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Investigating the Distribution of Clodinafop-propargyl Resistant Wild Oat (*Avena ludoviciana*) Populations in South Western Iran

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Abstract

To evaluate the distribution of clodinafop-propargyl resistant wild oat to in south western Iran (Khuzestan province), 50 fields which were sprayed with aryloxyphenoxypropionate herbicides were sampled. Those fields at which wild oat had been controlled efficiently by these herbicides were selected for the experiment. Populations (50 suspicious to resistance and 1 susceptible) were studied in a randomized complete block design with four replications in 2005. Populations of wild oat were sprayed during two- to four- leaves stage using the recommended dose of herbicide. Shoot biomass, survived plant and EWRC visual rating, were recorded four weeks after herbicides application. Longitude and latitude of different sampling locations were registered using GPS. Grouping populations using cluster analysis showed that 52% of populations were resistant, 28% were suspicious to resistance and only 18% of populations were susceptible and semi susceptible. Furthermore resistant populations were detected in all of parts of khouzestan province.

Keywords: clodinafop–propargyl, distribution, resistant wild oat.

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بررسی توزیع توده‌های یولاف وحشی (*Avena Indoviciana*) مقاوم به علف‌کش کلودینافوپ پروپارژیل در جنوب غربی ایران

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چکیده

به منظور ارزیابی و بررسی توزیع توده‌های یولاف وحشی مقاوم به علف‌کش کلودینافوپ پروپارژیل در استان خوزستان ۵۰ مزرعه که توسط علف‌کش‌های خانواده آریلوکسی فنوکسی پروپیونات سمپاشی شده بودند، انتخاب گردیدند. مزارعی بدین منظور انتخاب شدند که در آنها یولاف وحشی به گونه‌ای مطلوب توسط این علف‌کش‌ها کنترل شده بودند. توده‌های انتخاب شده (۵۰ توده مشکوک به مقاومت و ۱ توده حساس) در قاب طرح بلوک‌های کامل تصادفی با چهار تکرار مورد مطالعه قرار گرفتند. سمپاشی یولاف وحشی در مرحله دو تا چهار برگی گیاه، بر پایه در توصیه علف کن صورت پذیرفت و بیوماس اندام هوایی، درصد زنده ماندن و معیار چشمی EWRC، چهار هفته پس از سمپاشی مورد اندازه‌گیری و بررسی قرار گرفتند. طول و عرض جغرافیایی هر منطقه نمونه‌برداری با استفاده از دستگاه GPS ثبت گردید. نتایج نشان داد که ۵۲٪ توده‌ها مقاوم، ۲۸٪ توده‌ها مشکوک به مقاوم و ۱۸٪ آنها حساس و نیمه حساس بودند. در مجموع توده‌های مقاوم در سراسر استان توزیع شده‌اند.

کلید-واژه‌ها: کلودینافوپ - پروپارژیل - توزیع یولاف وحشی مقاوم.

Introduction

Wild oat is the most important grass weed in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and other cereals throughout the world, from Iceland and Alaska to the southern hemisphere (Beckie *et al.*, 2005). It also grows as a problematic weed throughout wheat -growing regions of most provinces in Iran (Cobb and Kirkwood, 2000; Deihim fard and Zand, 2005); therefore satisfactory control of this weed helps improve the crop yield. In Iran, herbicides have been the main means of wild oat control for more than 30 years. Different herbicides have been registered for wild oat control in Iran including difenzoquat, diclofop-methyl, flameprop-m-isopropil, fenoxaprop-p-ethyl, clodinafop propargyl, imazamethabenz-methyl and tralkoxydim (Cavan and Moss, 2001). Among these herbicide options, ACCase inhibitors have caused significant improvements in weed control efficacy, as a result, wheat growers have become highly reliant on these herbicides. As ACCase inhibitors, clodinafop propargyl, fenoxaprop-p-ethyl and fenoxaprop-p-ethyl have shows higher efficacy in the control of wild oat (Zand *et al.*, 2003; Zand and Baghestani, 2002). It is for more than a decade that Iranian farmers have intensively used these three herbicides to control wild oat. With regard to the mid-term period of 7 years necessary for evolution of weed resistance to these herbicides (Kashani *et al.*, 2006; Ross and Lembi, 1999). the first resistant wild oat (*A. ludoviciana*) population was reported in 2003 from some wheat fields in south western Iran (Kashani *et al.*, 2007). Thereafter, the abundance of resistant wild oat has increased. Kashani *et al.* (2007) studied wild oat resistance in south western Iran using whole plant and seed bioassay. While most populations in this region were found resistant, they differed in the level of resistance. Resistance to ACCase inhibitors has also been reported in other countries including Canada, Australia, France, South Africa, United State and Chile (Heap, 2006).

