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Evaluation of *Trichoderma* Isolates for Biological Control of *Rhizoctonia* Root Rot of Bean in Zanjan

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Introduction: *Rhizoctonia solani* is one of the major pathogens causing root rot in the main bean-growing regions in Zanjan province. Under favorable conditions, yield losses in commercial bean fields due to *Rhizoctonia* root rot have exceeded 50 percent reduction in pod and seed numbers per plants. In 2012 most isolates of the pathogen from severely infected bean fields in Zanjan were assessed to AG-4. *R. solani* AG-4 can attack other commercial crops such as potato, alfalfa, barley, tomato, cabbage, etc. which are grown in rotation with bean in the area. Thus, the disease is unlikely to be controlled by crop rotation. Moreover, there is no registered resistant bean cultivar against the disease in Iran. Although soil treatment with fungicides is the only effective control method in the region, according to environmental and side effects of fungicides, alternative approaches such as biocontrol method using *Trichoderma* species is considered.

Materials and Methods: The study was conducted using five isolates of *Trichoderma* (T_{12-0}, T_{12-N}, T_{19}, T_6, T_{95}) received from the Department of Plant Pathology, Ferdowsi University of Mashhad (Dr. H. Rouhani) and six isolates of *Trichoderma* (T_{36},T_{125}, T_{131}, T_{93}, T_{89}, T_{25}) collected in 2011 from rhizosphere of bean plants in the commercial bean fields of Zanjan province (Table 1). Trichoderma isolates were evaluated for their potential to antagonize in vitro the plant pathogenic fungus *R. solani* using three different tests. In the first test, each isolate of *Trichoderma* was grown in a dual culture with *R. solani* AG-4 strain Rh7 on PDA and incubated at 25˚C. Radii of colony of *R. solani* were measured after 72 h. In the second test the ability of *Trichoderma* isolates to produce volatile inhibitors was measured. This experiment was conducted in two conditions involving the same time culturing of *Trichoderma* and *Rhizoctonia* and isolating 72 h early growing *Trichoderma*. For both tests the percentage of inhibition was measured by dividing the difference between the radial growth of control and antagonized cultures of the pathogen by the radial growth of the control and multiplied by 100. All experiments were replicated three times. The third test was conducted with growing of *Trichoderma* and *Rhizoctonia* on microscopic slides covered with PDA and the interaction of hypha was studied using a microscope. For assessment of in vivo antagonistic activity, a greenhouse experiment was designed in 11 treatments with 3 replicates. Inoculum of *R. solani* AG-4 was prepared by inoculation of sterilized wheat grains with the fungus and the soil was inoculated with infected wheat grains. Bean seeds (red bean, cultivar Naz) were treated with conidial suspension of *Trichoderma* (10^7 ml^{-1} conidia) and planted in pots. Disease severity was recorded using a scale (1 to 5) according to Nelson and coworkers (1996) 30 days after sowing. Shoot length, root length, shoot weight and root weight also were measured. For all data the analysis of variance (ANOVA) was performed using SPSS Version 18.

Table 1- *Trichoderma* isolates used for their antagonistic activity against *Rhizoctonia solani*

isolates	species
T_{36}	*T.viride*
T_{125}	*T.viride*
T_{131}	*T.viride*
T_{93}	*T.virens*
T_{89}	*T.parseomosum*
T_{25}	*T.harzianum*
T_{12-0}	*T.virens*
T_{12-N}	*T.harzianum*
T_{19}	*T.sp.*
T_6	*T.sp.*
T_{95}	*T.harzianum*

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Results and Discussion: The results indicated that the mycoparasitism was the main mechanism accounting for antagonistic activity of *Trichoderma* isolates. In a competitive saprophytic ability test in a dual culture on PDA, T_{25}, T_{93}, $T_{12\text{-}N}$ and $T_{12\text{-}0}$ performed more effective than the other isolates, as T_{25} covered the whole colony surface of *R. solani*, grew and sproulated on the pathogen mycelia after five days. Volatile metabolites of all *Trichoderma* isolates inhibited mycelial growth, whereas $T_{12\text{-}N}$ with % 32/73 at the same time culture and T_6 with %56/08 at 72 h early culture of *Trichoderma* isolates had the highest effect on mycelial growth of *R. solani*. Microscopic studies also showed that *Trichoderma* isolates could inhibit hyphal growth of *R. solani* and parasitize the pathogen by hyphal contact, penetration, hyphal coiling around the pathogen hypha which finally resulted in hyphal fragmentation and caused death in the pathogen. These effects were typically caused by all successful *Trichoderma* isolates specifically $T_{12\text{-}0}$ and T_{93}. Although all *Trichoderma* isolates used in this study, had significant reduction in disease severity of bean root rot, caused by *R. solani* under greenhouse conditions (P = 0.01), but the most effective disease control occurred when the seeds treated with one of the following *Trichoderma* isolates: $T_{12\text{-}0}$, T_{131}, T_{95}, $T_{12\text{-}N}$ and T_{93}, among them $T_{12\text{-}0}$ belonging to *T. virens*, was the most effective isolate and the plants treated with this isolate, showed no disease symptoms. T_{93} also is belonged to *T. virens*. Two other species of *Trichoderma* were also observed among the successful isolates: *T. harzianum* ($T_{12\text{-}N}$, T_{95}) and *T. viride* (T_{131}). There are several publications on the biocontrol of *R. solani* by these species. Out of five successful isolates in greenhouse experiment, two isolates (T_{131}, T_{93}) were collected from the rhizosphere of bean in the studied area (indigenous isolates), thus could have priority for usage in future field assays. Seed treatment with T_6, T_{36}, T_{93}, $T_{12\text{-}N}$ and $T_{12\text{-}0}$ resulted in significantly increasing of root length, shoot weight and shoot length (P =0.01). Significant increasing of plant growth parameters caused by *Trichoderma* isolates has been reported by many other researchers on bean and other plants.

Conclusion: According to our results, several *Trichoderma* isolates not only antagonize in vitro the pathogen, but also controlled the disease under greenhouse conditions, thus they possibly use the same biocontrol mechanisms in the soil as in vitro conditions. Although field conditions are more complex and the many biotic and abiotic environmental factors will have an influence on the biocontrol potential of *Trichoderma* species, but it is essential to use successful indigenous and non-indigenous isolates under field conditions in the studied area to assess their biocontrol potential against the disease in fields for future commercial usages.

Keywords: Antagonist, Biocontrol, Competitive saprophytic ability, Seed treatment, *Trichoderma*

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Evaluation the Effect of some Vegetable Oils on Pinoxaden (Axial®) Efficacy in Little Canarygrass (*Phalaris minor Retz.*) Control

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Introduction: Adjuvants are used worldwide in order to improve the efficacy of foliage-applied pesticides and minimize the environmental damaging effects. It is generally agreed that there are two main ways by which adjuvants can enhance ultimate biological performance of herbicides. Initially, by increasing the amount of active ingredient retained by the target, and the second promoting their uptake.

Materials and Methods: Therefore, a greenhouse experiment was conducted to detect a suitable adjuvant for pinoxaden herbicide against littleseed canarygrass. Experiment was done as factorial in completely randomized design with 12 replications in Research Greenhouse of Ferdowsi University of Mashhad in 1390. The treatments consisted herbicide factor in 6 levels (0, 2.81, 5.62, 11.25, 22.5 and 45 g a.i. h^{-1}) and adjuvant at four levels (without adjuvant, coconut, sesame and almond oil as a vegetable oil at 0.5 percentages by volume (% v/v). Seeds treated by 98% sulfuric acid for a period of 6 minutes then were planted in trays that were filled with peat moss. Trays were irrigated daily. After emergence, seedlings were planted in pots. The spray treatment was done at three to four-leaf stage by using an overhead trolley sprayer (Matabi 121030 Super Agro 20 L sprayer; Agratech Services-Crop Spraying Equipment, Rossendale, UK), equipped with an 8002 flat fan nozzle tip delivering 200 L ha^{-1} at 2 bar spray pressure. Four weeks after spraying, the plants of the experimental units were harvested and oven-dried at 75°C for 48 h, then weighed. The greenhouse temperature varied from 18°C to 25 °C during the day and 14°C to 21°C at night.

Results Discussion Analysis of variance indicated that usage of vegetable oils and pinoxaden herbicide affected littleseed canarygrass biomass significantly at 1%. The results of the average comparison also showed that with increasing of amount of herbicide littleseed canarygrass biomass decreased significantly. It seems, increasing the amount of herbicide enhanced herbicide concentration gradient and resulted in the effectiveness of pinoxaden herbicide on littleseed canary grass. In all treatments, concentration of 45 g active ingredient had the greatest effect. Results of adjuvant main effects also showed that the use of vegetable oils had significant positive impact on pinoxaden herbicides. Coconut, sesame and almond oils respectively had the best performance in increasing the efficacy of pinoxaden herbicide compared to control without the adjuvants. The average amount of biomass for coconut, sesame, almond oils and control without adjuvants were 1.21, 1.41, 1.48 and 1.65 grams, respectively. Based on results of the regression analysis and comparison of the mean ($P\leq 0.05$), all adjuvants increased pinoxaden herbicide efficacy. The amount of ED_{50} decreased and relative potency (R) increased. Data analysis showed that coconut oil increased pinoxaden herbicide efficacy more than other Adjuvants and ranking of other treatment was such as sesame oil> peanut oil> herbicide without additive. ED_{50} of usage coconut oil, sesame seeds, almonds and pinoxaden alone was 29.98, 35.62, 45.93 and 57.80 g a.i.h^{-1} respectively. Average dry weight for pinoxaden alone, peanut, sesame, coconut oil was 1.65, 1.48, 1.41 and 1.12 grams, respectively. Probably high ratio of saturated to unsaturated fatty acids is reason greater efficiency of coconut oil than other adjuvants. In addition to the above reasons, It seems to abundant of shorter fatty acids in sesame oil and particularly coconut oil (Caprylic 8:0, Capric 10:0 and Lauric 12:0) compared with almond oil enhanced efficacy of herbicides. Vegetable oils that have more saturate/un-saturate fatty acids ratio were more effective in increasing the performance of herbicides. Instance, coconut oil with highest amount of this ratio (11.8) had the most impact on performance of herbicides. A positive relationship existed between lack of oleic acid and effectiveness of herbicides. Coconut and sesame oils with smaller oleic acid amount increased herbicide performance more that almond oil.

