

Evaluation Effect of Adjuvant on Mesosulfuron+Iodosulfuron Herbicide Performance on Littleseed Canarygrass Control

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Introduction: Adjuvant application is one of the most important ways to increase herbicide efficacy and decrease environmental damaging effects of herbicides. In general, It has displayed that a very few of the spray droplets retained on the surface of leaf plants and the majority of them bounce off the leaf surface. Therefore, in spraying processes, adjuvant designed to enhance the absorbing, emulsifying, dispersing, spreading, sticking, wetting, or penetrating properties of pesticides. Adjuvant are most often used with herbicides to help a pesticide spread over a leaf surface and penetrate the waxy cuticle of a leaf or to penetrate through the small hairs present on a leaf surface. Surfactants and crop oils are two types of adjuvant that are used for increasing efficacy of herbicides. In many cases, significant increases have been observed in biological activity with the addition of surfactants or crop oils. For example, the performance of specific graminicides and some sulfonylureas is usually increased by the addition of tank-mix oils. It is generally accepted that the benefit of oils is related to their ability to increase the drying period of droplets during their fly time before their impact on the plants, to improve the spreading of the deposit on difficult-to-wet targets (mainly Gramineae), to act as solubilizing agents, and above all to enhance the penetration of herbicides into the plants. Among commercially available adjuvants, emulsified vegetable oils have been shown to increase droplet retention and spreading, and enhance absorption and translocation of active ingredients. It has been reported that efficacy of atrazine, bentazone, phenmedipham and rimsulfuron on various weeds were increased by the addition of rapeseed oils to solution spray.

Materials and Methods: In order to evaluate the effect of adjuvant concentrations on surface tension of aqueous solutions, an experiment was conducted as completely randomized design with 4 replications at 8 levels of adjuvants (0, 0.01, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 (% v/v)). Moreover, the effects of surfactant and vegetable oil on the efficacy of mesosulfuron + iodosulfuron commercial mixture on littleseed canarygrass (*Phalaris minor* Retz.) were investigated under greenhouse conditions in Agriculture Faculty of Ferdowsi University of Mashhad. Greenhouse study was conducted in 6 concentration levels of mesosulfuron-methyl+idosulfuron (0, 5.62, 11.25, 22.5, 33.75, and 45 g ai ha⁻¹). This herbicide was applied alone and with these adjuvants including: (i) citogate (a nonionic surfactant), (ii) castor oil and (iii) rapeseed oil each one of them at two levels 0.1 and 0.2 %, the experiments were arranged in a completely randomize design with a factorial arrangement of treatments with four replications.

Results and Discussion: When adjuvants alone were applied against littleseed canarygrass, none of them had not phytotoxic effect so that fresh weight and dry weight of plants did not decrease significantly as compared to the control. The results of lab experiment showed the lowest and highest surface tension belonged to Citogate and rapeseed oil, respectively. Also the results of greenhouse experiment indicated all adjuvant were able to increase the efficacy of mesosulfuron + iodosulfuron herbicide. Relatively potency (R) of dry weight increased with usage of Citogate surfactant, castor and rapeseed oils to 1.4, 1.35 and 1.13 respectively. The Citogate surfactant leads to the greatest enhancement of herbicide efficacy. The foliar activity of the tested herbicide enhanced with increasing adjuvant concentration from 0.1 to 0.2 (% v/v). According to the accessible information, surfactants (nonionic Citogate) are suitable for reduction surface tension. Previous studies showed that nonionic surfactant was too effective in decreasing surface tension of spray solution. Despite, it has been observed that vegetable-derived oils could not greatly decrease surface tension. For example, castor oil decreased surface tension of water from 73 to 71.5 mN/m. Whereas, nonionic surfactants can lower the surface tension of the spray solution to 33 to 34 mN/m. It seems that drop in surface tension by the citogate surfactant is considered sufficient to decrease the contact angle of spray solution proplets and makes an extension of droplet on leaf plant. Ultimately, spray droplet spread can be very important in obtaining the desired foliar coverage for contact pesticides and appears to play a role in moving the active ingredient to more absorption sites on the plant.

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Conclusion: Based on the results of this study; the following conclusions can be made: (1) the ranking of adjuvants to enhance the tested herbicides efficacy was alike this order: citogate > castor oil> rapeseed oil.

Keywords: Surface tension, Surfactant, Vegetable oil



Antifungal Effect of 27 Medicinal Plant Species Crude Extracts Against Tomato Early Blight Agent *Alternaria solani*

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Introduction: In recent years, synthetic pesticides are known to be the most effective method of the pest and disease control. However, they are not considered as a long-term solution due to the concerns associated with pesticides application such as problems of public health, environmental pollution, reduction in crop quality, toxic effect on non-target organisms and causing resistance in pest and disease agents. Nowadays, using plant secondary compounds has been increased and these compounds are used for plant protect against post and pre-harvest diseases. Therefore, considerable research to search for biocides that are environmentally safe and easily biodegradable have been carried out during last two decades. So, using extracts from plants containing natural antifungal compounds for plant disease control is considered to be one of the desirable methods for plant protection in agriculture. The main goal of this report was studying the antifungal effect of 27 medicinal plant species crude extracts against *Alternaria solani* were investigated.