The objectives of this study were to diagnose clodinafop-propargyl resistant wild oat populations in south western Iran and determining the distribution of these populations using GIS mapping technique to propose management strategies for preventing more evolution of resistant populations.

Materials and methods

Plant Material

Fifty suspected resistant *A. ludoviciana* populations were collected in 2005 from fifty wheat fields in south western Iran (Khuzestan province). Seeds of the suspected resistant populations were collected from plants survived an annual treatment of aryloxyphenoxypropionate herbicides that had been used for at least 4-5 successive years. A susceptible population (S) was also collected from locations which had never been treated previously by any graminicide (Tal *et al.*, 1996). Populations were coded based on their place of collection and susceptibility or suspicious to resistant. VR, SOR, NR, ZR, STR, AR and DR were suspicious to resistance populations which were collected from Ahvaz, Shoush, Andimeshk, Ramhormoz, Shoushtar, Dasht Azadegan and Dezfoul counties, respectively.

Diagnosis of Resistant Populations

The experiment was laid out in a randomized complete block design with four replications. An individual pot containing 10 seeds was considered as a treatment unit. Prior to planting, and in order to break the seed dormancy, *A. ludoviciana* seeds were dehulled by hand and germinated on filter paper moistened with 8ml distilled water in 9cm plastic Petri plates. Plates were transferred to a refrigerator at +5°C in the dark for 24 h, and then placed in a germinator at +20/10°C with a 16/8 h day/night to germinate the seeds. Ten germinated seeds of wild oat were planted at a depth of 1cm in 12cm diameter pots filled with a loam:sand:peat:perlit mixture as 0.5:1:1:0.5. Pots were transferred to the greenhouse at 25/18°C day/night temperature regime. Pots were watered daily to field capacity.

Clodinafop-propargyl at 64 g ai/ha was applied on wild oat at the two- to three-leaf stage. Herbicide was sprayed in a cabinet sprayer equipped with a flat-fan nozzle calibrated to deliver $200L^{-1}$ of spray solution at a pressure of 2 bars. Visual percent wild oat control was rated 28 days after herbicide applications (DAHA) using EWRC rating system (Sandral *et al.*, 1997). Four weeks after treatment, number of survived plants in each pot was recorded, then the plants were harvested and oven dried at 75°C for 48h and weighed. Percent wild oat biomass was calculated by dividing plant biomass in the treated pot by plant biomass in the untreated pot and multiplying by 100.

Data analysis and Distribution mapping

All data were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute, 2000). The assumptions of the variance analysis were tested by ensuring that the residuals were random, and homogeneous with a normal distribution about a mean of zero. If the assumptions of variance were not adequately met, data were subjected to an arcsine square root transformation (for data calculated as percent of the check treatment) or square root transformation (for visual rating scores). In order to group studied populations based on their response to clodinafop propargyl application, cluster analysis was performed following standardization. Populations were clustered by using a dissimilarity matrix of squared Euclidean distance using Mean procedure.

In order to map the distribution of the studied populations, longitude and latitude of sampled areas were first recorded using GPS (Etrax Vista). An information bank was designed based on cluster analysis results using Access software. Data was then analyzed using Arcview (ESRI, Raddlands, CA). Finally, two maps were produced, one for resistant and semi-resistant and another for susceptible and semi-susceptible populations.

Results and discussion

Table 1 shows percent wild oat biomass reduction and survival compared to the check treatment (hereafter referred to as percent wild oat biomass and percent plant survival, respectively), and visual percent weed control 4 weeks after herbicide applications. With respect to the great number of wild oat populations studied and in order to have a better comparison, cluster analysis was used. The dendrog am of cluster analysis is illustrated in Figure 1. As observed, populations were clustered into four groups.