Conclusion: The results indicated that the relative potency of coconut oil has increased more than the other oils. All the adjuvants were effective in increasing the efficacy of the herbicide and it is recommended that after

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farm tests if these materials have positive effects, they are used to increase the effectiveness of the herbicide.

Keywords: Adjuvants, dose response, relative potency

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Study the Reaction of Some Barley Cultivars to *Rhizoctonia solani* AG-8, the Causal Agent of Root Rot Disease

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Introduction: Barley is one of the important agricultural products, mostly as livestock feed, and secondly for its important role in human nutrition as bread, soups, baby food and etc. It has the second-largest rank of cultivation area and yield of the national grain production and the Isfahan province, with production 5% of total barley yield, has been ranked eighth in 2010. Because its consumption exceed over the production, barley is one of the major imports to the country. In addition to, agronomy operations, plant diseases are important factors in yield loss. Rhizoctonia root rot (caused by soil-inhabiting fungus *Rhizoctonia solani*) is one of the important diseases of cereals include barley over the worldwide cultivation area. Apropriate soil fertility, delaying planting dates, crop rotation with insensitive crops such as legumes, planting resistant varieties and fungicide seed dressing are recommended methods to reduce disease damage. Chemical control of this disease is difficult because of its soil-born the pathogen. Therefore, reducing disease level requires application of other methods especially resistance cultivars.

Materials and Methods In this research, the reaction of 8 barley cultivars were examined against root rot disease in greenhouse conditions, in the winter of 2009. Fifteen isolates of the fungus were isolated from infected barley fields in the Isfahan province and their pathogenicity was examined on barley. One isolate with the highest pathogenicity potential was selected and special tests showed that the isolate was *Rhizoctonia solani* AG-8. The experiment was conducted in a completely randomized design with 4 replications. The test plants were harvested at two times of 4 & 8 weeks after planting. Following parameters were measured: 1- dry weight of plant root and aerial part, 2- disease severity as an index of subcrown internodes infection.

Results and discussion Statistical analysis of recorded data showed that there were significant differences among cultivars with respect to the dry weight of plant parts and disease reactions. Seedling shoot dry weight loss showed significant differences in the level of 5% among the tested cultivars of barley and compared to the healthy controls. The shoot dry weight of Bedonepushine cultivar seedlings treated with pathogen showed the highest loss (75% loss compared to the control) and had a significant difference at level of 1% compared to the healthy controls, thus it has been the most sensitive cultivar with due regard to shoot dry weight. In contrast, the Yoseph, Fajr 3 and Rudasht cultivars, with the shoot dry weight loss of 5%, 7% and 19%, respectively, compared to control, appeared to be more resistance than others. Pathogen effect as reducing root dry weight of seedlings had also significant differences at level of 1% among cultivars and compared to control. The maximum loss of root dry weight among cultivars was found in Bedonepushine cultivar (73% weight reduction compared to the control) while Yoseph, Rudasht and Fajr 3 cultivars, with the root dry weight loss of 6%, 7% and 11% respectively, compared to control, had the lowest weight loss. With a view to roots and crown infection rate, the Fajr 3 cultivar (with an average of 1.6 degree) had the lowest rate of root and crown infection, compared to control, and Bedonepushine cultivar (with an average of 4.6 degree) showed the highest degree of infection. In other cultivars, infection rates were measured between 2 and 4 degrees.

Conclusion In the final data analysis of tested barley cultivars, those with root and crown infection rate less than grade 3 were selected as tolerant cultivars. According to the study, the Yoseph, Fajr 3 and Rudasht cultivars, with comparatively lowest infection index and minimum weight loss, were more tolerant to disease in comparison to others. On the other hand, Bedonepushine, Bahman and Makuy cultivars are causes of highest infection index and maximum weight loss, were introduced as more susceptible cultivars against Rhizoctonia root rot disease. These results provide the possibility of the use of tolerant cultivars to reduce disease damage, and the possibility of transmission of resistance genes to *R. solani* of tolerant or resistant genotypes to susceptible commercial ones.

Keywords: Root rot, Barley, *Rhizoctonia solani*

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Insecticidal Effects of Crude Extracts of Six Officinal Plants on the Cowpea Weevil, *Callosobruchus maculates* F. (Col.: Bruchidae)

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Introduction: The cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae), is one of the most important stored product pests of several pulse crops including chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* (L.) Walp.), lentil (*Lens culinaris* Medik.), common bean (*Phaseolus vulgaris* L.) and soya (*Glycine max* Mer.). This pest cause considerable economic damage especially under conditions of tropical and subtropical subsistence agriculture. Infestation quickly becomes obvious as a result of the presence of eggs stuck to the outside of seed together. In the recent years, plant extracts have received much attention as pest control chemical agents. The application active compounds, which are less persistent, are harmless for the environment.

Materials and Methods: In this study, the contact and fumigant toxicity of extracts of *Ziziphora clinopodioides* Lam., *Lavandula officinalis* Chaix., *Laurus nobilis* L., *Rosmarinus officinalis* L., *Satureja hortensis* L. and *Salvia officinalis* were investigated against adult repellency, egg hatching, and oviposition rate of the cowpea weevil, *Callosobrochus maculatus* F. The extract of the examined plants was provided by the polar (methanol) and non-polar (N- hexan) solvents. The adults of the cowpea weevil were originally collected form Esfahan's grain warehouses and colonized at the favorite experimental conditions. The experiments were conducted using a completely randomized design. All experiments were carried out at 27±2˚C, 55±5% RH and 12L: 12D photoperiods. Insecticidal effects of the extracts were determined as mortality percentage 24, 48 and 72 hours after treatment. The data of mortality percentages were corrected by Abbott equation. Repellency effects of the extracts were determined one hour after treatment and were continued until five hours.

Results and Discussion: The contact toxicity at the highest concentrations of *Z. clinopodioides, L. vera, L. nobilis, R. officinalis, S. hortensis* and *S. officinalis* caused 82.21, 94.07, 73.74, 77.76, 88.23 and 92.65% mortality of adults, respectively. The calculated LC_{50} in contact toxicity was 14.87, 9.72, 32.30, 19.20, 21.48 and 23.59% for extract of *Z. clinopodioides*, *L. officinalis*, *L. nobilis*, *R. officinalis*, *S. hortensis* and *S. officinalis*, respectively. in fumigant toxicity, the highest concentration of extracts caused 63.48, 97.72, 55.96, 81.17, 86.47 and 90.85% mortality of adults and 83.15, 88.88, 71.73, 41.84, 53.60 and 76.74% mortality of eggs. The calculated LC_{50} in fumigant toxicity of various plant extracts on the adults of cowpea weevil was 47.07, 4.91, 61.77, 11.07, 6.01 and 20.42% for *Z. clinopodioides*, *L. officinalis*, *L. nobilis*, *R. officinalis*, *S. hortensis* and *S. officinalis*, respectively. At the highest concentration, (1µl/ml) oviposition deterrence rate was recorded 92.81, 99.20, 97.22, 100.00, 91.60 and 95.80% by extracts of *Z. clinopodioides, L. vera, L. nobilis, R. officinalis, S. hortensis* and *S. officinalis*, respectively. The crude extracts of *L. vera* and *R. officinalis* repelled 100 and 64.00% of insects at the highest concentration (30µl), respectively. The extracts of *Z. clinopodioides* and *S. officinalis* showed the high repellency potential on the adults of cowpea weevil and both of them repelled 80% of studied insects after the 5-h exposure time to the highest concentration (30 µl/ ml). The highest and lowest egg mortality was obtained by the extracts of *L. officinalis* and *R. officinalis* as 89 and 42%, respectively. The present study revealed the high efficiency of these plant extracts in non-chemical control of *C. maculates*. Based on our findings, at the low concentration of all examined plant extracts, more than 50% mortality was observed on the population of cowpea weevil in the laboratory conditions.

Conclusion: Among the examined plants, *L. officinalis* and *Z. clinopodioides* are introduced as the most effective plant species in control of the adults of cowpea weevil based on the low values of LC_{50} which are

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obtained in the experiment of contact toxicity.

