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Full Length Research Paper

Influence of temperature and genotype on *Pythium* damping-off in safflower

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Improvement of genetic potential in safflower (*Carthamus tinctorius*) against Pythium species would be an efficient means of control of this major seed and seedling fungal pathogen. The type and content of reaction for plant to pathogen could be severely affected by environmental conditions such as temperature. In this study seed rot and seedling damping-off of fourteen safflower genotypes that came from different origins, were evaluated using *Pythium ultimum* infected and sterile paper towels at temperatures 10, 15, 20, 25 and 30°C. Both factors including the temperatures and the genotypes and their interaction affected seed germination of safflower. The results showed that temperature had a significant effect on number of normal and diseased seedlings in Pythium-infected media. Among the five different levels of treated temperatures, the lowest number of normal seedlings occurred at 25 and 30°C, and the lowest number of diseased seedlings were also observed at 10 and 15°C. There was a considerable difference among the fourteen studied genotypes for number of normal seedlings and number of diseased seedlings in infected media under laboratory conditions. The effect of genotype × temperature interaction on both number of normal seedlings and number of diseased seedlings was no significant. Cultivar CW-74 had the lowest, and cultivars LRV-51-51 and LRV-55-259 had the highest number of normal seedlings under *Pythium* - infected conditions. And also, Line 34072 had the lowest, and cultivar CW- 74 had the highest number of diseased seedlings in Pythium-infected media. In fields infesting with *P. ultimum*, sowing safflower seed when temperature is more than 15°C is likely to have poor stand establishment due to seed rot and seedling damping-off. Therefore it is advisable to plant safflower early when soil temperature is cool.

Key words: *Pythium ultimum*, zoospore, seed, seedling, rot.

INTRODUCTION

Safflower, *Carthamus tinctorius* L., is an annual, broad leaf crop which belongs to the family of Compositeae. Safflower is cultivated worldwide as an oilseed or ornamental crop. In Iran, this crop is grown for its seeds to extract oil or feed home birds, and also for its flowers to use in medicine or ornamental purposes, and is being cultivated on approximately 1000 ha annually (FAO, 2008). Safflower suffers severely from soil pathogens, which may attack seed, germinating seed, and young seedlings or at time of seed formation, causing directly or indirectly yield and quality losses. Seed and seedling rots by *Pythium* as well as *Phytophthora* rots are among the more devastating soil borne diseases of safflower (Heritage et al., 1984; Huang et al., 1992). Studies showed that *Pythium ultimum* Trow. is the causal agent of seed rot and seedling damping- off of safflower in Iran and other countries (Ahmadi et al., 2008; Ahmadinejad and Okhovat, 1976; Huang et al., 1992, Mundel et al., 1995). It is not only made some limitations for safflower production in Iran, but also for the other producing areas in the world. The pathogen parasitizes seeds and invades the hypocotyl or first internode tissues of safflower seedlings

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and causes rotting and collapse of infected tissues and finally decays seeds and seedlings (Kolte, 1985; Thomas, 1970). Although different chemical fungicides are used to control damping-off, but similar to other fungi diseases the best way for decrease the losses is planting the resistant cultivars.

The condition of infection, seed decay and seedling death caused by Pythium has been studied in safflower and other crop plants (Ben-Yephet and Nelson, 1999; Fortnum et al., 2000; Mundel et al., 1995; Thomas, 1970). Like other soil borne pathogenic fungi, severity of infection by *Pythium*, incidence of damping-off and loses in crop production is a function of environmental factors and how the plants can use from their genetic potential to resist against the pathogen (Ahmadi et al., 2008). Among the most important environmental factors which can favor the disease, temperature plays a key role. The optimum temperatures for infection of citrus fruits to brown rot that caused by *Phytophthora palmivora* were 27 to 30°C (Timmer et al., 2000). Ben- Yephet and Nelson (1999) studied the effects of 20, 24, 28 and 30^0C on differential suppression of *Pythium* irregulare and showed that the pathogen caused damping- off in cucumber only at 20 and 24^0C. Temperature had a profound impact on root rot development, plant growth and infection of carambola (Averrhoa carambola) roots by *Pythium* splendens (Ploetz, 2004). Infection of apples and pears with Phytophthora cactorum required 3 to 7 h of wetness at temperature 15 to 30^0C (Grove et al., 1985). *Pythium aphanidermatum* and *Pythium myriotylum* are considered to be broad host range species favored by very warm conditions, whereas others such as *P. ultimum* and *P. irregu-are* are considered to be broad host range species favored by cool conditions (Van der, 1981).