Cluster 1 consisted of populations SOR_1, SOR_5, SOR_6, SOR_8, NR_{10}, NR_{14} ,AR_2, AR_5 DR_4, ZR_4 (Figure 1) with biomass more than 75% of the check treatment, and 100% plant survival. The score of visual wild oat injury was 9 (Table 1). For example, precent wild oat biomass, percent elant survival and visual injury were 91.38%, 100%, and 9%, respectively, for AR_5 which indicate that this population was not affected by clodinafop propargyl (Beckie *et al.*, 2004). If percent plant survival and biomass compared with the check treatment are more than 50% and 80%, respectively, the population could be served as resistant to that herbicide. In the present study, except for the 4 populations in which percent wild oat biomass was near 80%, biomass of all other populations was more than 80% of the check treatment. All populations showed complete survival when treated with clodinafop propargyl. So, it could be concluded that all these populations have evolved resistance to clodinafop propargyl, among which 4 populations belonged to Shoush, 2 to each of Andimeshk and Azadegan, and 1 to each Dezfoul and Ramhormoz (Figure 2). As observed, Shoush had the highest portion of wild oat resistant populations in Khouzestan (Figure 3).

Cluster 2 consisted of populations NR_8, NR_{13}, STR_1, STR_2, DR_1, DR_3, DR_5, VR_4, VR_5, ZR_3, ZR_5, AR_4, SOR_2, SOR_7, NR_3, and NR_5 (Figure 1), which almost survived when treated by clodinafop propargyl, but their biomass reduced to 60-70% of the check treatment. In this cluster no wild oat damage was detected, however, because plant growth was a little bit ceased due to herbicide application, the score of visual rating was 8 (Cavan *et al.*, 2001). ZR_5 could be considered exampled as representative of this cluster (Table 1).