Keywords: *Callosobruchus maculates*, Crude extract, Medicinal plants, Insecticidal activity

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Chemical weed control in Spinach (*Spiniacia oleracea*)

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Introduction Spinach *(Spinacia oleracea)* is an annual plant of family Chenopodiaceae. It is cultivated in temperate and cold regions in Khouzestan in autumn and winter. Weeds are the main problems that limit the production of vegetables. Competition ability of spinach against weeds is very low and weeds cause the loss of quality and quantity in this plant. Weeds reduce germination and establishment and growth of spinach. Weed management in spinach should be done at the beginning of the season. Hand weeding is the best way to control weeds spinach, although due to the high cost it is not cost effective, but is steel common in large areas. Weed control spinach, using chemical methods, the number of weeds are kept below the threshold of economic damage.

Materials and Methods The experiment was conducted in a randomized complete block design with 15 treatments and three replications. Treatments included pre-plant application of EPTC at 5 and 6 lit ha^{-1}, pre-plant application of Trifluralin at 2 lit ha^{-1}, pre-plant and pre-emergence application of Pendimethalin at 3 lit ha^{-1}, pre-emergence and post-emergence application of Meteribouzin at 300 g ha^{-1}, pre-emergence and post-emergence application of Meteribouzin at 400 g ha^{-1}, pre-emergence and post-emergence application of Imazethapyr at 0.7 lit ha^{-1}, pre-emergence and post-emergence application of Imazethapyr at 1 lit ha^{-1}, weedy and weed free checks. Each plot the size of 2.5 × 2 meters and 10 row cultivation with distances between rows of 15 cm and the distance between the plants 25 cm and the sowing depth was 3 cm. The herbicide treatments were applied to the back sprayer with Flat fan nozzle with volume of consumption of 240 lit ha^{-1} solution. The final harvest was about 50 days after emergence. Sampling of weeds 10 days before harvest was performed with using quadrate 0.5 × 0.5.

Results Discussion Important broad-leaf and narrow leaf weeds observed in the field, included field bindweed *(Convolvulus arvensis)*, Common lambsquarters *(Chenopodium album)*, Malva (Malva spp.), Chamomile *(Anthemis altissima)*, Purple nutsedge *(Cyperus rotundus)*, canary *grass (Phalaris minor)*, mouse barley *(Hordeum morinum)* and Japan brome *(Bromus japonicus)*, respectively. The results of variance analysis showed that the effects of treatments on the number of broadleaf and weed narrow leaves were significant. Meteribouzin and Pendimethalin herbicides (pre-emergence), had better control on broadleaf weed than other herbicides. Low amounts of herbicides EPTC (5 lit ha^{-1}) and imazethapyr (0.7 lit ha^{-1}) were the least effective broadleaf weed control. Trifluralin herbicide reduced approximately 44% broadleaf weed density compared to control plots without control. The minimum weight of broadleaf weed at all doses studied allocated to herbicides Pendimethalin and Meteribouzin. Most of reducing the number narrow leaves was belonged to Meteribouzin and Pendimethalin herbicides as pre-emergence with doses of 300 g and 3 lit ha-1, respectively. The effect of treatments on petiole length, number of leaves per plant and the spinach fresh yield was significant in 1% probability level. Meteribouzin damage in spinach was 100%. It was reported that the half-life in soil herbicide Meteribouzin is about 30-60 days. It seems spinach a high sensitivity to the herbicide and relatively long survival in the soil that causing damage spinach was perfect, while maximum weed control amounts in all methods of used allocated to this herbicide. Number of leaves per plant trait was that less affected by weed interference. Both components of leaves per plant (r= 69.0**) and petiole length (r= 87.0**) showed significant positive correlation with the spinach fresh yield. The highest spinach yield was obtained in Trifluralin herbicide after treatments control. The difference between spinach yield in Trifluralin and control treatments was not significant.

Conclusion: In general, the results showed that the broad and narrow leaf weeds were well controlled by different rates of pre-emergence and post-emergence application of meteribouzin, but this was followed by severe damage in spinach. Trifluralin had the lowest damage effect on spinach leaves. Pre-plant application of trifluralin at 2 lit ha^{-1} reduced approximately 45% of weed density and increased yield of spinach by 26.6% compared with control treatment. Therefore, this herbicide was recommended to use for weed control in spinach

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fields. According to the results it seems that the use of herbicide meteribouzin not advisable in spinach whereas causing damage, but due to weed well control is recommended increased resistance spinach to the herbicide through breeding programs. However, it should be noted that spinach leaves are consumed fresh, therefore, investigating the presence of herbicide residues in plant is necessary.

Keywords: Herbicides, Narrow and broad leaf weeds

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The Study Efficiency of Reduced Rate of Pendimethalin Integrated with Mulch and Hand-Weeding in Fennel *(Foeniculum vulgare* Mill.*)*.

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Introduction: Fennel has been cultivated as a medicinal and spice plant for a long time in different areas of Iran. However, its characterizes such as slow-growing, weak establishment, owning low LAI index and long seasonal plant, make it a poor competitor against weed, therefore, protection from weed is essential for successful cropping. The combination of management practices, such as mulching with herbicide could improve weed control efficacy of reduced rates. Limited information is available on control of weeds in fennel as important medicinal plant. Using a combination of physical control and herbicides at reduced rate may increase the efficacy of both treatments while reducing the cost of the weed control program and environmental impact of over reliance on herbicide applications. Therefore, the objective of this study was to evaluate the effects of reduced rates of the pendimethalin alone and in combination with wheat straw mulch and one hand-weeding on the growth of weed and grain yield of cultivated fennel.

Materials and Methods: this experiment was carried out at the Research Farm of Faculty of Agriculture, University of Zanjan in the spring 2012 to introduce an integrated weed control method in fennel. In this experiment the efficacy of different rates of pendimethalin alone and with combination with one hand weeding and straw mulch for selective control of grass and broadleaf weed species were evaluated. Field experiment was laid out as factorial based on randomized complete block design with three replications. Factors were application dose (0, 660, 990 and 1320 g a.i. ha^{-1}) and supplemental weed control (none, one hand-weeding, mulching). A plot with whole season hand weeding was also included as control. The recommended doses of pendimethalin were 1320 g ai ha^{-1}. Fennel was planted at 10 seeds m^{-2}, at 0.5 m row spacing, on May 6, 2012. Pendimethalin was applied pre-emergence soon after planting. In the mulch treatments, wheat straw were spread evenly after fennel planting at the rate of 2 kg m^{-2}. In the weed control treatments which one hand weeding used as supplemental physical control, a hand-weeding was performed at 50 days after planting. At fennel maturity, weeds were harvested from a 0.5 m^2 area and dried in an oven for 48 h at 75 ^oC and biomass was recorded. For assessing the effect of the treatments on fennel seed yield a 2 m^2 centre area of the plots was harvested manually. Data were subjected to an analysis of variance using PROC GLM in SAS Software. The means were compared using a Fisher's Protected Least Significance Difference test ($P< 0.05$).

Results and Discussion: Biomass of broad leaf weeds was significantly affected by herbicide doses and supplemental control. However, application dose and supplemental control interaction were not significant on broad leaf weeds biomass. Maximum broadleaf weed biomass control (85.9%) was recorded by recommended dose of pendimethalin. For a given dose of herbicide, weed control efficiency was higher when the physical methods were used as supplemental control and generally one hand-weeding was effective than mulching. All weed management treatments reduced biomass of grass weeds compared to weedy control. No differences were observed between the grass weed biomass in plots which received 75% and 100% of the labeled recommended rate of pendimethalin. Significant differences ($p < 0.05$) among herbicide rates and 50% of the labeled recommended rate (660 g a.i. $ha^{-1)}$ was observed for grass weed biomass. Biomass of grass weeds was also significantly affected by physical control. Maximum grass weed control (86%) was observed with one hand-weeding than the mulch. Same to broadleaf, the interaction effect of application dose × supplemental control treatments was not significant for grass weeds biomass. Total weeds biomass was significantly affected by herbicide. The interaction effect of application dose × supplemental control treatments were significant for total weeds biomass. Maximum weed biomass (1837.5 $g.m^{-2}$) was recorded in weedy control. When herbicide combined with one hand-weeding, the rate of 660 g a.i. ha^{-1} of pendimethalin was sufficient to provide 81% total weed control, which was statistically similar to the best control achieved for all dose and physical combination treatments. Fennel seed yield and yield component was significantly affected by application dose and physical control. The greatest seed yield loss (97.6%) was recorded in no weed control plots. and the lowest seed yield loss (13%) was seen in the recommended dose of pendimethalin + one hand-weeding plot. When one hand-

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weeding was used as a supplemental control, increased herbicide application rates from 990 to 1320 g a.i. ha^{-1} did not significantly influence the level of fennel seed yield.

Conclusions Overall, results of this study showed that pendimethalin in labeled recommended rate and applied alone was not sufficient to provide season long weed control. The recommended dose in combination with one hand weeding could not prevent of economic yield loss. As a result, it is recommended to test efficient herbicide or other methods of weed management of this plant. To improve the level of weed control with mulch, application of herbicides at the same time as the mulch or supplemental hand-weeding could be suggested as a part of integrated weed management in fennel.