The incidence of damping off of safflower caused by *P. splendens* was reported to increase with temperature from 10 to 25^0C (Thomas, 1970). Mundel et al. (1995) performed an experiment on infected soil with *P. ultimum* and indicated that temperature level affected emergence of safflower seedlings and incidence of damping-off. They showed that safflower should be seeded early when soil temperature is low, even though emergence may be slow. Also they concluded that if seeding is delayed until soil temperatures are higher than 10ºC, growers should consider not planting safflower if soil moisture levels are high (Mundel et al., 1995). On the other hand the optimum temperature for safflower seed germination is about 25°C (Gu and Xu, 1984). As noted, the effect of temperature on seed and seedling growth and incidence of *Pythium* damping-off in safflower has been investigated by researchers. But the best temperature for both pathogen and host has not been investigated. So, finding the optimum temperature in which favorable conditions provide to both fungal infection and expression of disease resistance in host is an important aim of safflower breeders. This study was undertaken to determine the tempe-rature conditions decreasing seed rots and seedlings damping-off caused by *P. ultimum* in different safflower genotypes. The objectives were to find temperature in which the lowest seed and seedling death takes place, recognize the most resistant genotype to the pathogen; and determine the effect of temperature × genotype interaction on the disease.

MATERIALS AND METHODS

This study was performed at Gorgan University of Agricultural Sciences and Natural Resources (GUASNR), Gogan, Iran in 2008. In this study germination of fourteen safflower genotypes was evaluated using a test by *Pythium*-infected and sterile paper towels at five temperatures in four replications.

Isolate of pathogen

Isolate of *P. ultimum* used in this study was recovered from rotted seeds and dead seedlings of safflower showing typical disease symptoms. The 3 to 5 mm pieces of diseased tissues were surface-sterilized by immersing in 0.5% hypochlorite sodium for 1 min. Then, the sterilized pieces were grown on a CMA selective medium that contained penicillin, streptomycin sulfate, pimaricin antibiotic and benomil (Singleton et al., 1992). After incubation on agar plates for 4 days at 25^0C, some pieces of fungi cultures transferred to another plates containing 2% water agar. These plates keep for 24 h at room temperature and the pathogen purified by single hyphal tip isolation technique as describe by Singleton et al. (1992). Identification of *Pythium ultimum* was performed using previously published criteria (Dick, 1990; Van der Plaats-Niterink, 1981).

Zoospore production of *P. ultimum* was activated using a method described by Rahimian and Banihashemi (1979). To prepare zoospore suspension, 4 × 4 mm2 piece of fully grown agar plates were flooded in 500 ml flask containing sterilized distilled water and kept in light conditions for 72 h. These flasks were incubated for 10 min at 5^0C and followed by keeping for 2 h at room temperature for releasing of zoospores. Zoospores concentrations were estimated with a hemacytometer, and the appropriate dilution was made with sterilized water to a final concentration of 10^5 zoospores ml-1.

Genotypes of safflower

Safflower genotypes tested in this experiment formed part of safflower collection held at the GUASNR, Gorgan, Iran. These genotypes were included cultivars, promising lines and plant introductions from Iran and other countries and to keep their genetic purity were grown along with controlling cross pollinations at least for three years in Research Farm of the GUASNR. The main reasons for choosing them to be in this study were their good performance, high seed production and considerable variability in seed size and oil content. The names and origins of selected genotypes are shown in Table 3.