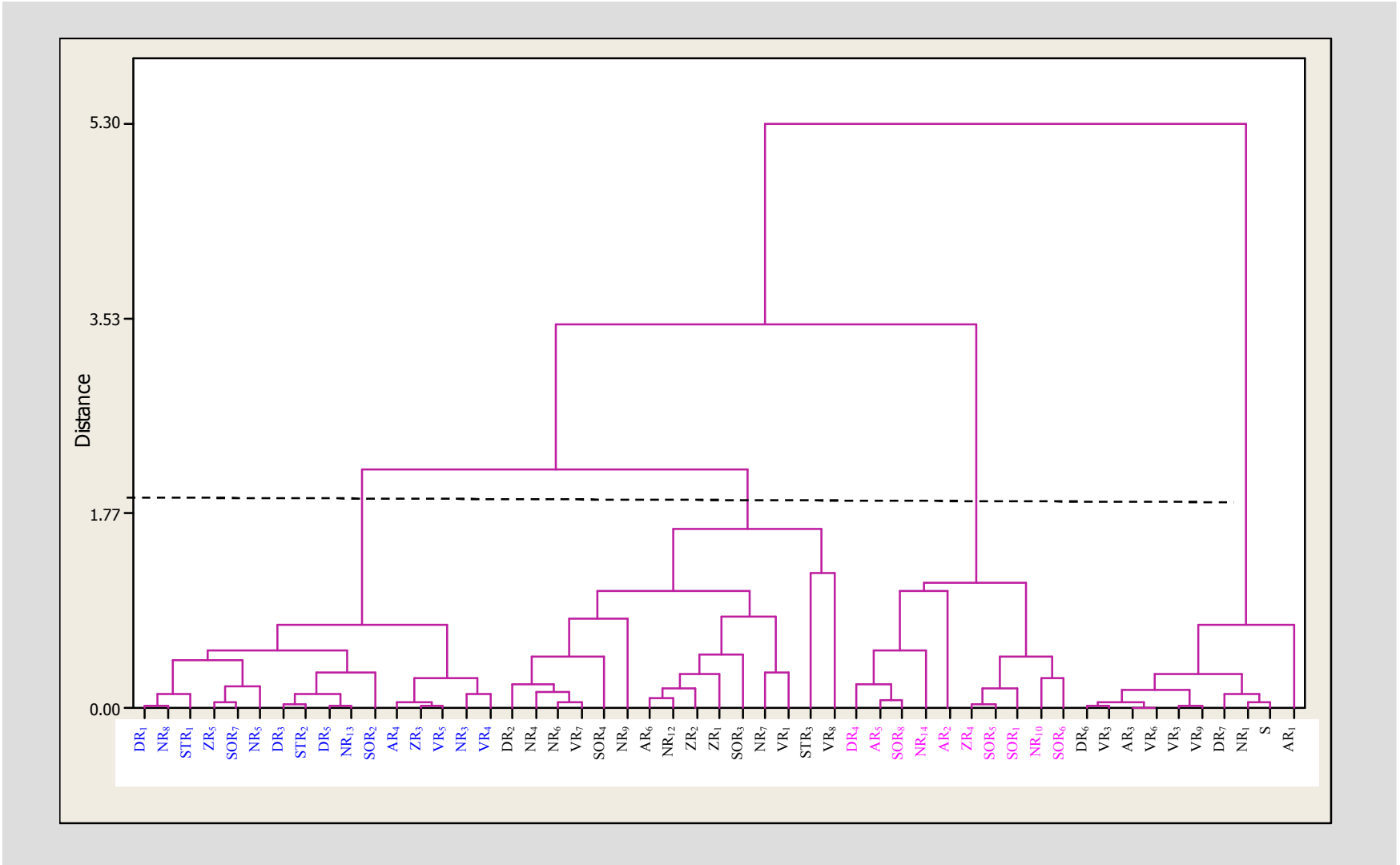

Figure 1- Dendogeram of cluster analysis of *A.ludoviciana* biotypes treated by clodinafop-propargyl.

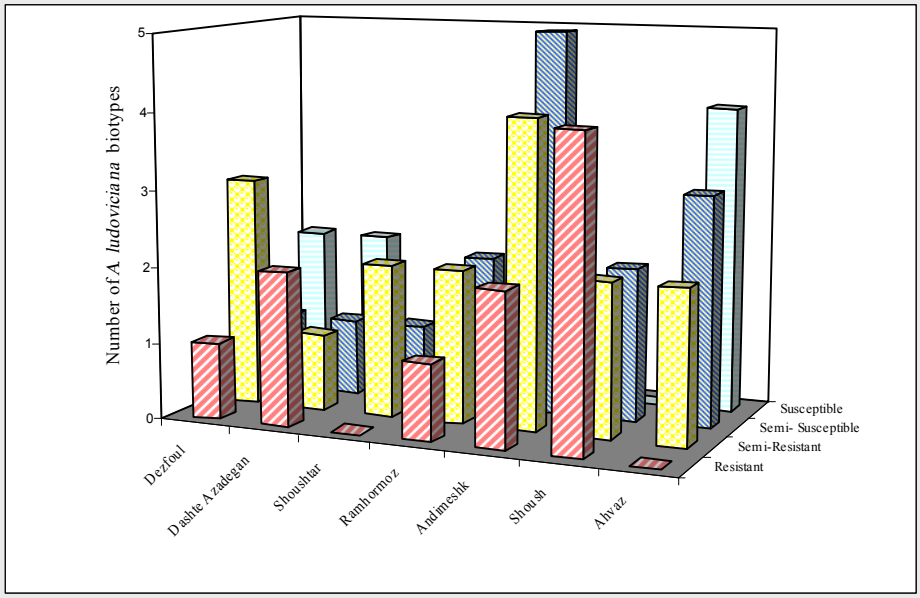

Figure 2- A number of resistant, semi-resistant, semi susceptible and susceptible *A. ludoviciana* biotypes of different counties of south western Iran.

