

## EVALUATION OF SAINFOIN POPULATIONS IN REACTION TO POWDERY MILDEW DISEASE IN DIFFERENT CLIMATIC CONDITIONS OF IRAN

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### SUMMARY

In order to evaluate the sainfoin populations, the powdery mildew disease in different climatic conditions, 40 populations were cultivated in the research stations including : Zanjan (Zanjan province), Semrom (Esfahan province), Khoramabad (Lorestan province), Tabriz (East Azarbyjan), and Sanandaj (Kordestan province). The infected plants were selected and the fungi of *Leveillula taurica* was identified as an agent of powdery mildew disease. Results of disease severity index on the populations with natural infection showed that all populations had different degree disease severity index. Combined analysis of variance over locations showed that there were significant differences between locations, populations, populations x locations interaction effects for disease severity index, disease percentage rate and forage yield. Mean comparison of populations showed that disease severity index of two populations including Poly cross and Oshnavieh were as 0 to 25 per cent and both of them were considered as tolerant populations to powdery mildew disease.

**Key words :** Powdery mildew disease, sainfoin, disease severity index

The genus of sainfoin (*Onobrychis* spp.) extends from the Mediterranean region to Caucasia, the Zagros Mountains of Iran and Asia. The genus is concentrated in Iran (60 species) (Rechinger, 1984; Mozafarian, 2007) and Turkey (52 species) (Emre *et al.*, 2007; Çelik *et al.*, 2011). The sainfoin is a nutritive plant which is growing as perennial plant and also it was noticed due to *Hypera postica* Gyll pest tolerant of alfalfa plant (Allen and Allen, 1981). This plant is growing as irrigated and dry farming in some parts of Iran including Char Mohal bakhtiary, Lorestan, Fars, Kerman, Kordestan, Kermansah, Zanjan and Mazandaran (Hidarian and Mollaei, 2001). The sanfion plant would be able to cultivate in the farming which clover and alfalfa plant could not be grown. This plant would be able to fix nitrogen and therefore it does not need more chemical fertilizer (Delorit *et al.*, 1984).

This plant has special properties which would be used for improvement of dryland and rangeland area and it would resist to weak nutritive soil and severely winter cold. The powdery mildew disease is the most important disease of *Onobrychis sativa* in Iran and its epidemic is distributed in Zanjan, Esphahan, Lorestan, and Charmohal Bakhtiari province. This disease will appear

in the end of season growth and have high damage in the end of cutting. The agent of this disease is a fungi as *Leveillula taurica*, and form of un-sexual identified as Oidiopsis. This genus of fungi which attack in many plant species including sainfoin, alfalfa, sunflower, safflower and hemp. On the base of diversity and distribution of these plant as honest of powdery mildew disease, Behdad (1996) reported the epidemic of this disease in Esphahan, east and west of Azarbayjan, Charmohal Bakhtiari, Lorestan, Phars, Kerman, Kordestan, Kermansah, Zanjan and Mazandaran. Similarly, Bamdadian (1990) reported that the symptom of *Leveillula taurica* as leaf tissue under spot area and white covering fungi appeared as necrotic. The infected plant by powdery mildew disease caused low growth and reduced forage yield. The extension of sainfoin in many parts of Iran is limited by powdery mildew and it decreased forage yield during second and third harvest (Majidi, 2010). The plant parts severely affected by this disease were dried and fell off. The disease caused by *Leveillula taurica* started in August to September in Iran (Sharifnabi and Banihashemi, 1990; Ershad, 1995). Although sainfoin is one of the important legume forages cultivated as high quality hay in Iran, the information on

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the effect of powdery mildew disease on production and recognized dormant populations is limited. Therefore, with regard to this point that one of the limitation factors of sainfoin cultivation is powdery mildew disease, therefore, for evaluation of 40 populations in response to powdery mildew disease, this research project was conducted in five provinces of Iran.