Material and Methods: Twenty-seven medicinal plant species including *Anethum graveolens*, *Ocimum basilicum*, *Quercus* sp., *Heracleum persicum*, *Cinnamomum* sp., *Caryophyllium aromaticum*, *Mentha pulegium*, *Mentha piperita*, *Lavandula* sp., *Valeriana* sp., *Borago officinalis*, *Urtica dioica*, *Petroselinum* sp., *Salvia officinalis*, *Trigonella foenum-graecum*, *Thymus* sp., *Foeniculum vulgare*, *Pimpinella anisum*, *Allium ampeloprasum*, *Adiantum capillus – veneris*, *Viola odorata*, *Papaver* sp., *Rosa hybrida*, *Malva sylvestris*, *Artemisia dracunculoides*, *Matricaria recutita* were collected. These plants were randomly collected to increase the chance of finding plants with bioactive extracts. Each sample was cleaned, air dried in the shade and ground to a fine powder. The powdered plant materials were extracted using ethanol and water. In the ethanolic extraction, 5 g ground sample was extracted with 100 ml ethanol for 24 h by shaking on an orbital shaker at 300 rpm. Then 30 ml distilled water was added to 70 ml of the ethanolic extract and lipids were removed with 100 ml n-Hexane mixed at 250 rpm for 2 h. Finally, Ethanolic phase was concentrated using a rotary evaporator. In aqueous extraction, 5 g ground sample was extracted with 100 ml distilled, and sterilized water warmed on a magnetic hot plate. The obtained extract was filtered over Whatman No.1.Paper filter and the filtrates were collected and dried in 50 °C oven. The inhibitory effect of these extracts was examined against *Alternaria solani* via paper disc method. In paper disc method, each disc was loaded with 50 mg/paper disc of crude extract. The discs were dried well after loading to remove the solvent. Loaded paper discs were placed on the plate. Plates were incubated at 25°C and the radius zone of inhibition (distance between the center of the paper disc and margin of the inhibited mycelium) was recorded. The experiments were performed in four replicates.

Results and Discussion: Results showed that the most inhibition on *A. solani* belonged to aqueous extract of, *Papaver* sp., *Allium ampeloprasum*, and *Ocimum basilicum* with 10.22 ± 0.22 , 10 ± 0.39 and 9.87 ± 0.17 mm inhibition, respectively. Ethanolic extract of *Foeniculum vulgare* had remarkable inhibitory effect against *A. solani* with 8.33 ± 0.51 mm inhibitory radius zone. Results indicated the presence of antifungal compounds in different plant extracts and that was in agreement with the results reported by authors who tested the plant extracts on different plant pathogens using paper disc method. In this study, preliminary phytochemical screening of the extracts showed the different groups of components such as Alkaloids, terpenoids, flavonoids, sterols, carbohydrates and tannins. These results showed that medicinal plants can be considered as rich sources of plants with antifungal activity. Given the effect of the plant species origin and genetic diversity on chemical composition, studies screening for novel antifungal compounds in plants grown in different parts of the world are needed. Therefore, further screening of other plant species, identifying active fractions or metabolites and in vivo application of active extracts are warranted. Moreover, they could form the basis for further investigation of fractionation for finding active fractions, the effect of origin of growing on the quality and quantity of active compounds, a number of bioactive compounds in different plant parts and finally in vivo application of extracts will be considered.

Conclusion: Medicinal plants are valuable resources of bioactive compounds that can be utilized to control

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phytopathogens through comprehensive studies and complementary experiments.

Keywords: Extract, Paper disc, Solvent

Antagonistic Activities of *Streptomyces* against Root Knot Nematode of Kiwifruit

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Introduction: Iran is among the world leading kiwifruit producers with 2.816 ha cultivated and 31.567 tones production. Plant parasitic nematodes cause damages to a variety of agricultural crops throughout the world. Interest in biological control of nematodes has increased because of the need for alternative methods to fumigant and non-fumigant nematicides and overall improvement of IPM programs. Bacterial species with nematocidal activity have also been used with some success for controlling root-knot diseases, including *Streptomyces* spp., *Serratia* spp., *Bacillus* spp. and *Pseudomonas* spp. The goal of the current study was to isolate, identify and investigate the potential of local *Streptomyces* bacteria for controlling and reducing root-knot nematode population in the north of Iran.

Materials and Methods: In order to evaluate the effect of antagonistic bacteria on control of root-knot nematode of Kiwifruit, 100 isolates of bacteria were collected from Kiwifruit rhizosphere in the north of Iran and screened for pigmented microorganisms especially *Streptomyces* by applying standard serial dilution plate technique, using starch casein nitrate agar and glycerol asparagine agar. Morphological characterizations were achieved by the microscopic method. The microscopic characterization was done by cover slip culture method. The mycelium structure, color and arrangement of conidiospore and arthrospore on the mycelium were observed through the oil immersion (100X). The observed structure was compared with Bergey's Manual of Determinative Bacteriology and the organism was identified. Various biochemical tests performed for the identification of the potent isolates are as follows: casein hydrolysis, starch hydrolysis, urea hydrolysis, esculin hydrolysis, acid production from sugar, NaCl resistance, temperature tolerance. Soil samples (100g) were collected, and then processed for nematode egg and larvae extraction Hussey method. The suspension was pipetted into counting Petri plate and examined under a stereomicroscope. Nematode larvae were identified to generic level and were counted. Evaluation of Actinomycetes isolates against root-knot nematodes in vitro performed according to Sun *et al.* Seedlings (Six-month-old) of kiwifruit (*Actinidia deliciosa*) were sown in 30 cm³ pots containing autoclaved sandy loam soil (1:1). Pots were divided into three groups by three replicates. Bioagents were individually incorporated into the soil at a dose rate of 10 cm³ (Heavy cell suspension of all isolates was prepared at rate 10⁵ spores ml⁻¹) were added to the soil. After seven days, (when bacterial cells reach its maximum growth peak) plants were inoculated with 2000 freshly hatched second stage juveniles (J2) of *Meloidogyne* spp. Pots were fertilized with recommended dose and kept at 25°C ± 3°C in complete randomized design. After two months plants were uprooted then galls and egg masses were counted and their indices were recorded according to Sharma *et al.* Fresh weight of roots was also registered. Treatments means were compared by the Duncan Multiple Range Test at 0.05 level of probability. The growth responses of kiwifruit (roots weight and number of galls and egg mass) were also recorded. Statistical analyses were achieved using SAS.