Seeding, incubation, germination and disease assessments

50 seeds of each genotype were surface-sterilized in 2% sodium hypochlorite for 3 min, placed on a 50 × 50 cm2 paper towel. Then another paper towel was placed on seeds and former paper towel and all were rolled. All rolled towels were wetted with distilled water for control and with 10^5 zoospore suspension in *Pythium*-infected treatments. To keep the humidity on the paper towels (experimental units), all of them put in plastic bags. The experimental units were separately incubated at 10, 15, 20, 25 and 30°C. After 7 days keeping experimental units in incubator, number of germinated seeds (NGS), number of normal seedlings (NNS) and number of diseased

Figure 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.

seedlings (NDS) were counted. Seedlings were considered germinated when the 3 mm of rootlets went out of the seed coat. The normal seedlings were all apparently healthy (symptomless) seedlings and diseased seedlings were those showed typical symptoms of Pythium damping-off such as brown discoloration (Figure 1).

To verify the cause of seed rots and seedlings damping-off, samples of germinated seed, diseased seedlings and ungerminated seeds were washed in sterile water, dried on paper towel, transferred to 2% water agar in petridishes. The petridishes were incubated at room temperature for 2 to 3 days, and examined for the preence of the pathogen, resembling those identified as *P. ultimum* which has been used for production of the zoospore suspension.

Experimental design and statistical analyses

The temperature levels (main plot; 10, 15, 20, 25 and 30°C), media (sub plot; *Pythium*-infected and control) and genotypes (sub- sub plot, fourteen safflower genotypes) were run as a split-split plot design with four replications (Snedecor and Cochran, 1980). Each experimental unit was a two rolled paper towels containing 50 seeds. For each experimental unit NGS, NNS and NDS were recorded. Analysis of variance were carried out on NGS, NNS and NDS data to determine if temperatures, infection, genotypes and their interactions have significant effects on the recorded traits.

After examining data with a Kolmogorov- Smirnov (KS) test, data were put through a log transformation to stabilize the variance. Although data transformation decreased error in the coefficient of variation in analysis of variance table but had no significant effects on results, so the raw data were used in all analyses. Analysis of variance, least significant differences (LSD) test and KS test were carried out using the GLM procedure of SAS (SAS, 2004).

RESULTS

Some *Pythium*-infected seeds were rotted and small gray or black spots had been observed on their seed coat, so showed reduced germination (Figure. 1). The pathogen, *P. ultimum*, invades the hypocotyls, cotyledons or some parts of germinating seedlings and caused rotting and collapse of infected tissues and so showed damped-off seedlings. Temperature had a highly significant effect on number of germinated seeds (NGS) (Table 1). The highest NGS occurred at temperatures 10, 15, 20 and 25°C and the lowest observed at 30°C (Table 2). The difference between *Pythium*-infected and control media for NGS was significant at 1% level and NGS was greater in control than *Pythium*-infected media (Tables 1 and 2). Genotypes differed significantly in their ability to germinate ($P < 0.01$) (Table 1). The results also showed that there is no significant interaction between genotype and media in this experiment (Table 1). The NGS of the genotypes had a variation between 39.1 and 47.8 (Table 3). In general, in every 50 seed plot, more than 45 seeds were germinated at all temperatures for all genotypes. Hartman, Dinger, LRV-51-51 and Arak-2811 with more than 45 germinated seeds were those genotypes that got letter 'a' in LSD test grouping at all temperatures (Table 3).

Because the interaction between genotype and temperature was highly significant for NGS (Table 1), the LSD test among genotypes was separately performed at each temperature (Table 3). At 10°C, the greatest NGS belonged to a group of genotypes including all except genotypes CW-74 and 34074 (Table 3). At 15°C, the highest NGS was observed in a group of genotypes including Acetria, LRV-55-295, Hartman, Dinger, LRV-51-51, Arak-2811 and Zarghan- 259 (Table 3). At 20°C, the greatest NGS belonged to a group of genotypes including Acetria, LRV-55- 295, Hartman, Dinger, LRV-51-51, Arak-2811, Isfahan, Zarghan-259 and 34074 (Table 3). At 25°C, the highest NGS were observed in a group of genotypes including Acetria, LRV-55-295, Hartman, Dinger, LRV-51-51, Arak- 2811, Isfahan and Zarghan-259 (Table 3). And finally, at 30°C the greatest NGS belonged to a group of genotypes including LRV-55-295, Hartman, Dinger, LRV-51-51, Arak-2811 and Zarghan-259. Temperature had a significant effect on number of normal seedlings (NNS) in *Pythium*-infected media (Table 1). Among the 5 different levels of treated temperature, the highest NNS were observed at 10, 15 and 20°C (Table 2). Genotype had little effect on NNS, and all genotypes were similar and had a considerable NNS except genotypes CW-74 and 5-541 (Tables 1 and 3). The range of NNS for the genotypes was 14.90 to 22.20 (Table 3). Also the interaction bet-

