study, identification of resistant and susceptible genotypes was performed based on the results of resistance index (RI), in that, genotypes 34040 and Arak2811 were relatively resistant and LRV-55-295 was susceptible. And genotypes Arak2811 and 541-5 could be used in breeding program of safflower to produce cultivars with resistance to seed rot and seedling damping-off (Table 3).

Also, our results indicate that variation exists among safflower genotypes in their level of susceptibility to *P. ultimum.* No relationship was found between emergence at sterile soil and percent of seedling emergence at *P. ultimum* infested media. Genotypes 34040, Arak2811, Isfahan and 34074 were the most resistant to *P. ultimum* infection among the safflower genotyprs evaluated in this study. Further research will be conducted to determine whether the resistance detected in these genotypes is heritable, and whether the screening method used here are associated with tolerance in the field. Genetic mapping populations will be developed in an attempt to identify DNA markers for resistance gene candidates. Data presented here may prove useful when selecting crossing parents for use in safflower variety enhancement.

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