### MATERIALS AND METHODS

The complete infected sainfoin plant samples with mycelium and unisexual spores were collected from the research field of some province. In order to multiply and maintain the inoculums for inoculation of host plant, two seed samples were cultured on 50 pods in the research field stations of five provinces. After two months, when the seedling developed to 10 leaves, the inoculation was carried out by spray suspension with concentration spores  $10^6$ , in 1 ml, of sterile water with Tween 80 (0.05%), (De Souza and Café-Filho, 2003). The inoculation of plantlet was carried out by shaking of plantlet within the suspension. The identification of unisexual of powdery mildew disease which isolate from collected leaves of the field, was carried out by method

of Mukerji (1968). Evaluation on the field was carried out in the five locations including Esphahan, Zanjan, Lorestan, Azarbayjan and Kordestan. The geographical and ecological characteristics of locations are shown in Table 1. In field experiment, seeds were sown in four drilled lines as long as 2 m with 25 cm distance in sward condition using randomized complete block design with three replications in spring 2008. The amount of seed was used as 60 kg/ha. After elimination of border effect, the 10 plants were selected randomly from the two middle rows and yield, disease severity index and disease percentage rate of each plot were recorded. For measurement of forage yield, fresh foliage was cut and air-dried. Then a sample was placed in oven in  $100^{\circ}\text{C}$  for 48 h and dry forage yield was estimated as t/ha. Evaluation of disease severity index was assessed in the second cutting and it was scored as 0 to 4 and the way of assessment was as follows : (1) score of 0-2 with mycelium covering of 0-25, it was considered as tolerant to powdery mildew disease and (2) score 2 to 3 with mycelium covering of 26-50, it was considered as semi-susceptible, score of 3-4 with mycelium covering of 50-100 was considered as susceptible.

The total annual DM yield summed over three

TABLE 1  
Ecological and geographical characteristics of five experimental stations

Name of station	Name of province	Height of sea level (m)	Maximum temperature ( $^{\circ}\text{C}$ )	Minimum temperature ( $^{\circ}\text{C}$ )	Mean temperature ( $^{\circ}\text{C}$ )	Altitude	Latitude	Annual precipitation (mm)
Sanandaj	Kordestan	1373.4	22.30	6.30	16.0	47°00'	35°20'	374.50
Koramabad	Lorestan	1147.8	25.26	8.92	17.2	48°17'	33°26'	447.78
Tabriz	East Azarbijan	1361.0	19.15	8.82	14.0	46°17'	38°05'	229.34
Semirom	Esphahan	2400.0	17.70	3.50	12.3	51°17'	30°43'	398.10
Zanjan	Zanjan	1663.0	18.41	4.35	11.0	48°29'	36°41'	267.30

TABLE 2  
Compound analysis variance of 40 sainfoin populations

Source of variation	Forage yield		Disease severity index		Disease per cent	
	Df	MS	Df	MS	Df	MS
Locations	4	37.10**	4	38.10**	4	342.58**
Error 1	10	9.35	10	0.70	4	92.41
Populations	39	4.06**	39	1.42**	39	625.7**
Populations x Locations	142	2.71**	142	0.53**	39	325.9**
Error 2	358	1.32	358	0.32	153	111.37
CV (%)		16.26		16.29		14.62

\*\*Significant at P=0.01 level.

cuts over two years per plot and the values of disease severity index were averaged over years and consequently were used for combined analysis over five locations. Disease infection per cent was recorded only in two locations Esphahan and Zanjan. The SAS9 software (SAS Institute Inc.) was used for ANOVA.

## RESULTS AND DISCUSSION

With regard to obligate parasite of powdery mildew disease, there was no any symptoms by artificial inoculation. Results of analysis of variance showed significant differences between locations, populations

TABLE 3  
Mean comparison of forage dry matter yield of 40 sainfoin populations in five locations