Results and Discussion: Among 25 isolates identified as *Streptomyces* genus, 9 Actinomycetes isolates showed the antagonistic potential in vitro and reduced the rate of egg hatching in seven days and larval mortality in four days. *Streptomyces* sp3, *Streptomyces* sp4., *Streptomyces* sp5., *Streptomyces* sp9. And *Streptomyces* sp12. were able to reduce egg hatching 16.29%, 19.99%, 27.11%, 20.22% and 18.41% and increased the percentage of larval mortality 45%, 33.3%, 37.53%, 35.01% and 37.50%, respectively. They showed the greatest effect and selected for evaluating in greenhouse condition. In addition, *Streptomyces* sp9. and *Streptomyces* sp4. reduced galls by 65.35% and 64.56% compared with the phenamiphus 57.47% had good performance. Results showed *Streptomyces* can be considered as an alternative for control of root-knot nematode. This is the first report of biocontrol of root-knot nematode in Kiwifruit by *Streptomyces*. The production of most antibiotics is species specific, and these secondary metabolites are important so the *Streptomyces* spp. can compete with other microorganisms that may come in contact, or even within the same genus. Another important process involving the production of antibiotics is the symbiosis between *Streptomyces* and plants, as the antibiotic protects the plant against pathogens and plant exudates allows the development of *Streptomyces*. Almost 80% of the world's antibiotics are known to come from Actinomycetes, mostly from the genus *Streptomyces*.

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Conclusion: The present study clearly indicates that the use of *Streptomyces* sp9. And *Streptomyces* sp4. that significantly enhanced kiwifruit growth and reduced root-knot nematode populations and that it could be proposed for eco-friendly bionematicide use. Strains belonging to genus *Streptomyces* can, therefore, act as biocontrol agent with plant growth promoting ability. Furthermore, their potential metabolic diversity, mycelia growth habit, rapid growth rate, colonization of semi-selective substrates and ability to be genetically manipulated make them well-suited for soil inoculation. Additionally, ability to form desiccation-resistant spores which assists their spread, persistence and formulation make them preferred biocontrol agents. Biological control agents offer one of the best alternatives to reduce the use of pesticides.

Keywords: *Actinidia deliciosa*, Biocontrol, *Meloidogyne*, Rhizobacteria

Identification and Prevalence of *Grapevine fanleaf virus* in Khorasan-Razavi Vineyards

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Introduction Grapevine *fanleaf virus* (GFLV) is one of the devastating viruses of grapevine cause severe crop loss in vineyards. GFLV is a member of the genus *Nepovirus* in the family *Secoviridae*. The GFLV genome consists of two positive-sense single-stranded RNAs. The genome has a poly (A) tail at the 3' terminus and a covalently linked VPg protein at the 5' terminus. Each genomic RNA encodes a polyprotein from which functional proteins are released by proteolytic processing by the virus-specific protease. GFLV isolates differing in the type of leaf symptoms, ranging from fanleaf, yellow mosaic, vein banding and mottle in different grapevine varieties. GFLV is specifically transmitted from grapevine to grapevine by the ectoparasitic nematode species *Xiphinema index*. It is also transmitted by grafting, vegetative propagation or mechanical inoculation onto herbaceous plants. GFLV has restricted natural host range, grapevine is the dominant natural host of GFLV and, however, it has been reported on several weeds in Iran. It is thought that the old Persia, especially the region located between the Caspian Sea and the Black Sea, might be the origin of GFLV. Grapevine wide cultures in Khorasan-Razavi province, northeast of Iran, but little information is available for the incidence of GFLV in this region. In the present work, we are interested in the study of the Prevalence of the *Grapevine fanleaf virus* in Khorasan-Razavi province.

Materials and Methods: To identify the distribution of GFLV in Khorasan-Razavi, 280 leaf samples were randomly collected during the growing season of 2011-2012. GFLV was detected in leaf samples by enzyme-linked immunosorbent assay (ELISA) using specific antibodies raised against Iranian isolate of the virus (Zakiaghl and Izadpanah 2003). *Chenopodium quinoa* plants were used as systemic herbaceous host for the propagation of GFLV. The carborundum dusted seedlings were inoculated by extracts of ELISA positive samples in phosphate buffer. Total plant RNA were extracted from fresh leaves using silicon dioxide (Boom et al. 1990). The cDNA strand was synthesized using Moloney murine leukemia virus (MMuLV) reverse transcriptase. The partial length of coat protein gene of GFLV isolates was further amplified using DetF (CGGCAGACTGGCAAGCTGT) and DetR (GGTCCAGITTAATTGCCATCCA) specific primer pair by RT-PCR in leaf samples that were positive in DAS-ELISA. PCR products were run on 1% agarose gel containing 0.5 µg/ml DNA Green Viewer, and visualized under UV irradiation. The PCR products were purified using the Qiaquick PCR purification kit (Qiagen), then were sequenced bidirectionally using DetF/DetR specific primer pair. Consensus sequences were verified using the BLAST program in NCBI database. Multiple sequence alignments of the nucleotide sequences of the coat protein gene Phylogenetic analysis were carried out by the Neighbour-joining method implemented in MEGA v.5