Keywords: Biomass, Herbicide, Recommended rate, Weed

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The Investigation of pH Variation of Water in Spray Tank on Glyphosate and Nicosulfuron Performance on Barnyardgrass and Velvetleaf Control

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Introduction: Many factors affect the absorption, transport and performance of herbicides, include; physical factors (such as the orientation, shape, size, cuticle thickness, and its amount downy of the plant leaves), physiological factors (such as the growth stage and its succulence), environmental factors (like rainfall after spraying, relative humidity, wind, and temperature), as well as water quality in spray tank (32). The quality of natural water resources is very important, because the water passes through soil and rocks and dissolve natural salts and transfer them to groundwater reserves (12). pH is a chemical scale for measuring the concentration of hydrogen ions (H^+) in the water (21). When pH of solution is less than herbicides pKa (ionic dissociation constant), increasing pH can increase the solubility of herbicides, especially when the absorption limitation of herbicide is because of its solubility (14). To investigate the effect of water pH in herbicide spray tank, testing the effectiveness of weed control is appropriate method. The different species of weeds may have different amounts of ions in the tissue that showed different responses to herbicide solution (14). Accordingly, these basic experiment conducted to study the pH variation of water in spray tank on glyphosate (Roundup®) and nicosulfuron (Cruse®) performance on barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] **and velvetleaf** (*Abutilon theophrasti* Medicus.) control in the greenhouse condition.

Materials and Methods: Two separate **e**xperiments were performed as factorial arrangement of treatments 2×7 based on completely randomized design with six replications at Research Greenhouse of the Ferdowsi University of Mashhad in 2010. Factors included were: pH at 7 levels (4, 5, 6, 7, 8, 9, and 10) obtained by using buffer prepared solutions (+3 control pots for each pH level), and two weeds (barnyardgrass and velvetleaf). Glyphosate and nicosulfuron herbicides were applied post emergent 158 and 22 g ai ha-1 (based on ED50 outcome preliminary test (11)), recpectively, at the 3-4 leaf stage of the weeds in a spray volume of 250 L ha-1. Four weeks after treatment, survival, plant height, leaf area, shoot fresh and dry weight of weeds (% control) were calculated. The data of experiment were subjected to ANOVA using MSTATC software. Means of the treatments were separated using Duncan's Multiple Range Test at $\alpha = 0.05$. Based on the distribution of data, regression analysis was used as two, third, and four-degree polynomial.

Result and Discussion: The results showed significant effect ($P \leq 0.01$) of water pH variation in spray tank of herbicides on survival, plant height, leaf area, shoot fresh and dry weight of weeds (% control). Nicosulfurone herbicide in water pH equal 7 and 8 showed the highest effect on barnyardgrass weed, so that, the highest activity of nicosulfuron herbicide on velvetleaf was found in water pH= 8. Whereas, glyphosate herbicide in water pH equal 6-7 and 6 showed the highest effect on barnyardgrass and velvetleaf, recpectively. The total, more alkaline pH of water in spray tank was suitable for nicosulfuron compared to glyphosate performance (9, 28). Comparison between two weeds was showed that nicosulfuron effect on barnyardgrass control was more than of velvetleaf. So that in the best performance of the herbicide (water pH= 8), values of survival, plant height, leaf area, fresh weight and dry weight (% control) were estimated equal 25.8%, 16.7%, 21.6%, 4.6%, and 2.6% for barnyardgrass, respectively. The corresponding values for velvetleaf were 50.0%, 61.1%, 53.6%, 32.9%, and 24.5%, respectively.

In general, the solubility of sulfonylurea herbicides in water decreases with decreasing pH. Therefore, an acidic solvent prevent from their optimal distribution and their efficiency are reduced (18, 22). In this regard, Matocha and Senseman (15) reviewed the half-life of Trifloxysulfuron herbicide in water pH 5, 7 and 9 and found that this herbicide hydrolyzed faster in acidic pH than neutral/ alkaline pH. Green and Cahill (9) also were showed when alkaline agents added to spray tank, increased the pH of nicosulfuron solution and finger grass (*Digitaria sanguinalis*) was well controlled by this herbicide. The researchers, increasing the nicosulforon performance on weed knew because of its higher solubility at high pH. In research conducted by Buhler and Burnside (4) concluded that an increase in the water pH of glyphosate spray tank (400 g ai ha^{-1}) by from 2.4 to 7

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and 9, reduced herbicide performance on oat (*Avena sativa*) 14 days after spray at the greenhouse experiment ($P \leq 0.05$). So that, shoot fresh weight of oat was lost by 69 percent at pH= 2.4 (compared to the control without spraying). The corresponding values for pH 7 and 9, were estimated 62% and 55%, respectively. Field test results also coincided with greenhouse experiment (4).

Conclusion: Results of current study emphasized the role of water pH in spray tank of glyphosate and nicosulfuron on barnyardgrass and velvetleaf control.

Keywords: Biomass, Post emergence, Solution quality, Surfactant

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The Effect of PSII Inhibitors on Kautsky Curve and Chlorophyll Fluorescence in Common Lambsquarters (*Chenopodium album* L.) and Common Purslane (*Portulaca oleracea* L.)

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Introduction: Desmedipham + phenmedipham + ethofumesate, phenylcarbamates + benzofuranyl alkanesulfonate herbicides, is widely used for post-emergence broad-leaved weed control in sugar beet. Chloridazon, a pyridazinone herbicide, is used as a pre- and post- emergence herbicide in sugar beet. Desmedipham, phenmedipham and chloridazon, are photosystem II (PSII) inhibitors, their translocation via xylem are slow, mostly absorbed not only by roots, but also by foliage. Their mode of action is through the blocking of electron transfer between the primary and secondary quinones (Q_A and Q_B) of PSII by binding to the Q_B-binding site and accepting electrons from Q_A in the chloroplasts. Measures of changes to the chlorophyll fluorescence induction curve (Kautsky curve), is a rapid, non-invasive and simple method for monitoring the physiological status of the photosynthetic apparatus in the plant. There are three phases found on the O, J, I and P steps. These phases primarily point out photochemical events relevant to PSII. The three phases are described as follows: at the O-J phase complete reduction of the primary electron acceptor Q_A of PSII takes place from 50 µs to 2 ms, the J-I phase corresponds to electron transfer from Q_A to Q_B happens between 2 to 30 ms and the I-P phase corresponds to the release of fluorescence quenching by the oxidized plastoquinone pool taking place within 30-500 ms.

Materials and Methods: In order to determine how exposure affects the fluorescence induction curve (Kautsky curve) and its parameters, two dose-response experiments carried out for chlorophyll fluorescence measuring. The treatments involved desmedipham + phenmedipham + ethofumesate at 0, 51.38, 102.75, 205.5, 308.25, 411, 616.5 and 822 g a.i. ha-1 and chloridazon at 0, 81.25, 162.5, 325, 650, 1300, 1950 and 2600 g a.i. ha-1 on common lambsquarters (*Chenopodium album* L.) and common purslane (*Portulaca oleracea* L.) at the research glasshouse of Agricultural Faculty of Ferdowsi University of Mashhad, Iran. Spraying was performed by overhead trolley sprayer (Matabi 121030 Super Agro 20 litre sprayer), 8002 flat-fan nozzle at 300 kPa and a spray volume of 200 Lha^{-1}. The plants were treated at 21 days (at the four- to six-true leaf stage) after planting. Chlorophyll fluorescence measurements were carried out on dark-adapted leaves at the same stages of development among pots. Fluorescence emissions were measured using a portable chlorophyll fluorometer (Handy-PEA), which emits light of 650 nm wavelength with an intensity of 3000 µmol photons m−2 s−1 for 10s. Leaves were dark adapted for a minimum of 30 min prior to measurement. The fluorescence measurements were taken 4 hours after spraying (HAS) for common lambsquarters and common purslane and again at 24, 48, 72 and 168 HAS. The Kautsky curves were visually examined for the effects of time and dose by the BIOLYZER program with OJIP steps as fix points.

Results Discussion: The results showed that the parameters of measured had different sensitivity to the herbicides application, So that, four hours after desmedipham + phenmedipham + ethofumesate application on weeds mentioned, maximum quantum efficiency of PSII (F_v/F_m), did not show any change whereas the relative change in J stage fluorescence (F_{vj}) and the area between the curve and Kautsky (Area) F_m strongly reduced during this period, but chloridazon usage was not changes on Kautsky curve and their parameters during this period due to lower solubility, and sediment deposition in the sprayer tank mix. Common purslane was more affected by desmedipham + phenmedipham + ethofumesate application because of sponge leaves and stems while common lambsquarters had lesser sensitivity to both of herbicides application due to powdery and white cover over leaves and stems. Among chlorophyll fluorescence parameters measured, F_{vj} was appropriate parameter and quicker to detect the herbicides effects on both of experiments plants.

Conclusion: In conclusion, various fluorescence parameters can be used to describe the shape and change of Kautsky curves in different plant species. In this paper we focused on common fluorescence parameters for two

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tested herbicides. The parameter F_v/F_m seems to be less sensitive to detecting changes than are F_{vj} and area. Since chlorophyll fluorescence parameters were affected after herbicide application immediately; therefore, it could be used as a practical tool for assessing the efficacy of herbicides in the early hours after spraying herbicides in the greenhouse and field experiments.

Keywords: Chloridazon, desmedipham + phenmedipham + ethofumesate, herbicide effective dose, photosynthetic apparatus.

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The Lethal Effect of Nanosilica (SiO_2) and Nanocopperoxide (CuO) against *Ephestia kuehniella* Zeller

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Introduction According to the international organizations, stored-product insects can cause serious postharvest losses. The Mediterranean flour moth, *Ephestia kuehniella* Zeller is a world-wide pest of the main stored products. This pest was attracted to a large variety of food types and grain in stores where its larvae can cause serious damages. The feces and webbings of the larvae cause additional quantitative degradations of the product. Fumigants are commonly used for control of the stored product pests, but awareness of the health hazard from pesticide residue and the growing problem of insect resistance to the conventional insecticides have provoked the discussion among the researchers to explore the alternative strategies for protection of the stored products. Using of nanoparticles as pesticide is another alternative for conventional pesticides. Nanoparticles due to being less dangerous for the environment, the warm-blooded animals can be used as an alternative for conventional pesticides. Nanoparticles are ultra- fine particles which have at least one dimension which is less than 100 nm. Because of having increased ratio of surface to volume, nano-particles are more reactive than their bulk counterpart. So, the present study was performed to investigate the potential effect of nanosilica and nanocopper oxide against larvae of *E. kuehniella*.