Population	Origin	Esphahan	Kordestan	Lorestan	Tabriz	Zanjan	Total mean
Khalkhal	Ardebil	4.91 h-i	5.83 g-i	8.58 a-d	5.55 c-d	7.03 a-d	6.38 g-i
Gorjan	Ardebil	5.77 c-i	7.95 a-h	5.85 j	5.13d	6.55 a-e	6.25 g-i
Sarab	East Azarbijan	6.01 b-i	8.02 a-g	8.72 a-d	8.12 a-d	6.30 a-e	7.43 a-f
Ahar	East Azarbijan	6.10 a-h	7.00 c-i	7.73 c-i	6.80 a-d	6.66 a-e	6.86 a-g
Haris	East Azarbijan	6.71 a-g	5.92 f-i	8.04 b-g	5.76 c-d	7.57 a	6.80 b-g
Asdabad	Hamadan	6.96 a-e	7.32 a-i	6.42 g-j	5.10 d	7.14 a-d	6.59 e-h
Arak	Central	6.62 a-g	8.67 a-d	6.19 h-j	5.64 c-d	6.65 a-e	6.75 b-g
Komian1	Central	6.43 a-h	8.10 a-f	7.40 d-j	7.06 a-d	6.90 a-d	7.18 a-g
Banab	East Azabijan	6.81 a-g	8.11 a-f	8.00 b-h	6.22 b-d	7.31 a-c	7.29 a-g
Marand	East Azabijan	6.30 a-h	nd	nd	6.46 a-d	6.52 a-e	6.42 f-h
Khansar1	Esphahan	6.56 a-h	9.54 a	6.73 e-j	6.82 a-d	6.75 a-e	7.28 a-g
Damavand	Theran	6.68 a-g	nd	8.13 a-g	6.40 a-d	6.81 a-e	7.00 a-g
Varazghan	East Azabijan	6.34 a-h	6.08 f-i	9.76 a-b	7.72 a-d	6.91 a-d	7.36 a-f
Khoramabad	Loresatan	7.72 a	6.20 e-i	8.52 a-d	6.61 a-d	5.92 c-e	7.00 a-g
Fridonshar	Esphahan	7.04 a-d	9.18 a-c	9.55 a-c	7.29 a-d	6.51 a-e	7.91 a
Aligodarz1	Lorestan	6.97 a-d	5.82 g-i	6.10 i-j	8.05 a-d	6.97 a-d	6.78 b-g
Azna1	Lorestan	7.53 a-b	5.59 i	9.00 a-d	6.94 a-d	6.43 a-e	7.10 a-g
Kabotaabad1	Esphahan	6.16 a-h	6.18 e-i	7.53d-j	7.91 a-d	6.60 a-e	6.88 a-g
Kohleran	Ardebil	7.02 a-d	nd	nd	6.18 b-d	7.13 a-d	6.78 b-g
Koramabad	Lorestan	7.14 a-d	nd	nd	7.58 a-d	6.99 a-d	7.24 a-g
Aligodaraz2	Lorestan	6.85 a-f	9.34 ab	8.04 b-g	6.79 a-d	7.13 a-d	7.63 a-e
Fridonshar2	Esphahan	6.85 a-f	6.24 e-i	7.91 b-i	5.43c-d	7.00 a-d	6.69 d-h
Fridan	Esphahan	6.73 a-g	9.09 a-c	9.28 a-d	7.43 a-d	6.78 a-e	7.86 a-b
Khansar2	Esphahan	7.44 a-c	7.41 a-i	7.94 b-h	7.12 a-d	7.59 a	7.50 a-f
Khomain2	Central	6.90 a-e	9.41 a-b	8.70 a-d	7.28 a-d	6.81 a-e	7.82 a-c
Eromieh	West-Azarbijan	6.44 a-h	9.24 a-b	8.29 a-f	7.49 a-d	7.39 a-b	7.77 a-d
Miandoab	West Azarbijan	6.15 a-h	8.02 a-g	8.23 a-g	10.02 a	6.82 a-e	7.85 a-b
Silvana	West-Azarbijan	5.28 e-i	7.28 b-i	8.50 a-d	9.75 a-b	6.09 b-e	7.38 a-f
Sanandaj	Kordestan	5.19 f-i	5.95 f-i	8.76 a-d	7.97 a-d	5.74 d-e	6.72 c-h
Poly cross	RIFR	7.78 a	6.82 d-i	7.45 d-j	9.51 a-b	6.37 a-e	7.59 a-e
Aligodarz3	Lorestan	5.97 b-i	6.21 e-i	6.52 f-j	7.67 a-d	6.60 a-e	6.59 e-h
Azna2	Lorestan	4.45 i	5.74 h-i	8.74 a-d	6.70 a-d	6.54 a-e	6.43 f-h
Kermanshah	Kermanshah	5.76 c-i	8.32 a-e	9.93 a	6.33 a-d	6.99 a-d	7.46 a-f
Oshnavieh	West -Azarbijan	5.15 g-i	nd	8.05 b-g	8.02 a-d	6.82 a-e	7.01 a-g
Kabotarabad2	Esphahan	6.52 a-h	nd	nd	8.92 a-c	7.26 a-c	7.57 a-e
Azarshar	East Azarbijan	5.83 c-i	6.75 d-i	6.51 f-j	5.23 c-d	4.22 f	5.71 h-i
Divandareh	Kordestan	6.62 a-g	8.09 a-f	7.95 b-h	7.20 a-d	7.00 a-d	7.37 a-f
Songhor	Kermanshah	7.21 a-c	7.57 a-i	7.83 c-i	5.75 c-d	6.79 a-e	7.03 a-g
Meshkinshar	Ardebil	5.49 d-i	nd	nd	5.12 d	5.39 e	5.33 i
Sejas	Zanjan	7.20 a-c	nd	nd	7.21 a-d	6.29 a-e	6.90 a-g
Total mean		6.44D	7.41B	7.97A	7.01C	6.68D	7.04C

nd= not determined.