Table 1. Analysis of variance of the effect of temperature and genotype on number of germinated seeds (NGS), number of normal seedlings (NNS) and number of diseased seedlings (NDS) in infected media with *Pythium ultimum* in safflower.

Sv	df	NGS	Sv	df	NNS	NDS
Temperature (T)	4	49.51**	Temperature (T)	4	4856.96**	3668.04**
Error 1	15	9.69	Genotype (G)	13	78.38	40.89
Media (M)	1	75.78**	G × T	52	39.20	48.02
M × T	4	5.54	Error	210	63.27	57.27
Error 2	15	6.82	Total	279	—	—
Genotype (G)	13	65.37**				
G × T	52	10.60**				
G × M	13	6.48				
G × M × T	52	4.17				
Error 3	390	6.01				
Total	559	—				

**; significant at % level.

Table 2. Effect of temperature and *Pythium*-infection on seed germination, number of normal and diseased seedlings in safflower.

Temperature (^{O} C)	NGS	NNS	NDS	Media	NGS
10	45.4±2.59	25.7±4.91	19.7±4.81	Sterile	45.6±2.78
15	44.7±2.76	27.5±6.37	17.2±5.69	infected	44.9±2.91
20	45.8±2.37	25.2±11.31	20.5±10.32	LSD(0.05)	0.471
25	44.9±2.79	9.0±6.17	34.9±6.84		
30	43.6±3.43	9.5±8.14	33.3±8.05		
LSD (0.05)	0.888	2.963	2.819		

Means followed by the same letter within a column are not significantly different (P> 0.05) according to the least significant difference (LSD) test; NGS: number of germinated seeds; NNS: number of normal seedlings; NDS: number of diseased seedlings.

Table 3. Effect of safflower genotype on seed germination, number of normal and diseased seedlings in infected media with *Pythium ultimum* at temperatures 10, 15, 20, 25 and 30ºC.

Genotype	Origin	NGS					NNS	NDS
		10 ° C	15 ° C	20 ° C	25 ° C	30 ° C		
Arak-2811	Iran	46.1±2.87	46.5±3.59	46.2±1.91	47.3±2.62	47.8±0.81	20.3±12.21	26.0±11.56
Isfahan	Iran	45.6±1.41	43.6±2.08	46.1±0.81	45.0±3.69	43.6±2.62	18.8±11.01	25.5±8.87
Zarghan-259	Iran	45.5±2.70	46.3±2.68	45.5±1.70	47.0±2.38	45.8±3.68	20.3±11.75	25.0±9.04
LRV-51-51	Iran	45.5±4.76	46.2±2.44	45.5±2.62	46.3±3.50	45.7±2.75	22.2±9.21	23.3±7.86
LRV-55-295	Iran	45.5±1.73	46.8±2.51	45.5±1.82	46.3±2.38	45.6±4.08	21.8±10.79	23.7±10.27
IL-111	Iran	45.8±2.50	44.1±0.95	44.7±1.91	43.1±3.86	41.8±1.73	18.8±11.39	25.6±9.00
Dinger	Turkey	46.6±2.62	45.3±2.06	45.8±1.29	45.3±1.70	46.0±2.62	19.8±11.50	25.4±11.94
Syrian	Syria	46.7±2.21	44.1±1.41	45.0±1.41	45.0±1.50	43.7±0.95	20.6±12.72	24.5±11.94
CW-74	USA	45.7±3.46	42.0±3.20	45.1±3.69	45.0±0.95	43.8±2.44	14.9±11.53	27.7±11.93
Hartman	USA	47.7±1.70	47.6±0.81	47.7±2.06	47.7±1.50	47.2±0.57	20.4±10.53	26.6±10.53
Aceteria	Canada	45.3±1.70	46.8±2.38	47.2±4.69	45.3±1.15	44.5±1.50	18.1±12.75	26.6±13.40
PI-250537	Unknown	46.8±2.51	44.7±2.61	47.2±2.36	44.8±2.94	42.6±2.50	19.5±10.80	24.0±10.42
5-541	Unknown	45.5±3.30	44.1±1.73	44.2±3.69	41.5±1.29	39.1±1.73	16.2±11.10	25.2±10.99
34074	Unknown	43.5±2.06	43.5±1.91	45.7±3.68	43.5±3.59	41.3±2.061	19.3±12.16	22.3±10.25
LSD (0.05)	—	2.283	2.390	2.534	2.377	2.653	—	—