Materials and Methods *Ephestia kuehniella* larvae were reared on broken wheat grain at 27± 1°C, 50% ± 5 RH with a photoperiod of 14L: 10D. Nanosilica (SiO_2) and nanocopperoxide (CuO) was synthesized in the laboratory by ultrasonication method (ref: Rao et al. Size of nanoparticles was measured by scanning electron microscope. To study the oral toxicity of the nanoparticles, different concentrations of nanosilica (0.200, 0.287, 0.412, 0.592 and 0.850 mg/kg) and nanocopper oxide (1.20, 1.60, 2.135, 2.848 and 3.80 mg/kg) was mixed with a certain amount of diet and then 30 three-day old larvae were kept in each treatment. Larval mortality was recorded after 7 and 21 days and adult emergence was counted after 51 days. The experiments were carried out with four replications. Distilled water was used in the control treatment. To study the contact toxicity of nanosilica, fifteen-day-old larvae (third instar) were transferred into Petri dishes (n=10 larvae). Larvae were sprayed with 750 ml of aqueous emulsions of different concentrations (0.800, 1.640, 1.414, 1.880 and 2.500 mg/L) of nanosilica. The spray was applied by using Potter Precision Spray Tower. The experiments were carried out with nine replications. Distilled water was used in the control treatment. Larval mortality was recorded over five days. Statistical analysis was performed using SPSS 18 software followed by Duncan's multiple range test (P<0.01). The results were expressed as means (±SE) of data. Probit analysis was used for estimation of LC_{50} by Probit-Analysis-MSchart.

Results and Discussion The results of SEM showed that nanoparticles of 70%Zn-28%TiO_2-2%Ag and 70%Zn-29%TiO_2-1%Ag were successfully made with particle size of 48 and 49 nm, respectively. In nanosilica and nanocopper oxide treatments, mortality of the immature stages was increased with the concentration of the nanoparticles and the exposure time also increased (Table 1). The lowest adult emergence (5.00% for nanosilica and 31.67% for nanocopper oxide) was recorded at the highest concentration of the nanosilica (850 mg/l) and nanocopper oxide (3800 mg/l). Differences between the mortality of the nanoparticles and control were also significant. In nanosilica, LC_{50} values after 7, 21 and 51 days were 805, 518 and 403 mg/l, respectively. The LC_{50} values for nanocopperoxide after 7, 21 and 51 days were 3988, 3820 and 3036 mg/l, respectively (Table 2). Probit analysis revealed that nanosilica was more toxic to the larvae of the Mediterranean flour moth than nanocopper oxide. Toxicity of nanosilica against mortality of third instar larvae was increased in proportion with increasing of the nanoparticles concentration and reached to the highest level of 72% at the highest concentration of 2500 mg/l. However, probit analysis showed that nanosilica with LC_{50} value of 1870 mg/l has relatively low contact toxicity.

Conclusion: Our results indicated that nanosilica in comparison with nanocopper oxide was more effective

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against immature stages of *E. kuehniella*. It could also be concluded from our results that oral toxicity of nanosilica is more than its contact toxicity. Nanoparticles and specially nanosilica could be used as an alternative of the conventional pesticides in the management of the stored product pests.

Keywords: *Ephestia kuehniella*, Nanoparticles, Bioassay.

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Identification of *Aphelenchoides* Fischer, 1849 Species (Nematoda: Aphelenchoididae) Collected from Rapeseed Fields in North Khorasan Province

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Introduction: Rapeseed is seeded in the fall, from late september through late october. Seed should be broadcast across the garden. Plants are left to grow through the winter and tilled into the soil in march. Rapeseed crops have a high sulfur requirement, a nutrient that is commonly deficient in sandy soils. When growing rapeseed for nematode control, be sure to take a sample of your soil to your local cooperative extension office for testing to find out if you need to add additional sulfur. If sulfur levels are too low, the rapeseed crop will not be able to generate the organic compounds that suppress nematode populations. Brassica and mustard cover crops are known for their rapid fall growth, great biomass production and nutrient scavenging ability. *Brassica napus* is the most important oil plant in the world. They must be planted earlier than winter cereal cover crops in most regions. There are many species of plant parasitic nematodes in the farms. High population densities of them cause economically significant crop reductions in most agricultural crops including *Brassica napus* production. The life cycles of different nematode species vary, and may include feeding on the outside of the roots or penetration and development within the roots. Nematodes survive between susceptible crops by feeding on other crops and weeds, and are spread in contaminated soil on machinery, windblown soil, and infested seed pieces. The different genera of plant parasitic nematodes such as *Heterodera*, *Meloidogyne* and *Pratylenchus* were recorded from Brassica fields in the world. Root-knot nematodes cause the round to spindle-shaped galls on the roots. Aboveground e-shaped galls on the roots. Aboveground symptoms of nematode injury appear as patches of stunted or dying plants. Infected plants may wilt when stressed by high temperature or moisture extremes. *Pratylenchus* spp. is a microscopic worm-like organisms less than one-millimetre in length, which feed on root tissues. All species of root lesion nematode have a wide host range. The root cortex (or outer root layer) is damaged and may disintegrate. Diagnosis is difficult and can only be confirmed with laboratory testing, particularly to identify the species as all RLN species cause identical, symptoms. The objective of this study was to investigate the plant parasitic nematodes associated with Rapeseed fields in North Khorasan province of Iran.

Materials and Methods: In order to identify the plant parasitic nematode fauna of Rapeseed fields in the North Khorasan province, 40 soils and root samples were collected during the years 2007-2008. From each field several small samples were taken from all areas of the field. Then the soil samples were put in a polyethylene bag with pertinent information about each sample and then brought to the laboratory and kept in the refrigerator at about 4ºC, until they were processed for nematode extraction and other activities. Nematodes were extracted using the centrifugal flotation technique and transferred to glycerine according to the modified Sein-Horst method (1959). The permanent slides were prepared from the extracted nematodes. The nematodes were observed by light microscopy and identified based on morphological and morphometrical characters.

Results and Discussion: In this study, five species of the genus *Aphelenchoides* namely*: A. limberi, A. daubichaensis, A. delhiensis, A. confusus, A. rutgersi* were identified. While the last four species are reported for the first time in Iran. *A. daubichaensis* is characterized by having cuticular papillae (mucron) tail end of the taper and the central part is sharp. *A. delhiensis*is characterized in having three lateral field in the body and the mucron centrally located on the tail. *A. confusus* characterized in having a stylet without end nodes, oval, large, offset spermatecha and full of sperm and the tail cone at the end of the round and without mucron. *A. rutgersi*, is characterized by having three lateral fields in body, stylet with small basal swellings, oval spermatecha and full of sperm and post-vulval sac short, usually one and a half vulval body- widths.

Conclusion: In this study, many species from different genera of plant parasitic nematodes belonging to suborder Tylenchina were identified. From genus *Aphelenchoides*, 5 species were identified, such as *A. limberi, A. daubichaensis, A. delhiensis, A. confusus, A. rutgersi.* Among these species, the four species *A. daubichaensis, A. delhiensis, A. confusus, A. rutgersi* are reported for the first time in Iran.

Keywords: Morphology, Morphometry, Plant Parasitic Nematodes

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Feasibility of Reducing Sulfosulforun Herbicide Application in Wheat (*Triticum aestivum* L.) by Nitrogen Application Management

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Introduction: Wheat is the main crop cultivated through out the world. Around 60 percent acreage of world farms belong to cereal crops from which, 33 percent belong to wheat production. In Iran wheat also is the dominant cereal crop accounting for almost 70 percent of cereal production. Iran wheat production in 2013 was about 14 million tones, and average grain yield 3750 Kg ha^{-1}, is much lower than developing countries such as France (6800 Kg ha^{-1}), Germany (7000 Kg ha^{-1}) UK (7500 Kg ha^{-1}) Japan (5000 Kg ha^{-1}) and China (4900 Kg ha^{-1}. The reasons for low yield include conventional planting methods, costly inputs, improper land preparation, imbalanced fertilizer application, lack of coordination between growers and mill owners, natural calamities and shortage of irrigation water and heavy weed infestation. Among these, poor management of fertilizers and weeds are the major cause of low wheat production in Iran. The use of herbicides for weed control in wheat is a common practice in management of weeds. But environmental and economical aspects, uncertainty in the long-term efficacy of chemical weed control methods are the most important issues that have caused researchers and farmers seeking to introduce an alternative to herbicides or to reduce the use of herbicides. Among herbicides, sulfonylurea's such as sulfosulforun is one of the most important herbicides in wheat field. However, sulfosulforun is an effective herbicide for weed control. But its active soil residual, may damage rotational crops. Due to importance of nitrogen fertilizer management in wheat yield improvement, present study was designed to investigate the effect of nitrogen fertilizer application methods on weed management of wheat and reducing sulfosulforun herbicide under agro-climatic conditions of Mashhad, Iran.