Means of each column followed by the same lowercase letters had no significant differences ( $P < 0.05$ ) based on DMRT method.

Means of last row followed by the same uppercase letters had no significant differences ( $P < 0.05$ ) based on DMRT method.

and populations by locations interaction effects (Table 2). Mean comparison of forage yield showed that population of Fridonshar, Fridan, Miandoab, Khomain2, Eromieh, Aligodaraz2, Poly cross, Kabotarabad2,

Khansar2, Kermanshah and Sarab with average values of 7.43 to 7.91 t/ha had higher total annual forage production over all of locations. The minimum forage yield was related to Meshkinshar as 5 t/ha (Table 3).

TABLE 4  
Mean comparison of disease severity index 40 sainfoin populations in five locations

Population	Origin	Espahan	Kordestan	Lorestan	Tabriz	Zanjan	Total mean
Khalkhal	Ardebil	3.40a-d	2.50ab	4.57ab	3.00abc	4.40a	3.50bc
Gorjan	Ardebil	3.72a	3.33a	3.61d-l	3.67ab	4.46a	3.75ab
Sarab	East Azarbijan	3.49abc	2.50ab	3.06j-m	4.00a	4.43a	3.49abc
Ahar	East Azarbijan	3.42a-d	3.33a	3.76b-k	3.33ab	4.09a	3.58ab
Haris	East Azarbijan	3.41a-d	2.50ab	2.97k-m	3.33ab	4.39a	3.32bc
Asdabad	Hamadan	3.48abc	2.50ab	3.82b-j	3.33ab	4.20a	3.47abc
Arak	Central	3.33a-f	3.33a	3.42f-l	3.33ab	4.20a	3.52abc
Komian1	Central	3.42a-d	3.33a	3.73c-l	3.33ab	4.16a	3.59ab
Banab	East Azabijan	3.53ab	2.50ab	2.93l-m	3.33ab	4.33a	3.32bc
Marand	East Azabijan	3.38a-d	nd	nd	3.33ab	4.50a	3.74ab
Khansar1	Espahan	3.35a-e	3.33a	4.02b-h	3.33ab	4.24a	3.65ab
Damavand	Theran	2.87b-g	nd	4.25a-e	3.33ab	4.25a	3.68ab
Varazghan	East Azabijan	3.37a-e	2.50ab	4.41a-d	4.00a	4.31a	3.71ab
Khoramabad	Loresatan	3.34a-e	2.50ab	3.92b-i	2.67bc	4.10a	3.33bc
Fridonshar	Espahan	3.42a-d	2.00ab	4.19a-f	3.00abc	4.32a	3.39abc
Aligodarz1	Lorestan	3.33a-f	2.50ab	3.64d-l	2.67bc	4.50a	3.33bc
Azna1	Lorestan	3.45abc	2.50ab	4.42a-d	2.67bc	4.07a	3.42abc
Kabotaabad1	Espahan	3.32a-f	3.33a	4.19a-f	3.33ab	4.14a	3.66ab
Kohleran	Ardebil	3.67a	nd	nd	3.33ab	4.22a	3.74ab
Koramabad	Lorestan	3.53ab	nd	nd	3.33ab	4.50a	3.79ab
Aligodaraz2	Lorestan	3.52ab	3.33a	3.99b-i	3.33ab	4.50a	3.73ab
Fridonshar2	Espahan	3.36a-e	2.83ab	3.19i-l	3.00abc	4.50a	3.38bc
Fridan	Espahan	3.46abc	3.33a	4.08b-g	3.00abc	4.38a	3.64ab
Khansar2	Espahan	3.43a-d	2.50ab	4.97a	3.00abc	4.46a	3.67ab
Khomain2	Central	2.71e-g	3.33a	4.20a-f	3.67ab	4.31a	3.64ab
Eromieh	West-Azarbijan	3.47abc	3.33a	3.43e-l	3.00abc	4.24a	3.49abc
Miandoab	West Azarbijan	2.69g-f	2.50ab	4.05b-g	3.67ab	4.39a	3.46abc
Silvana	West-Azarbijan	2.84c-f	0.83b	3.21h-l	3.67ab	4.50a	3.01c
Sanandaj	Kordestan	3.22a-f	2.50ab	4.30a-d	3.67ab	4.50a	3.64ab
Poly cross	RIFR	2.54g	0.67b	2.19n	2.00c	4.12a	2.30d
Aligodarz3	Lorestan	3.38a-d	2.50ab	3.78b-k	3.00abc	4.46a	3.42abc
Azna2	Lorestan	3.23a-f	3.33a	3.67d-l	3.33ab	4.44a	3.60ab
Kermanshah	Kermanshah	3.38a-d	3.33a	3.31g-l	3.00abc	4.35a	3.47abc
Oshnavieh	West -Azarbijan	2.77d-f	nd	2.35mn	1.00d	3.44b	2.39d
Kabotarabad2	Espahan	3.30a-f	nd	nd	4.00a	4.50a	3.93a
Azarshar	East Azarbijan	3.30a-f	4.17a	4.15b-f	3.00abc	4.35a	3.79ab
Divandareh	Kordestan	3.32a-f	2.83ab	3.92b-i	3.67ab	3.65b	3.47abc
Songhor	Kermanshah	3.42a-d	3.13a	4.54abc	3.00abc	4.46a	3.71ab
Meshkinshar	Ardebil	3.30a-f	nd	nd	3.03abc	4.24a	3.53ab
Sejas	Zanjan	3.35a-e	nd	nd	3.00abc	4.19a	3.51abc
Total mean		3.31C	2.78D	3.77B	3.19C	4.30A	3.49C