Means followed by the same letter within a column are not significantly different (P > 0.05) according to the least significant difference (LSD) test; NGS: number of germinated seeds; NNS: number of normal seedlings; NDS: number of diseased seedling.

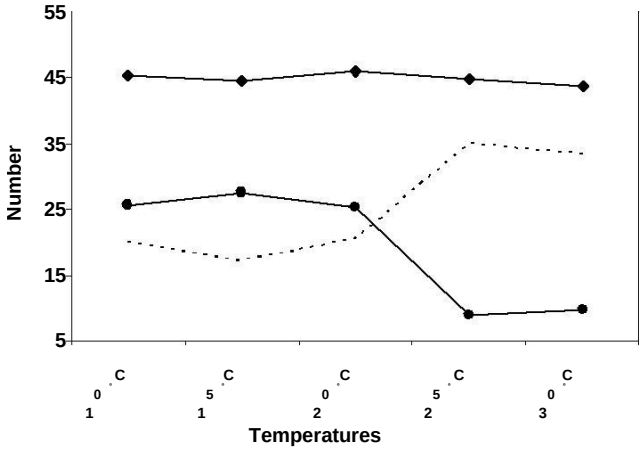

Figure 2. Effects of temperature on number of germinated seed (◆——◆), number of normal seedlings (●——●) and number of diseased seedlings (▬▬▬) of safflower at infected media with *Pythium ultimum*.

ween genotype and temperature was not significant for NNS (Table 1).

There was a significant difference between used temperatures for their effects on number of diseased seedlings (NDS) in *Pythium*- infected media (Table 1). NDS was greatest at 25 and 30°C (Table 2). But, genotypes were similar to each other for NDS, because there were no significant difference among them (Table 1). NDS of the genotypes varied between 22.3 and 27.7 (Table 3). The lowest NDS belonged to genotype 34072 (Table 3). Also the effect of interaction between genotype and temperature was not significant on NDS (Table 1).

The effect of temperature on NGS, NNS and NDS are also shown in Figure 2. As shown in this figure more NNS was observed at 10, 15 and 20°C (always 25) than at 25 and 30°C. The best temperatures for development of damping-off were higher temperatures, because more NDS occurred at 25 and 30°C (always 33) than 10, 15 and 20°C (Figure 2).

For better understanding of variability in NGS, NNS and NDS of the studied genotypes over the temperatures, 3 graphs were created separately for each temperature (Figures 3, 4 and 5). The levels of temperature had a more differential effect on both NNS and NDS than NGS. In case of NGS, as shown in Figure 3, lines representing temperatures have many cutting off or overlapping. However, both NNS and NDS lines for temperatures 25 and 30°C were interestingly distinct in relation to lines of other temperatures (Figures 4 and 5). Also, there were considerably similar trend among temperature lines in both NNS and NDS than NGS (Figures 3, 4 and 5).