Results and Discussion: 187 out of 280 samples were found to be infected with GFLV in indirect ELISA. Based on ELISA results, GFLV infection rate in Khorasan-Razavi ranging from 32% to 63%. Kashmar had the most infected vineyards with the prevalence of the virus in 90% of the samples. GFLV induced yellow mosaic and vein banding in infected leaves. Shorten internode, the zigzag growth of stem and double nude were observed in infected grapevines, however, most of the GFLV infected vines were symptomless. Mechanical inoculation with sap extracts from the GFLV positive leaf samples, induced chlorotic local lesions followed by vein clearing in systemic leaves of *Chenopodium quinoa* two weeks post inoculation. RT-PCR using specific primers amplify 1000 bp fragment corresponding to the GFLV coat protein gene. No fragment was observed in healthy control. Pairwise comparisons of the coat protein gene of four Iranian isolates showed 89%–97% nucleotide sequence identity and 90%–92% identity at the amino acid level with those of previously published GFLV isolates. The phylogenetic tree based on the coat protein gene of the four Iranian isolates and 15 other isolates, with 1000 bootstrap replicates, revealed that all the GFLV isolates were placed in two main clusters, the Iranian isolates being grouped in one distinct cluster and the other GFLV isolates in the other cluster. The Iranian

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cluster was sub-divided into two sub-clades, which could correspond to two distinct evolutionary lineages reflect their geographical separation.

Conclusion: *Grapevine fanleaf virus* is the major causal agent of the grapevine degeneration disease. It has been proposed that the origin of GFLV might be the old Persia, especially the region located between the Caspian Sea and the Black Sea. Evidence supporting this theory includes the high levels of divergence and distinct phylogenetic position of Iranian isolates. The sequencing data showed that geographical separation is an important determinant factor in the phylogenetic divergence of GFLV isolates.

Keywords: *Grapevine fanleaf virus*, Prevalence, Khorasan-Razavi, Iran



Investigating The Effect of Foramsulfuron and Rimsulfuron Residues in Soil on Growth, Nodulation and Nitrogen Fixation of Chickpea (*Cicer arietinum* L.)

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Introduction: Sulfonylurea (Su) is one of the important groups of herbicides which control weeds through inhibition of the acetolactase synthase enzyme, a key enzyme in the synthesis of amino acids. Their main character by which can be distinguished from other groups are their low cost, effectiveness against wide spectrum of weeds in cereal crops (mostly broad leaved plants), operator safety, and activity at low concentrations. However, many of the crops and vegetables which are grown in rotation with cereals are very sensitive to Su residues. Among crops, legumes are very sensitive at low levels (as low as 0.5 ppm). Therefore much less than 5% of recommended application rates for controlling weeds, will be applied cause not to damage other crops. Root growth is particularly sensitive to Su residues. Since the studies have not been conducted in the country in connection with possible effects of soil residue of foramsulfuron and rimsulfuron herbicides on growth, nodulation and biological fixation in chickpea (*Cicer arietinum* L.), The objective of this research was to study the effect of soil residue of mentioned herbicides in soil on chickpea, growth, nodulation and nitrogen fixation in control conditions.

Materials and Methods: A greenhouse experiment was conducted at the Research Greenhouse of Ferdowsi University of Mashhad in 2012. Experimental design was completely randomized design in a factorial arrangement with three replications. Treatments included chickpea genotypes (Hashem, Ilc482, Kaka and Kermanshah), herbicides (foramsulfuron and rimsulfuron) and herbicide residues in soil (0, 1, 2.5, 5, 10, 15, 20, and 30% of the recommended dose of application). At the beginning of reproductive stage (48 days after planting), plants shoot, root and nodule biomass, nodule number and plants total nitrogen amount were measured. For statistical analysis, data were changed to percentage of control. Data were subjected to ANOVA using SAS 9.1 software and treatment means were compared using Fisher's protected LSD at the 0.05 level of significance.

Non-linear regression analysis was employed using 3 (equation 1) and 4 (equation 2) logarithmic logistic dose-response model described by

$$\begin{aligned} f(x(b, c, d, e)) &= c + (d - c) / (1 + \exp\{b(\log(x) - \log(e))\}) \\ f(x(b, d, e)) &= c + d / (1 + \exp\{b(\log(x) - \log(e))\}) \\ f(x(b, c, d, e)) &= c + (d - c) / (1 + \exp\{b(\log(x) - \log(e))\}) \end{aligned} \quad \text{(Equation 1)}$$

where Y is the response (dry weight), c is the lower limit, d is the upper limit, b is the curve slope, e denotes the dose required to give a response halfway between the upper and lower limits (ED₅₀ (Effective dose required for 50% inhibition)); and x is the herbicide dose. The analysis of dose-response curves were determined by R, utilizing the *drc* package.

Results and Discussion: Results showed that residues of foramsulfuron and rimsulfuron herbicides in soil affected all traits of chickpea genotypes significantly. All chickpea genotypes trails decreased significantly with increasing of residue concentrations of foramsulfuron in soil. However, shoot biomass of Ilc482 and Kaka genotypes and nodulation of all genotypes except of Kaka were simulated at the lowest level of residues of foramsulfuron in soil. Kermanshahi genotype produced the lowest shoot and root biomass in rimsulfuron soil residue levels. Rimsulfuron decreased nodule number and biomass of Ilc482 and Kaka genotypes. Among the studied genotypes; total nitrogen of Kaka and Kermanshah genotypes negatively affect more than the other genotypes due to foramsulfuron residues in soil. Rimsulfuron soil residues levels also decreased nitrogen in all chickpea genotypes. According to ED₅₀ (Effective dose required for 50% inhibition) for shoot biomass, Kaka was the lowest and Hashem was the highest tolerant genotype. Kermanshah and Ilc482 were the lowest and the highest tolerant genotypes for root biomass production respectively. Kaka was the lowest and Ilc482 was the

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highest tolerant genotypes in nodulation, were identified to residues of foramsulfuron in soil.