nd=not determined.

Mean comparison of disease severity index showed that two populations of Poly cross and Oshnavieh with disease severity index as 2, disease percentage as 29 and 51 per cent were considered as tolerant populations to powdery mildew disease but population Kabotarabad2 (originated from Eshfahan) had maximum disease severity index as 3.93 (Table 4). The average forage production for Poly cross and Oshnavieh as tolerant and Kabotarabad2 as susceptible population was 7.59, 7.01 and 7.57 t/ha, respectively.

With regard to analysis of variance, differences between locations, populations and interaction of populations by locations were significant (Table 2). This phenomenon confirmed that the populations had good variability for tolerance of powdery mildew disease and forage yield. Mean comparisons based on combined analysis variance showed that maximum forage as 8 t/ha was related to population Fridonshar and the minimum forage yield to Meshkinshar (Table 3). Two populations of Poly cross and Oshnavieh with low disease severity index were considered as tolerant populations to powdery mildew disease but population Kabotarabad2 had maximum disease severity index and it was considered as susceptible (Table 4). This result was similar with result of Alizadeh and Jafari (2013), that evaluated of 56 populations of *Onobrychis sativa* in Alborz research station, Karaj, Iran and showed that the same populations : Polycross and Oshnavieh with low disease severity index were considered as tolerant to powdery mildew disease. Hydarian and Mollaie (2001), evaluated of 19 ecotypes populations of *Onobrychis sativa* and also they found that one population originated from Oshnavieh was considered as tolerant population compared with other populations. Naseri and Alizadeh (2012), Sepahvand *et al.* (2012), Sifollahi *et al.* (2012) in Zanjan, Lorestan and Esfahan went to the same conclusion that two populations of Poly cross and Oshnavieh were considered as tolerant populations to powdery mildew disease.

With regard to results obtained from five locations, it was proved that Fridonshar1 population was considered as high forage yield and two populations of Poly cross and Oshnavieh were considered as tolerant populations to powdery mildew disease.

Means of each columns followed by the same lowercase letters had no significant differences ( $P < 0.05$ ) based on DMRT method.

Means of last row followed by the same uppercase letters had no significant differences ( $P < 0.05$ ) based on DMRT method.

Means of each column followed by the same lowercase letters had no significant differences ( $P < 0.05$ ) based on DMRT method.

Means of last row followed by the same uppercase letters had no significant differences ( $P < 0.05$ ) based on DMRT method.

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