DISCUSSION

Both temperature and genotype affected number of germinated seeds (NGS) in safflower. Effect of temperature on seed germination has been reported several times in safflower (Ayan et al., 2005) and other crop plants (Nyachiro et al., 2002; Riley, 1981). Maftoun and Sepaskhah (1978) studied effects of different temperatures on safflower seed germination and showed that the temperatures in which maximum germination took place were 10 and 20°C which were similar to the result of this study. Observation of the significant difference among genotypes along with non significant mean squares of media × genotypes for seed germination showed that the evaluated genotypes kept their germination potential whether at sterile or Pythium-infected media. It could be concluded that these genotypes have good potential for seed germination because almost all of them showed NGS over 45 per each 50 seed-plots (Table 3).

There was a significant difference among the 14 studied genotypes for number of normal seedlings (NNS) and number of diseased seedlings (NDS) in infected media under laboratory conditions. Genotypes LRV-55-259 and LRV-51-51 showed the higher NNS whereas CW-74 and 5- 541 had the lowest NNS under *Pythium*-infected media. The lowest and highest NDS belonged to genotypes 34074 and CW-74, respectively. The variation in disease incidence among the genotypes could be attributed to differences in susceptibility to the pathogen. Presence of genotypic variation for response to infection with *Pythium* or other causal pathogens of damping -off have been reported by other researchers in safflower (Ahmadi et al., 2008; Heritage and Harrigan, 1984; Mundel et al., 1997). Also, it could be concluded that in *Pythium* free conditions, these genotypes should have good seedling establishment because all of them had a great percent of diseased seedlings under *Pythium*-infected conditions (Table 3). Breeding for resistance to damping-off in safflower is probably the most promising approach to minimizing

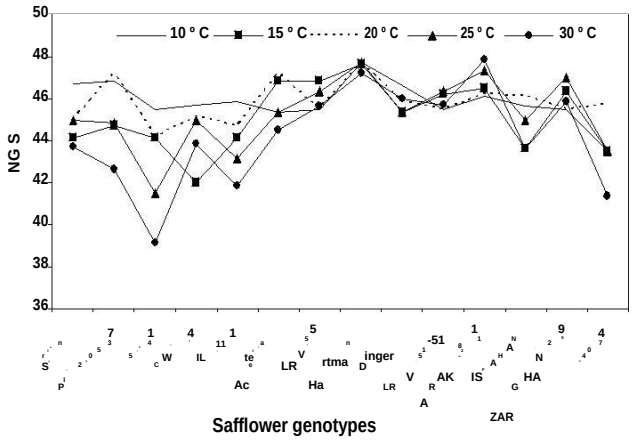

Figure 3. Effects of temperature on number of germinated seeds (NGS) in safflower at infected media with *Pythium ultimum*.

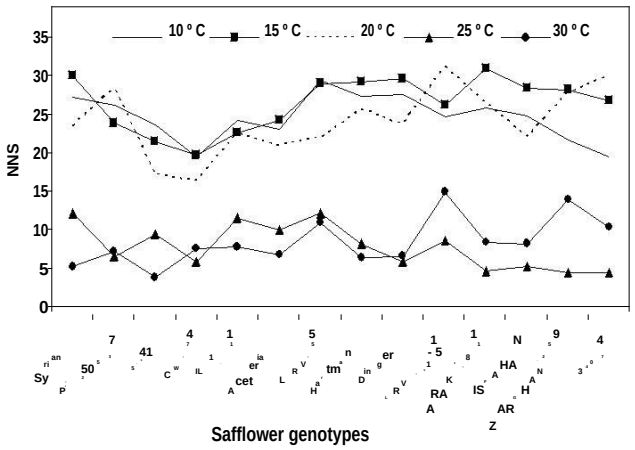

Figure 4. Effects of temperature on number of normal seed - lings (NNS) in safflower at infected media with *Pythium ultimum*

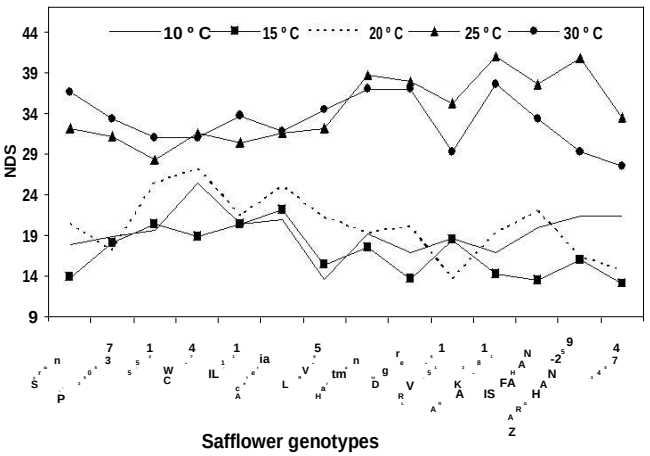

Figure 5. Effects of temperature on number of diseased seedlings (NDS) in safflower at infected media with *Pythium ultimum*.