Conclusion: In general, the results of this study showed that herbicide residues of foramsulfuron could be caused to high susceptibility in chickpea. Therefore, limitation of crop rotation is one of the most important problems for using foramsulfuron in crops before the peas. Since the residues of rimsulfuron were investigated in this study, had very low negative effect or even had estimulative effect on growth characteristics of chickpea genotypes, it would be introduced as selective herbicide in chickpea field.

Keywords: Genotype, Hashem, Ilc482, Kermanshahi and Kaka

The effect of species, planting date, and management of cover crops on weed community in hybrid sunflower (*Helianthus annuus*)

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Introduction: Studies showed that if mixed populations of annual weeds grow with the sunflower, for every 10% increase in weed biomass, seed yield would decrease by 13% (Van Gessel & Renner, 2000). In addition to control weeds using herbicides multi-stage spraying is required. In organic farming systems mulch is used to control weeds, protection, fertility and improve soil quality (Glab & Kulig, 2008; Kuchaki et al., 2001). Surface mulches from cover crops suppress weed growth by reducing light levels at the soil surface, thereby slowing photosynthesis. In return, these conditions reduce seed germination and act as a physical barrier to seedling emergence and growth (Teasdale et al., 2007).

Materials and Methods: The experiment was carried out in Ardabil Agricultural Research Station, as a factorial experiment based on randomized complete block design with three replications during 1390-1391. The first factor was considered four types of cover crops including winter rye (*Secale cereal*), spring barley (*Hordeum vulgare*), winter wheat (*Triticum aestivum*) and control (no cover crop, no weeding). The second factor was mulch management at two levels (living mulch and dead mulch) and the third factor was two planting dates for cover crops (synchronous with sunflower planting and 45 days after sunflower planting). Sunflower seeding performed manually on 23 May on the ridges with 50 cm row distance and spacing between plants was 25 cm in depth of 5 cm. Cover crops seeds, rye, barley and wheat, were planted between rows of sunflower. Due to the low density of weeds in study field, complete weeding and sampling of weeds in one session was performed (60 days after planting date sunflower). Statistical analysis of data performed using SAS software and mean comparison performed using Duncan's test with probability level of 5% and 1%. Diagrams drawn using Excel (Version 8.2).

Results and Discussion:

Density and dry weight of Field bindweed (*Convolvulus arvensis* L.): Results of the study indicated that the interaction between cover crop and planting date on density and dry weight of bindweed was significant at probability level of 5% and 1%, respectively. The best condition of reduced density of bindweed was related to the time of using rye cover crop where reduced Field bindweed density to 64% compared to control (without cover crop, no weeding). Date of simultaneous planting of cover crop, reduced bindweed density from mean 15/7% plant.m⁻² to 11/62 plant.m⁻², compared to 45 days after sunflower planting. Hasannejad and Alizadeh, (2005) reported that rye significantly controlled the weeds of redroot pigweed, common lambsquarters, knotgrass, russian thistle and field bindweed compared to controls with no cover crop. All three cover crops had significant effect on reducing the dry weight of bindweed. Cover crops showed the potential to reduce density and total dry weight of weeds compared to no control weeds and increase the plant yield. In a study, the density and dry weight of field bindweed in treated living rye and dead rye reduced 100% and 85% respectively (Samadani & Montazeri, 2009).

Density and dry weight of Pale bugloss (*Anchusa italica* Retz): The interaction (cover crop × planting date) on pale bugloss density and dry weight was meaningful in probability level of 1%. All three cover crops with simultaneous planting date showed lowest density of pale bugloss. Rye with proper ground cover in the beginning of season, and due to the long-term preservation of residues in the ground level, inhibited germination and growth of weeds for longer times. Results of the studies by (Abdollahyan Noghaby et al., 2011) on sugar beet showed that the effects of planting cover crops of triticale, wheat, rye and barley, between rows where sugar beet planted, to control weeds population would be the same as when herbicide used to control these weeds.

Density and dry weight of Russian thistle (*Salsola kali* L.): Results indicated that the interaction effect (cover crop × planting date) on density and dry weight of russian thistle was meaningful at probability levels of 1% and 5% respectively. The best result on the reduced density and dry weight of russian thistle observed when rye cover crop was used. Elmore, (1980) in a study observed that rye stubble, has more potential in reducing the biomass of wide spectrum of weeds, particularly the annuals.

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Density and dry weight of Common reed (*Phragmites australis* L.): Density of common reed on main effect of cover crop and its dry weight on main effect of cover crop and planting date showed meaningful difference with probability level of 1%. Cover crop of rye, due to the increased biomass, initial growth vigor, high tillering and in fact because of high allelopathy showed better performance in reducing density of perennial weeds such as common reed when planted simultaneously with sunflower, compared to wheat and barley. Samedani et al., (2005) reported that rye and wheat can better control the weeds due to high biomass.

Dry yield of sunflower seed: Regarding the results of data analysis, the yield of sunflower seed influenced by cover crop treatments ($P \leq 0.05$). Sunflower seed yield in treatments of rye and wheat cover crops with control 1 treatment (no cover crop, complete weeding) showed no meaningful difference. Among cover crops, highest yield of seed related to wheat with $3916/7 \text{ kg.ha}^{-1}$. Cover crop of barley showed poor yield compared to rye and wheat that was likely due to lower growth of barley and the lack of producing sufficient biomass and proper control of weeds. Cover crops can have positive or negative effects on grain crop yields, depending on environment, cover crop species and management (Miguez & Bollero, 2005).