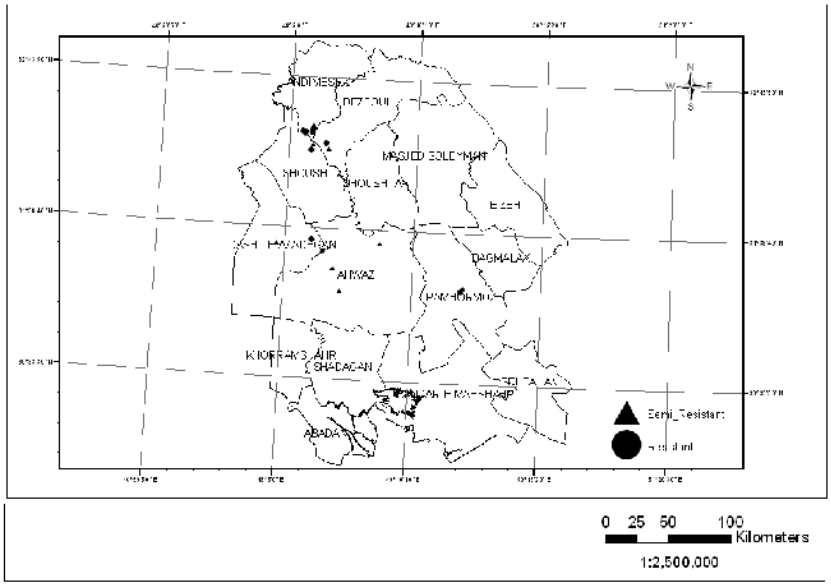

Figure 3- Distribution of resistant and semi-resistant *A. ludoviciana* in south western Iran.

Because percent wild oat biomass and percent survival of ZR_5 were more than 50% of the check treatment, these populations could be considered as semi-resistant to clodinafop propargyl. Totally, the results indicated the resistance of cluster 1 and 2 populations to clodinafop propargyl, however, the degree of resistance varied between clusters which should be determined using dose-response experiments. Among cluster 2 populations, 4 belonged to Andimeshk, 3 to Dezfoul, 2 to each Shoush, Shoushtar, Ramhormoz and Ahvaz, and 1 to Azadegan valley (Figure 2). Distribution of these populations is illustrated in Figure 3.

Cluster 3 consisted of populations NR_{12}, NR_9, NR_7, NR_6, NR_4, VR_8, VR_7, VR_1, ZR_2, ZR_1, SOR_4, SOR_3, DR_2., STR_3, and AR_6 (Fig. 1) where biomass was 40 to 50% of the check treatment. These populations were damaged by 50% when sprayed with clodinafop propargyl and rated 8 according to visual percent control (Cobb and Kirkwood, 2000). However, most of the plants survived in this cluster (Table 1). Nonetheless, and due to the high percent biomass reduction, these populations were classified as semi-susceptible to clodinafop propargyl. In this cluster, populations, 5 belonged to Andimeshk, 3 to Ahvaz, 2 to each of Shoush and Ramhormoz, and 1 to each of Azadegan valley, Dezfoul and Shoushtar (Figure 2). Totally, it could be concluded that continuous application of clodinafop propargyl at these locations could result in evolution of resistance in these populations in the near future. Distribution of these populations is illustrated in Figure 4.

Cluster 4 consisted of populations which were severely affected by clodinafop propargyl. Herbicide application resulted in chlorosis, necrosis and finally dieback of the plants. The score of visual injury was 1. Plant biomass reduced more than 75% compared with the check treatment (Table 1). Populations in this cluster were AR_3, AR_1, VR_9, VR_6, VR_3, VR_2, DR_7, DR_6, NR_1, and S. Altogether, these populations were classified as susceptible to clodinafop propargyl. In this cluster, 4 populations belonged to Ahvaz, 2 to each of Dezfoul and Azadegan valley, and 1 to Andimeshk (Table 2). The distribution of papaulations is shown in Figure 4.