mizing damages of the *P. ultimum*. Some of the studied genotypes had good potential of normal seedlings in *Pythium*- infected media and could be considered in breeding program for improvement of resistance to *Pythium* damping-off in safflower.

The number of diseased seedlings (NDS) caused by *P. ultimum* increased as incubation temperatures increased over 15°C and reached a maximum at 25 and 30°C (Figure 2). And, the maximum number of normal seedlings (NNS) were observed at 15°C and decreased as temperature increased up to 30°C (Figure 2). The increase in incidence of damping-off with temperature observed is in accordance with results of Thomas (1970) on *Pythium* splendens, and 24ºC is reported to be optimal for *P. irregulare* (Ben-Yephet and Nelson, 1999). Mundel et al. (1995) evaluated the effects of soil temperature, soil moisture and *P. ultimum* infection on the emergence of 12 safflower genotypes. They found that emergence in *Pythium*-infested soil was relatively high at 5, 10 and 15°C, but was dramatically lower at 20 and 25°C, particularly in soils with a moisture level of 30 kpa. They also observed post-emergence damping-off of seedlings in *Pythium*-infested soil, after maximum emergence at 15, 20 and 25°C. The results of this study agree with the above findings. The favorability of higher temperatures on damping-off of cucumber caused by *P. irregulare* was also showed by Ben- Yephet and Nelson (1999). Contrary to the observations of Martin and Loper (1999) and Ploetz (2004) showing *Pythium* infection occurs mainly at lower temperatures, we observed an increase in disease incidence with temperature. This discrepancy may be due to differences between isolates, since isolates of the same *Pythium* species vary in their optimum temperature (Ben-Yephet and Nelson, 1999). The observation of Lifshitz and Hancock (1983) offer another possible expla-nation. They found that the optimum temperature for *Py-thium* growth shifted to lower temperature when the fungus was added to nonsterile instead of sterile soil, indicating a difference between the physiological and ecological optima.

The results of this study indicate that to reduce the severity of damping- off in fields infested with the *Pythium* pathogen, safflower should be seeded early when soil temperature is low, even though germination may be slow or lower. In Golestan area, the mean temperature in March and April is 10.6 and 16.0°C, respectively. In the absence of major seedling diseases, mid-March to mid-April has been identified as optimum planting period for safflower in this area. If seeding is delayed until soil temperatures are higher than 15°C, safflower growers should consider not planting safflower if other conditions are favorable. Other studies with *Pythium* spp. have indicated that high soil temperature favours the development of seed and root rots (Ben-Yephet and Nelson, 1999; Thomas, 1970), and it may be easier to manipulate seeding date than to eliminate the pathogen. This study confirms these observations for *P. ultimum*, which is widespread inalmost all fields and is the main inciter of damping- off

of safflower in Iran (Ahmadi, 2008; Ahmadinejad and Okhovat, 1976).

Van der Plaats-Niterink (1981) in her monograph on *Pythium* noted that the role of *Pythium* spp. often depend on external factors. “When conditions are favorable for the fungus but less for the host, *Pythium* species can become very pathogenic”. In the interaction that is described in this paper, *P. ultimum* appears to be an opportunist in that it causes its greatest damage on safflower not under temperature conditions that are most favorable for it, but when the host genotype is susceptible. From this study it can be concluded that temperature and genotype conditions play a major role in *P. ultimum* damping-off of safflower. Therefore, implementation of proper sowing time as mentioned above and selection of less susceptible safflower cultivars should be part of *Pythium* management practices to reduce damping-off incidence and severity under field cultivation.

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