Conclusion: Results showed that cover crops, particularly rye, are very effective in reducing the density and dry weight of weeds so that the application of cover crops even resulted in increased yield of sunflower seed. Therefore, use of cover crops between planting rows of crops can be a good option to replace herbicides and conventional tillage and as a new and proper approach for sustainable management of weeds.

Keywords: Dry weight, Rye, Seed yield, Weeds density

Study on the Chitinase Changes in Interaction of Arbuscular Mycorrhizal Fungus (*Glomus mosseae*) and Root-Knot Nematode (*Meloidogyne javanica*) on Tomato

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Introduction: Root knot nematode (*Meloidogyne javanica*) is one of the most important pathogens for different crops such as tomato. There are various methods to control, but none of them are considered as definite and efficient. One alternative that is considered in recent years in the management of root-knot nematode is using of different micro-organisms that are effective against this nematode and decreasing its population. Several studies have indicated the positive role of mycorrhizal fungi in alleviating plant disease causing agents in different ways. One method is to involve the mechanisms in the control of plant pathogens by arbuscular mycorrhizal fungi which is promoting of chitinase activity in mycorrhizal plants. In some studies alleviating nematode losses with mycorrhizal fungi inoculation have been related to host plant growth promotion resulted from mineral nutrients absorption especially phosphorous as well as increasing photosynthesis rate. It is hypothesized that these fungi could be able to stimulate host plant defense response led to decreasing pathogenicity. Study on mechanisms involving in root-knot nematode control by the mycorrhizal fungi can be used for these fungi application in nematode management. The aim of this research is to investigate the chitinase activity changes rate in tomato inoculated host plant by mycorrhizal fungus as well as its role in the control of root-knot nematode. This method has been popular due to environmental pollution resulted from pesticides application. Several studies have indicated the positive role of mycorrhizal fungi in alleviating of plant disease causing agents in different ways. One of these involved mechanisms in the control of plant pathogens by arbuscular mycorrhizal fungi is promoting of chitinase activity in mycorrhizal plants.

Materials and Methods: Mycorrhizal fungus *Glomus mosseae* as well as *Meloidogyne javanica* nematode used in this research to study on chitinase activity changes in the interaction between mycorrhizal fungus and root-knot nematode in host tomato plant. Chitinase activity rate compared among control plants (non-mycorrhized) and mycorrhizal plants in four weeks (weekly). Next, the tomato was inoculated with mycorrhizal fungus and nematode separately, and in combination with each other. Non-inoculated plants with fungus and nematode (control) were carried out in greenhouse condition in an entirely randomized design with three replicates and enzyme activity in host plant roots were also measured during four steps every 48 hours followed by nematode inoculation. Chitinase activity rate was calculated by colorimetric and released N-acetyl glucosamine.

Results and Discussion: Results of chitinase activity rate in tomato roots showed a significant difference in enzyme activity among inoculated and non-inoculated (control) host plants at different times. The mean comparison of two treatments in all of four steps also showed the significant difference among inoculated and control host plants. In other words, the enzyme activity rate was more in mycorrhizal plants rather than control plants in all the measuring times.

Results of combined analysis of variance, as well as data mean comparison from chitinase activity showed the significant differences among treatments. However, differences were not significant among different times after inoculation. So, the enzyme activity rate did not show a considerable difference between control, nematode alone and mycorrhizal fungus treatments at different times. Furthermore there were no significant differences between control and nematode alone treatments across all the measuring times. In other words, root knot nematode had no significant effect on chitinase activity increasing in tomato roots. In mycorrhizal host plants inoculated with nematode, enzyme activity average was at highest amounts four days after inoculation with nematode and had a significant difference with other measured times.

Conclusion: Results of this study showed that inoculation of arbuscular mycorrhizal fungus in tomato roots leads to increasing chitinase activity rate. Investigation of chitinase activity in tomato roots inoculated with mycorrhizal fungus showed the positive effect on treated plants rather than non-inoculated ones. In this study

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inoculation only with nematode also could not induce the increasing of chitinase activity but in combination with mycorrhizal fungus could promote enzyme activity considerably in initial steps of nematode invasion. This study as well as other similar investigations showed that the induction of chitinase activity by mycorrhizal fungus led to the defense of host plant against nematode invasion and the decrease of the damages.

Keywords: Chitinase enzyme, *Glomus mosseae*, *Meloidogyne javanica*, Tomato

Effects of Aqueous and Powder Formulations of N’N-naphthaloylhydroxyamine on Feeding and Mortality of Laboratory Groups of *Microcerotermes diversus* Silvestri (Isoptera: Termitidae)

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Introduction: *Microcerotermes diversus* Silvestri is considered the most economically destructive pest of wood products in Khuzestan, Iran. This species lives in subterranean nests and gains access to buildings and vegetation by constructing underground galleries. Therefore, control or management of this termite is of serious public concern. Generally, design and implementation of effective management methods for subterranean termites have faced some limitations due to their cryptic life habits. Millions of dollars have been spent for controlling termites worldwide, and 80% of these expenses relate to structural damage repair and costs expended for termite management. In recent years, the use of new non-repellent and slow-acting termiticides has become an important approach for subterranean termite control and has been gaining popularity. In recent years, an extensive survey on the foraging behavior of the most damaging subterranean termites of Khuzestan province (Iran) has been undertaken with a view to the development of appropriate strategies for control of *M. diversus* as an extremely destructive structural wood pest, was considered to be the major species with a wide distribution throughout Iran. This species tends to form secondary nests (with reproductive and brood). When the water table is high, secondary nests are usually built in above-ground sites such as tree trunks (adjacent to houses) and wall voids. Termite management has primarily focused on the use of chemical methods such as insecticide applications to soil around and beneath wooden structures. Soil treatments with organophosphates such as Chlorpyrifos (Dursban) did not persist in the environment for long time and proved ineffective against aerial colonies. Therefore, it is important to evaluate new compounds that have potential application for termite management. The main purpose of this study was to evaluate possible delayed toxicity effects of N’N naphthaloylhydroxyamine (NHA; A. Johnson Metthey Company, USA) on feeding and mortality on *M. diversus*.