Materials and Methods: In order to evaluate sulfosulforun reducing dose, by nitrogen application management, an experiment was performed as split factorial plot, based on randomized complete block design with three replications at research farm, Faculty of Agriculture, Ferdowsi University of Mashhad, in 2012. Treatments included, nitrogen application methods (Broadcast and Band), the amount of nitrogen application (100, 200 and 400 kg ha^{-1})(46% urea source) equal to 46, 92 and 184 kg ha^{-1} nitrogen per hectare, as complete factorial plots and sulfosulforun doses (100, 75 and 50 % of recommended dose (26.6 g (75% EC (emulsifiable concentrate)) as sub plots, applied at wheat tillering stage. Weed density and relative frequency were recorded 149, 177 and 210 days after wheat planting. Wheat biomass and seed yield per 1 m^{-2} were recorded at the end of the experiments. Analysis of variance of data was carried out with SAS software and for means comparison LSD ($p\leq0.05$) test was used.

Results and Discussion: Results showed that band application of nitrogen, reduced weed density and weed biomass significantly. By increasing nitrogen dose, mentioned parameters were increased significantly in both methods on nitrogen application. The highest weed density (29.27 plant m^{-2}) were observed when sulfosulforun was not applied and the method of nitrogen application was broadcasted. The lowest weed density (12.78 plant m^{-2}) observed at solfosulforun recommended dose and nitrogen band application treatment. The method of nitrogen application affected the amount of solfosulforun application. Based on the results, the effect of sulfosulforun at 75% and 50% of the recommended dose, didn't effect significantly ($p\leq0.05$) on weed control when nitrogen applied in band with solfosulforun at recommended dose on weed control when nitrogen applied broadcast. Similar results were observed for wheat seed yield and wheat biomass. Wheat seed yield (343.40 g m^{-2}) and wheat biomass (1141.50 g m^{-2}) at solfosulforun recommended dose, when nitrogen applied in band, was not significantly different from 75% recommended dose of solfosulforun and nitrogen broadcast application method.

Conclusion: Based on the results of experiment, the amount of nitrogen and its application method concludes an acceptable approach for wheat integrated weed management. It can optimize nitrogen fertilizer and solfusulforun herbicide usage, which are the main concerns in wheat production economically and ecologically. However nitrogen band application reduced the amount of nitrogen fertilizer and solfusulforun herbicide. Due to

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various factors affecting the competitive relationship between weeds and crops, it is suggested that in future the use of other herbicides, in different places and different crops to be considered.

Keywords: Nitrogen band application, nitrogen broadcast application. Reducing of herbicide application

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Fixed precision Sequential Sampling of Aphids on Wheat fields in Ahvaz

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Introduction: Aphids are secondary pests in cereal fields but sometimes they outbreak and cause considerable damage to wheat and barley. Integrated pest management is one of the most important strategy in insect ecology and estimating population can be used to employ the strategy. Sampling of population is the most basic outdoor activities in ecology and as time goes need to fast and reliable sampling methods be felt more. Dispersion can be quantified by the comparison of observed frequency distribution data (based on a common sample unit) with mathematical models used to describe possible spatial distributions. Knowledge about dispersion pattern of an organism is required for understanding population biology, resource exploitation and dynamics of biological control agents. Moreover determination the dispersion pattern of a species is essential for developing an effective pest management program. . The degree of aggregation can be expressed by several indices of dispersion. Taylor's power law and Iwao's patchiness regression are two main models that also depend on the relationship between the sample mean and the variance of insect numbers per sampling unit. The slope of the regression model is used as an index of aggregation. Usually, sampling of insects is from the estimatation of some population parameter for research purposes or to make a pest control decision. However, the often large, fixed sample size necessary for research may be inappropriate where frequent and rapid monitoring is required to make a control decision. In such cases the sequential sampling method may be a better alternative as it characteristically has a variable sample number and can serve to classify a population in relation to a treatment level rather than provide actual estimates of population density. Designing sampling plans based on these indicators has been reported to reduce sampling effort, cost and minimize variation of sampling precision. As there were no any information about the spatial distribution and sequential sampling program of cereal aphids in Khuzestan province, this study was undertaken to determine dispersion pattern of cereal aphids in order to develop a suitable sampling plan for these pests.

Material and Methods: In order to assess the distribution pattern and density of aphid species on wheat the mixed population of aphids was sampled during 2012-2013 at three pesticide-free wheat fields (two wheat fields in Ahvaz and one in Mollasani) in Khuzestan province, southwestern of Iran. Each field was sampled twice a week throughout the growing season from initiation of tillering to grain ripening stage. Each sample included 25 plant, which were chosen randomly and the number of aphids was counted. Tillers were collected by traveling an X-shaped procedure. Spatial distribution of different developmental stages of wheat aphids were described by calculating dispersion indices (Taylor's and Iwao's indices of dispersion). A sequential sampling plan was also developed using the fixed precision method of Green for estimating the density of adults, Nymphs and total population.

Result and Discussion: Analysis of spatial distribution pattern using Taylor's power law and Iwao's regression model showed that Taylor's power law provides a better description of the aphids spatial distribution and based on this model dispersion pattern of wheat aphids population was aggregated for nymph and the total of life stage, but was random for adult life stage. Green's fixed precision sequential sampling plan at precision levels of 0.25 and 0.10 was designed for estimating the density of the adult, nymph and total population. The results showed that the required sample size increased dramatically with increasing levels of precision, and generally ranged from 1 to 34 and 8 to 210 tillers at the precision levels of 0.25 and 0.10 respectively, and the optimum sample size for the estimation of mean aphid density decreased approximately between 44% - 83%.

Keywords: Ahvaz, Aphids, sequential sampling plan, spatial distribution, wheat

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Comparison of some Vegetative and Reproductive Traits of Dominant Weeds in Cultivated Tomato as Influence by Metribuzin and Non-living Mulches

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Introduction: Tomato production, similar to other important crops, is affected by weed competition in the early season. Weed infestation can affect tomato yield significantly. So weed control can be one of the most important factors in improving of crop production. Nowadays pre-plant, post-plant and post-emergence herbicides are widely used for controlling weeds in tomato fields, but it can lead to increasing environmental hazards, making weed resistant to herbicides and injuring tomato plants. Therefore, the use of non-living mulch (organic mulch and inorganic mulch) could be a suitable substitutional way to weed control.

Material and method: In this study, in order to investigating the time of emergence, flowering, seed shattering, length of vegetative and reproductive growth period of weeds under six types of non-living mulches (black plastic mulch, clear plastic mulch, wheat straw, sawdust, coco peat and peat moss) and mtribuzin in tomato, research was conducted in a randomized complete block design with three replications at College of Agriculture, Shiraz University in 2012. The plots were fertilized with 20:20:20 (N, P_2O_5 and K_2O) with concentration of 166 kg ha^{-1}. In this study, the appearance of plumule (for narrow leaf weeds) and hypocotyl (for broad leaf weeds) from weed seeds in soil medium was considered as the time of emergence. The weeds were visited once a week since transplanting tomato seedlings to the farm and the related date was recorded. Flowering time recording for each weed species was carried out after flowering. Plots without mulch (weedy and weed free) were used as control. Statistical analysis of the collected data was performed by using SAS 9.1 software and comparing of the mean of characteristics was done by Tukey's test at 5 % significant level.

Result and discussion: The longest and shortest time of weed emergence respectively belonged to the clear plastic mulch (28 days after transplanting) and organic mulches and metribuzin (21 days after transplanting). Meanwhile clear plastic mulch effect was significantly different from other treatments, but there was no significant difference among the organic mulches and metribuzin. Also, black plastic mulch and wheat straw respectively showed the highest (66.80 days after transplanting) and the lowest (52.69 days after transplanting) time of weed flowering and both treatments stimulated weed flowering compared to metribuzin (95.33 after transplanting). Weed seed shattering was another important characteristic that was affected by mulch treatment, so that wheat straw showed weed seed shattering at 87.33 days after transplanting while black plastic delayed weed seed shattering until 101.73 days after transplanting. Mulch treatment and metribuzin showed significant differences in their effects on weed seed shattering and metribuzin with seed shattering at 131.29 days after transplanting had the highest effect on it. Also according to the results of current study, there were statistically differences among applied treatments on the length of weed vegetative growth period and mulch treatments showed more efficiency in related to reducing the length of weed vegetative growth period than metribuzin. However wheat straw and clear plastic mulch respectively showed the longest (84.13 day) and the shortest (74.66 day) length of weed vegetative growth period among mulch treatments, but they respectively decreased the length of weed vegetative growth period by 9.20 % and 19.40 % compared to metribuzin. The Length of weed reproductive growth period under mulches, metribuzin and weedy treatments showed that there was no significant difference among all types of treatments. Finally, evaluating tomato yield showed that plots treated with black plastic mulch (20.93 kg/m^2), clear plastic mulch (8.31 kg/m^2) and metribuzin (6.06 kg/m^2) significantly influenced tomato yield. Meanwhile black plastic mulch significantly improved the yield. This is probably due to more effects of black plastic mulch on reducing of evaporation from the soil surface and minimizing of soil temperature and moisture fluctuations that cause better plant growth and yield.

Conclusion: According to the results, clear and black plastic mulches had more positive impacts on weed control and yield of tomato than the other treatment. Therefore, it seems that application of non-living mulch can lead to producing high production with higher quality in tomato.