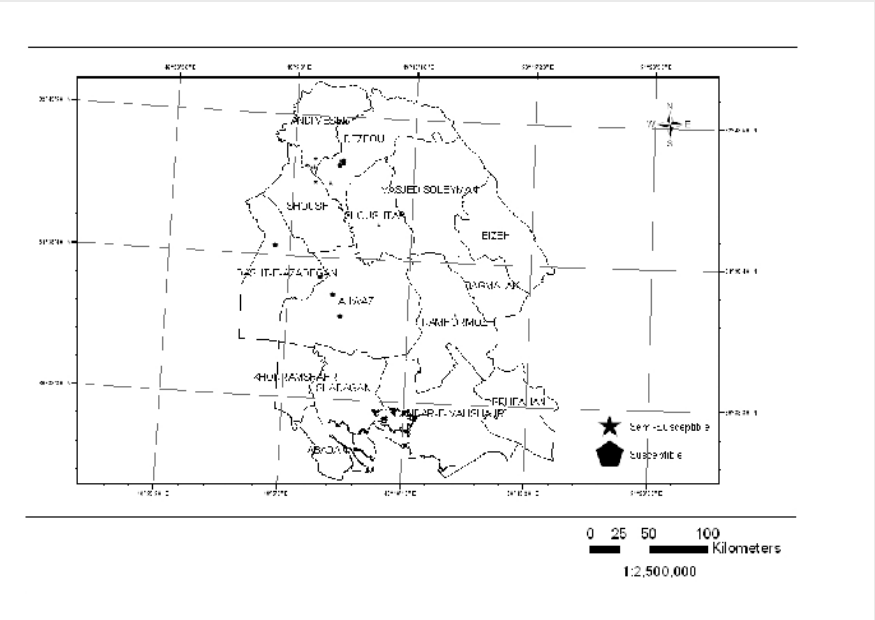

Figure 4- Distribution of susceptible and semi susceptible *A. ludovician*a in south western Iran.

Overal, the results indicated that 52% of wild oat populations in Khuzestan were resistant to clodinafop propargyl, 28% were semi-resistant, and 18% were susceptible to this herbicide (Figure 5). Resistant populations have also been distributed throughout Khuzestan (Figure 3) which indicates their rapid expansion in the province. The highest risk belonged to Shoush, with 6 resistant populations and 2 semi-resistant populations. Andimeshk was ranked second with 6 resistant and 5 semi-resistant populations. The lowest resistance was observed in Shoushtar and Ahvaz (Figure 2). Bena Kashani (2006 and 2007) reported 3 wild oat populations which were resistant to clodinafop propargyl and fenoxaprop-p-ethyl in Khouzestan province. Generally, appropriate application of herbicides and using alternative grass weed herbicide options are quite necessary to inhibit further evolution of resistant populations in Khuzestan.

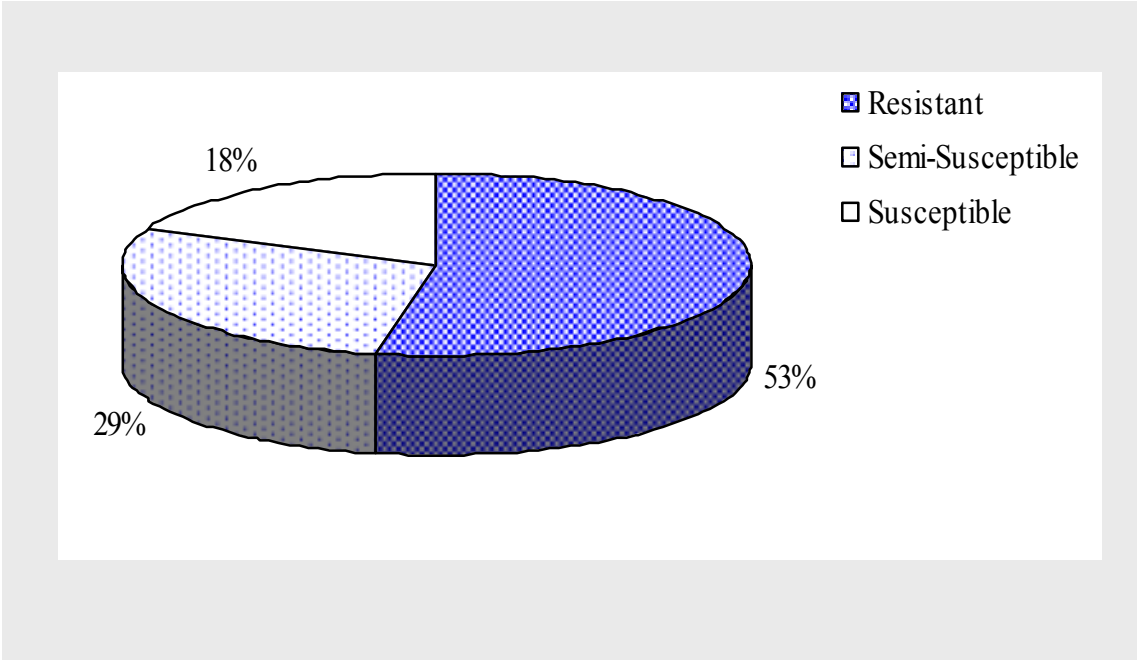

Figure 5- Percentage of resistant, semi-susceptible and susceptible *A. ludoviciana* in south western Iran.

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