Materials and Methods: Termites (*M. diversus*), which were collected from infested wooden blocks previously buried in soil, transferred to the laboratory and placed in plastic boxes in a dark incubator at $28\pm 2^{\circ}\text{C}$ and $90\pm 5\%$ relative humidity. Only active and healthy termites were used for tests. Feeding inhibition and mortality of workers treated with NHA were evaluated using both ‘choice’ and ‘no-choice’ test. In no-choice tests, termites were provided by only NHA-treated soil, or NHA-treated filter paper alone. Therefore, they could not escape the treated soil, or had only treated filter paper for food. In choice tests, termites were provided both treated and non-treated soil or filter paper, and could escape or avoid the treated substrates concentration. Technical NHA (99% wt:wt) was used. Two formulations, which were powdery and aqueous, were evaluated to investigate their efficacy for management of *M. diversus*. Termites were exposed to NHA at concentrations which included ranging from 0.5 to 4.0% (wt:wt) in aqueous formulation, and from 0.5 to 8.0% (wt:wt) in powdered formulation. Termites were exposed to sterilized soil or filter paper treated with mentioned concentrations of NHA. Termites’ behaviors monitored daily and their mortality recorded. Mortality data were corrected using Abbott’s formula. ANOVA was performed in conjunction with a least significant difference (LSD) test, and Duncan’s Multiple Range Test at the 5% level using SAS software (ver. 9.1). A probit analysis was also conducted. The related diagrams were drawn using Microsoft Excel 2007. Noted that the tests were carried out with regard to the standards E1-06 (2006) of the USA and prEN117(2003) of the Europe.

Results and Discussion: NHA-treated-soil indicated that over 21 days, mortality rate gradually increased with increasing concentration levels. Trials with filter paper impregnated with the aqueous formulation showed that within 14 days mortality also gradually increased with increasing concentration. Probit analysis indicated that the level of mortality was dependent on concentration. There was an inverse relationship between time to 90% mortality (LT_{90}) and concentration. LC_{50} and LC_{90} values when for compared between different tests showed that in no-choice tests where termites were forced to contact NHA, these values are lower compared with the choice tests. This indicates greater lethality in no-choice tests. Overall, at doses of 0.5, 1.0, 2.0, and

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4.0% aqueous formulation (treated-filter paper), within 7 to 14-days mortality was 63%. Powdered formulation at doses of 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0% (treated-soil) caused 67% mortality within 14 to 21 days, indicating that NHA is a slow-acting toxin once ingested.

Conclusion: With regard to our results, we suggest NHA as an effective non-repellent termiticide for controlling *M. diversus*, and perhaps other subterranean termites in soil. We suggest that application of NHA may be an effective strategy for the control of *M. diversus*. Further, field testing is warranted to determine NHA efficacy in natural conditions.

Keywords: *Microcerotermes diversus*, N'N naphthaloylhydroxyamine (NHA), feeding inhibition, termiticide

The Influence of Different Amounts of Nitrogen and Weed Interference on Yield and Yield Components of Corn under Two Irrigation Systems

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Introduction: Corn (*Zea mays* L.) is one of the most important crops in the world that need high water requirement during its developmental stages. Improvement of water management system increases crop production all over the world, especially in arid and semiarid climates. For instance, drip irrigation system can be used to provide accurate and adequately nitrogenous fertilizers while conventional systems such as flood irrigation has lower efficiency in the use of nitrogen fertilizers. An improved understanding of the effects of nitrogen (N) on crop-weed interactions is needed for development of integrated weed management systems where reasonable application of N fertilizers is considered too. Moreover, maize competition with weeds is another important factor that affects its yield. So this study was conducted to evaluate the effect of nitrogen on yield and yield components of corn and weed interference under two systems of flood and drip irrigation.

Materials and Methods: In order to evaluate the effect of irrigation methods and nitrogen under weed-infest condition on yield and yield components of maize, a field experiment was conducted in split-split plot design based on randomized complete blocks during 2012 growing season at the Collage of Agriculture, Shiraz University. Factors were irrigation type as main plots (flooding and drip), nitrogen levels as sub plots (0, 75 and 150 kg ha⁻¹) and weed interference as sub-sub plots (weed infest and weed free). Drip irrigation system was carried out by using strips with 20 cm output, 0.175 mm thickness and 16.5 mm internal diameter. Urea 46% was used to supply nitrogen. A control (weed free) was used in this experiment. Statistical analysis of the data was performed by using SAS 9.1 software and comparing of the means was done by the least significant difference test (LSD) at 5% level.