Keywords: Seed shattering, Emergence, Flowering

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Purple Nutsedge (*Cyperus rotundus* L.) Response to Type and Rate of Ammonium and Nitrate Sources as Nitrogen Fertilizer

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Introduction: Crop nutritional management is one of the most important components of integrated weed management which maintains crop yield and reduces weeds performance over time. Nitrogen fertilizer is one of the major factors that has a significant effect on plant growth characteristics so that an optimized growth and yield could be achievable by choosing an appropriate type and amount of nitrogen.

Materials and Methods: In order to study the effect of different types and rates of nitrogen fertilizer on growth characteristics of purple nutsedge (*Cyperus rotundus* L.), an experiment based on CRD (Completely Randomized Design) was conducted with three replications at the Research Greenhouse of University of Birjand in 2013. 12 different treatment levels consisting of calcium nitrate, ammonium sulphate and urea as well as nitrogen rates of these fertilizers (0, 50, 100, 200 mg N kg^{-1} soil) were used to conduct the research.

Results and Discussion: ANOVA results showed that nitrogen fertilizer treatments had a significant effect ($P \leq 0.01$) on all study traits of nutsedge. The results of this study showed that different growth characteristics of purple nutsedge responded differently to the nitrogen fertilizer treatments The greatest height of purple nutsedge (94 cm) was observed with 50 mg N kg^{-1} soil of ammonium sulfate source, which provided a 27.3% height increase compared to the control. The greatest stem number of weed was achieved with 50 mg N kg^{-1} soil of calcium nitrate, which provided a 37.50% increase in stem number compared to the control. Application of 50 mg N kg^{-1} soil of ammonium sulfate increased the weed leaf number by 62.37% compared to the control and showed the greatest value of this trait amongst all study treatments. Results showed that increasing urea consumption resulted in a significant increase in plant leaf area, so that using 50 mg N kg^{-1} soil of this source showed the highest leaf area, which accounted for 59.88% increase compared to the control. Increasing ammonium sulphate usage significantly increased the pre-tuber number, so that utilization of 50 mg N kg^{-1} of this fertilizer resulted in the highest pre-tuber number and 52.60% increase compared to the control. Application of ammonium sulphate and calcium nitrate fertilizers enhanced the tuber number, so that the maximum tuber number was obtained where 50 mg N kg^{-1} of these fertilizers were applied. Results showed that applying 50 mg N kg^{-1} soil of ammonium sulfate provided a 76.3% increase in tuber dry weight compared to the control, and the highest tuber dry weight (3.780 g $plant^{-1}$) was observed with this treatment. Utilization of 100 mg N kg^{-1} soil of urea resulted in the highest rhizome dry weight (60.46% increase compared to the control). Moreover, the greatest shoot dry weight (9.729 g $plant^{-1}$) and underground dry weight (4.634 g $plant^{-1}$) was obtained with the usage of 50 mg N kg^{-1} soil of ammonium sulfate. On the other hand, the lowest height (62 cm), stems number (2.66 stem pot^{-1}), leaf number (15 leaf pot^{-1}), leaf area (2310.85 cm^2 pot^{-1}), shoot dry weight (1.014 g $plant^{-1}$), pre-tuber number (2.33 pre-tuber pot^{-1}), tuber number (4.33 tuber pot^{-1}), tuber dry weight (0.218 g $plant^{-1}$), rhizome dry weight (0.330 g $plant^{-1}$) and underground dry weight (0.785 g $plant^{-1}$) were obtained high rates (200 mg N kg^{-1} soil) of calcium nitrate and urea fertilizers were used.

Conclusion: Overall, the results showed that the application of ammonium sulfate and urea fertilizers at moderate rates (50 and 100 mg N kg^{-1} soil) can increase growth characteristics and competitive ability of purple nutsedge. It seems that farmers should consider these results and apply fertilizer types and rates resulting in the lowest growth where face purple nutsedge infestation. In this regard, the response of natural and horticultural crops competing with purple nutsedge should also be taken into consideration that will need further studies.

Keywords: Nutrients, Perennial weed species, Pre-tuber, Tube

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Comparison of Resistance Index Some Potato Cultivars to the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae)

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Introduction: Potato, *Solanum tuberosum* L. is an important crop with 5.24 million tons of production on 210,000 hectares of irrigated land in Iran. Several pests attack the potato crop which among them the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most important defoliating pest of potato throughout the world and Iran and considerably reduce crop yield. Growers rely on pesticides to control this pest in the field but this insect is well known for its rapid resistance development to pesticides, so that it has developed field resistance to nearly all insecticides used against it and it is now resistant to more than 40 chemical insecticides. The problems of insecticide resistance, combined with continuing environmental concerns associated with chemical pesticide use, have provided a considerable stimulus over the past 50 years for the development of alternative control methods. Host plant resistance is considered to be an important part of integrated pest management (IPM) system of this pest, which is compatible with sustainable control methods and can reduce the use of chemical insecticides .With the aim of identifying the existence of resistance resources, a study was conducted to evaluate and comparison the resistance index (PRI) of 33 potatocultivarsto the Colorado potato beetle. Detected resistant variety (ies) could be used as a resistance source for IPM programs of this pest.

Materials and Methods: Greenhouse and field experiments were conducted to compare resistance index (PRI) of 33 potato cultivars to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in Naghadeh region during 2007-2008. In a choice test, the numbers of attracted beetles to each cultivar was determined as antixenosis index in the field. Also, Percentage of larval and pupal mortality were determined and used as the antibiosis index under greenhouse conditions. To evaluate the tolerance index, infested and non infested plots were planted and arranged based on a randomized complete block design under field conditions. In infested plots, each plant was infested by 40 medium larvae (second and early third instars) 15 days prior to the blooming of plants. At the end of season, yield loss in infested plots were determined and compared with non infested plots for each cultivar and used as the tolerance index. Normalized values for each category were computed on a zero-to-one scale by dividing the mean value of each variety evaluated by the maximum mean value of all entries. Normalized tolerance values were calculated as percent reductions of plant biomass relative to a non-infested control. Normalized indices for antibiosis (X), antixenosis (Y) and tolerance (Z) were used to calculate PRI in the formula: PRI = 1/(XYZ) (Inayatullah *et al*., 1990).

Results and Discussion: Combined analysis of variance showed that interactive effect of cultivar × year was significant for all of the indices at P=0.01. The cultivars Sinja, Bridjet and Cardinal with the least antixenosis index (Y), had the highest antixenosis resistance to the Colorado potato beetle in 2007, also the cultivars Cardinal, Carlita and Elles with the least antixenosis index (Y), had the highest antixenosis resistance to the pest in comparison to other cultivars in 2008. The highest antibiosis (X) resistance was observed in the cultivars Delikat, Carlita, Armada and Sinja with the least antibiosis index, in two studied years. The cultivars Satina, Cardinal, Santana and Nicola with the least tolerance index (Z), had the highest tolrence to the Colorado potato beetle in comparison to the other cultivars during 2007-2008. The cultivars Delikat,Bridjet and Cardinal ere the most resistant cultivars with 52.63, 36.30 and 29.87 PRI, respectively in 2007. In this year, the cultivars Diamont, Aparet and Velox were the most sensitive cultivars with 1.90, 2.06 and 2.26 PRI, respectively in comparison to other cultivars. In 2008, the cultivars Santana, Bridjet and Sinja were the most resistant cultivars with 48.56, 28.36 and 25.80 PRI, respectively and the cultivars Velox, Agria and Bright were the most sensitive cultivars with 1.90, 2.71 and 3.20 PRI, respectively in comparison to the other cultivars in 2008.

Conclusion: Significant positive correlation was observed between the plant resistance indices (PRI) in two study years (r= 0.499, P= 0.01). Results of this research revealed that the cultivars Bridjet, Delikat, Cardinal,

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Carlita, Sinja and Nicola with the highest PRI were the most resistant cultivars to the Colorado potato beetle in comparison to other cultivars during 2007-2008, suggesting that these cultivars can be used in the IPM program of the pest and resistance transfer to high yield varieties through plant breeding programs.

Keywords: Antixenosis, Antibiosis, Tolerance, Plant resistance index

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Problems Encountered with Nested PCR to Diagnosis of Phytoplasmas

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Introduction: Phytoplasmas are phytopathogenic bacteria without cell walls that can be found in plant phloem, have been found associated with numerous diseases of plants worldwide. A sensitive and precise diagnostic test for detection of phytoplasma-infected plant is critical to avoid using infected planting material and dispersal of these agents. The detection of phytoplasma from plant tissues by PCR, requires using DNA extraction methods that extract high level phytoplasmas DNA with less plant inhibitors. Usually Phytoplasma diagnostics have been based on the 16S rRNA gene and the 16S–23S rRNA spacer region because universal primers that design for replication these regions could detect different groups of phytoplasmas but diagnostics based on these primers can be problematic, with occasional false positives, through amplification of some bacterial genomes that might be present in a plant sample. These primers also have sequence homology to chloroplasts and plastids and increase the risk of false positives so it is important to guard against false negatives and positive during such detection techniques.

Materials and Methods: Health and infected Lime samples with *Candidatus* phytoplasma aurantifolia were used respectively as negative and positive samples. DNA was extracted by the CTAB methods, SDS method, Fermentas DNA extraction kit, column based Method (Genet Bio genomic DNA isolation kit) and compared to remove inhibitors and reduced false negative reactions in nested PCR detection method. DNA samples were tested for phytoplasma infection by direct PCR using the universal phytoplasma primer pair P1/P7 and nested PCR using primer pairs P1/P7-R16F2n/R16R2 and P1/P7-fU5/rU3. The PCR products were sequenced and subsequent analysis using GenBank database information at the national center for biotechnology was employed.

Results and Discussion: Comparison of different DNA extraction methods indicated using suitable method can significantly reduce false negative reaction, but even in successful column-based DNA extraction method, false negative reactions were reported that were due to low phytoplasma concentration and irregular distribution within host tissues or could be caused by inhibitor presence in DNA samples. Based on results of sequencing, false positives were obtained sporadically, using primer pairs combination P1/P7- R16F2n/R16R2 that may be arising from cross over contamination or sequence homology with plant genome, so some primers can react probably with sequences of plant genome and false positives could be observed. Since false positives are also a major problem in PCR protocols, especially in nested PCR so single tube nested PCR (STNP) was optimized to avoid false positive reaction but regardless advantages of this method such as facility, cost and time effective and ability to detect low concentration of pathogen, false positive reactions were observed in a few samples. The advantage of STNP is that tubes do not have to be opened, so the risk of contamination minimized.

Conclusion: PCR-based techniques for phytoplasma detection, appears to be the method of choice because of their high sensitivity and specificity. Using suitable method for extraction of DNA from infected plant tissues are getting more critical for precise detection through increasing DNA quality and quantity. In the other hand confirmation of phytoplasma presence must be accomplished at least by RFLP analyses or different primer pair combination to avoid false positive detection.

Keywords: Phytoplasma, Detection, Nested PCR

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Brief report

Evaluation of Suitable Host Plant, as Banker Plant for *Eretmocerus mundus*, Whiteflies Parasitoid

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Introduction: Whiteflies (Homoptera; Aleyrodidae) are the key pests of agricultural crops worldwide. Two species, which seriously damage crops, are the Silver leaf whitefly "*Bemisia tabaci* (Gennadius)" and the Greenhouse whitefly "*Trialeorodes vaporariorum* (Westwood)". These pests are active on the underside of the leaves where they are relatively protected from insecticide. Moreover, emergence of resistant biotypes of whitelies impose much more costs on its chemical control. Therefore, during falling the past population, several researches have been carried out to find and use efficient biocontrol methods. One of the biocontrol agents to control *B. tabaci* is "*Eretmocerus mundus"* (Hy., Aphelinidae). The parasitoid should be introduced to control pest in the greenhouses. One of the methods, which is used in introducing of biological control agents, is 'Banker Plant System'. In this system, the host plant has a population of reproducing natural enemy used as a reservoir. Therefore, as soon as the pest infection occurs, the parasitoids can find them and control the damage. İn a banker plant system, several aspects should be considered. Some of the behavioral and biological aspects are important. For example characteristics of leaf surface influence on the host finding time of the parasitoid. The biochemical characteristics of the host plants affect the parasitoid fitness. Therefore, we tried to compare these aspects of *E. mundus* in two host plants (eggplant and cotton) from banker plant point of view, to control *B. tabaci* in the greenhouses.

Materials and Methods: The two host plants (eggplant and cotton), were planted in the greenhouse and a culture of *B. tabaci* was reared on the plants. After two weeks, some of the infested plants were put in separate cages, and the parasitoid, *E. mundus*, that was collected from the egg plant and cotton fields of the Varamin region were released on the plants. Then, the biological characteristics of the parasitoid were evaluated on the two different host plants. The sex ratio was recorded in two different ways; 1) 100 pupae developed on each plants were put in vials, and 2) the wasps that emerged daily from collected leaves. In the next experiment, a leaflet of each host plant (2.5 × 3 cm), that was infested with the second and third stages of *B. tabaci*), was fixed upside down on a piece of wet cotton in a Petri dish. Then, a female parasitoid was release on each to record the host finding time for one minute. Finally, the four female parasitoids were released on an infested host plant for 48 hours, then the wasps were removed and the plants were kept in a climate room. Two weeks later, the leaves were collected and put up side down on cotton pieces in Petri dishes and the duration of the larval stages, the mortality and the number of females and males that come out from them were recorded for the two hosts.

Results and Discussion: In total, 438 wasps form the eggplant leaves and 297 from cotton leaves were collected, while 14 and 17 dead pupa were recorded on them, respectively. The data analysis did not show significant differences between them. In host finding experiment, 9 of 15 parasitoids could find the host during one minute on the eggplants leave, whereas, all female parasitoids could find the host on cottons leave, which was significantly different (P value of Fisher's exact test= 0.01689). In the biology parameter experiments on leaves, 192 parasitoid wasps form cotton and 158 from eggplant leaf were collected that were not significantly different (F=5.6; p=0.026). In contrast, on whole plant experiment, 156 wasps (76 females and 80males) from cotton plants and 134 wasps (66 females and 68 males) from eggplants were collected that were not significantly different (f=1.2; P= 0.32).

Conclusion: The results showed that the number of parasitoid and the sex ratio were not significantly different between two host plants, whereas the movement and searching activity of the parasitoid were better on cotton than eggplant. Base on the results and all of the factors that were considered in this work, eggplant could be a good host in a banker plant system for *Bemisia tabaci* and its parasitoid *Eretmocerus mundus*.

Keywords, Banker Plant, Whitefly, Biocontrol, Greenhouse

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Brief report

Etiological Study of Gladiolus Wilting Disease in Jiroft

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Introduction: Gladiolus belongs to the family Iridiaceae. It is commercially cultivated in all parts of the world. This plant is mainly affected by wilt disease caused by *Fusarium oxysporum* Schlecht Fr f .sp. *gladioli* (Massey) Snyd. and Hans. It results in death of plant and rotting of corms. *Fusarium* disease of gladiolus is commonly known as yellows, wilt or corm rot. The wilt disease of gladiolus is known to occur in almost all gladiolus growing areas. Wilt disease is one of limiting factors of cultivation and development of gladiolus in Jiroft, Kerman, Iran. Different fungi such as *Fusarium oxysporum* f.sp. *gladioli, F. moniliforme, F. sporotrichiella, F. hetrosporium* and *Penicillium gladioli, P. rubrum* have been reported as causal agents of gladiolus wilt, root and corm rot disease in many countries. The disease symptoms, including yellowing of leaves, weakness of plant, flower imperfect, root and corm rot, browning of infected root and corm, wilting and finally death of infected plants which observed in infected samples. No research has been carried out in Jiroft, therefore, this research aims to study and identify the causal agent of wilt disease on gladiolus.

Materials and Methods: During survey, characteristic symptoms of wilt disease were recorded and also samples were collected for isolation of pathogens that infect on Gladiolus. The infected corms showing typical symptoms of wilt disease were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. To identify the causal agent of gladiolus wilt, infected tissues from symptomatic plants were first surface disinfestations, dehydrated and cultured on Potato Dextrose Agar Medium and incubated at 25± 2^{oc}. Isolated fungi identified using morphological and microscopic characters. To pathogenicity test, corms were inoculated using the dip method in conidial suspension and then planted in pots containing sterilized soil. Inoculated plants were checked for disease symptoms. Distilled water used for inoculation of plants as a control. Observations were made regularly for the appearance and development of symptoms. After symptom development, re-isolation was done from the artificially infected corms.

Results and Discussion: The disease symptoms, including yellowing of leaves, weakness of plant, flower imperfect, root and corm rot, browning of infected root and corm, wilting and finally death of infected plants observed in infected samples. The type of disease symptoms was similar to others which have been reported before. A fungus with white aerial mycelium and little dense colony recovered from infected cultured samples. The produced micro conidia were single, sometimes two cells, oval to ellipsoid in shape, in diameter 3.75-5 * 8-10 micrometer which formed on single and short phialides. Macroconodia were mostly 3-4 celled, in diameter 3-5 * 18-35 micrometer and formed on spordochia. Chlamidospores were spherical to round shape, mostly single, sometimes in the short chain and formed intercalary and terminal. 10 to 14 days after inoculation the symptoms of the disease were seen as yellowing, plant weakness, and wilt. Based on morphological and pathogenicity test, the isolated fungus identified as *Fusarium oxysporum* f. sp. *gladioli* and confirmed by the Iranian Research Institute of Plant Protection. Infantino and Rumine (1993) reported that the *F. oxysporum* f .sp. *gladioli* infected other members of the Iridiaceae family. Other Fusarium species associated with the corms of gladiolus are *Fusarium solani* (Mart) Sacc., *Fusarium subglutinans* (Woolenweb and Reinking) *Fusarium heterosporum.* Nees ex Fr and *Fusarium sporotichoides* Sherb. (Georgieva and Peikova, 1976). This is the first report of Fusarium wilt of Gladiolus on three different cultivar (White, pink and red) in Jiroft, located in the south part of Kerman, Iran. Few resistant cultivars are available in the world. It found that Australian fair and Monsoer were tolerant to *F. oxysporum.* f .sp. *gladioli.*

Conclusion: based on this research project, the causal agents of gladiolus wilt disease in Jiroft is *Fusarium o*xysporum f. sp. gladioli. Disease incidence was different on three gladiolus cultivars which cultivated in this area. Field observation showed, the white cultivar was more tolerant in comparing to red and pink gladiolus

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cultivars. In gladiolus wilt disease control managements, more emphasis should be focused on disease resistance. The more resistant cultivars should be tested for resistance to several *Fos* isolates.

Keywords: Jiroft, *Fusarium*, Gladiolus and Wilt