Result and Discussion: The results showed that under weed interference, grain yield (51.0%) and yield components (number of rows and kernels per ear, ear length and diameter and seed weight) decreased significantly. In the former studies weed competition had been expressed a major limitation for corn production. It seems that weeds caused corn yield losses indirectly through their influence on the resources required for crop growth and competition for light and belowground resources. It was also shown that weeds are high nitrogen consumers cause to reduce the amount of available nitrogen for crops growth. Therefore when corn and weeds emerge simultaneously in a mixture, weeds competitiveness increases with increasing nitrogen supply. However, nitrogen addition has a positive effect on both corn and weeds growth, but weeds tends to respond more to nitrogen addition than corn. In this study we observed similar results and increasing of nitrogen application from 75 to 150 kg ha⁻¹ which increased grain yield by 44.4% and ear length by 4.9 %, but under weedy condition decreased ear length by 25.8%. Nitrogen application caused a different response in each irrigation system, so that the application of 75 and 150 kg ha⁻¹ nitrogen under drip irrigation system reduced weed biomass by 62.6 % and 64.4 % compared to flooding system respectively. Applying drip irrigation system also reduced weed density (56.8%) and biomass (54.3%) and increased corn grain yield compared to flooding irrigation system. Under weed free condition and using of drip irrigation system number of grain per ear increased by 50.1% significantly. In drip and flooding irrigation systems under weed free condition compared to weedy plots, number of grain per ear increased by 17.0% and 7.9% respectively whilst drip irrigation system compared to flooding irrigation system increased ear diameter by 50.5%. However hundred grain weights were affected by all treatments, under weed free condition compared to weedy condition, in drip irrigation system hundred grain weights increased by 18.2 %, but in flooding irrigation system no significant differences was observed. Performance comparison of grain yield under irrigation systems revealed that drip irrigation increased grain yield by 62.0 %.

Conclusion: The results showed that the effectiveness of nitrogen was much higher under drip irrigation compared to flooding irrigation. It can be because of that drip irrigation system directly provide water for plants and prevent from water availability for weeds. Therefore, appropriate irrigation system as an agronomical practice affects the growth and weed competition ability in corn

Keywords: Competition, Irrigation method, Weed biomass, Weed density

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Effect of Time and Burial Depth on Breaking Seed dormancy and Germination of Weed Seeds

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Introduction: Weeds limit crop growth, development and yield through competing. Seed bank of weeds in field is one of the sources which can affect weed management and their control methods. Environmental conditions during seed maturation and following dispersal interact to influence the germination phenology of many species. Disturbance plays a key role in the maintenance of habitat for many plant species, particularly referrals, for example, fire ephemerals, desert annuals, and arable weeds. Seed germination and emergence depend on endogenous and exogenous factors. Viable seeds are dormant when all environmental conditions are appropriate for germination but seeds fail to germinate. Thus, dormancy plays an important ecological role in preventing seed germination, being a major contributor to seed persistence of some species in soil. Buried seeds of annual weeds are certainly subjected to different soil moisture conditions during their dormancy release season (winter) according to the annual rainfall pattern and burial depth. Shallow buried seeds are exposed to soil moisture fluctuations that could affect their dormancy status. Laboratory studies showed that desiccation and subsequent re-hydration of seeds could stimulate germination and modify seed light requirements. Seeds buried in deeper layers of the soil would not be exposed to such fluctuations in soil moisture, but would be exposed to different soil moisture environments depending on weather and soil characteristics. The effects of interactions between temperature, and soil or seed moisture, on seed dormancy changes have been reported for several species. Therefore, the objectives of this study were to determine the effect of time and burial depth treatments on seed germination and seedling emergence of *Aegilops cylindrica*, *Agropyron repens*, *Avena fatua*, *Bromus dantoniae*, *Cynodon dactylon*, *Cyprus rotundus*, *Setaria viridis*, *Anthriscus sylvestris*, *Centurea cyanus*.

Materials and Methods: In order to evaluate the effects of time and burial depth on breaking seed dormancy and percentage of germination of 27 dominant weed in Shahrekord region, an experiment was conducted at the research field at Shahrekord University in 2013. The experimental design set as factorial based on completely randomized design with three replications. Treatments consisted of three burial time levels (1, 2 and 3 months) and 4 burial depth (0, 5, 15 and 25 cm from surface soil layer) after 1, 2 and 3 months of burial.

Results and Discussion: Results showed that the time and the depth of burial treatments had significant effect on breaking seed dormancy and germination percentage. Seeds retrieved from the soil surface showed highest dormancy percentage and breaking dormancy with increasing the depth and time of seed burial. The results showed that the effects of three burial times, burial depth and interaction of burial time and burial depth had significant effects on dormancy breaking and germination of weed seeds. The bitter herbs of expression and parsnip, the highest percentage of seed dormancy breaking. Germination of *Geobelia alopecuoides* and *Anthriscus sylvestris* was observed from seed burial depth of 15 cm. However, the germination percentage, between two and three seed burial months, did not show any significant difference. In this study, *Convolvulus arvensis*, *Rumex acetisella* and *Avena fatua* in the highest depth (25 cm) had the maximum seed germination. Tillage would bury weed seeds and may help to preserve some seeds, because the seeds on the soil surface or near it, are prone to hunting or decay that eventually, their number is reduced in the soil seed bank. Weed seeds of *Centurea cyanus*, *Geobelia alopecuoides*, *Turgeniala tifolia*, *Tragopogon collinus*, *Bromus dantoniae* and *Anthriscus sylvestris* had more germination percentage with increasing depth to 15 cm, but beyond this depth due to the negative impacts of increased depth, seed germination declined sharply. In fact, the results of this study showed that, based on vertical distribution of seeds, at depth of more than 15 cm, weeds seed germination would be prevented. Seeds for germination, requires special conditions of temperature and moisture, and for this seeds, probably favorable conditions for germination were obtained between soil surface and 15 cm of burial depth. Overall, after two months of burial depth of 15 cm, weed seed showed highest breaking dormancy and germination percentage.

Keywords: Seed depth, Seed burial time, Seed germination, Weed control

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Effect of Soybean and Wheat as Cover Crops on Corn Yield and Weed Control using Different Fertilizer Sources

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Introduction: According to the importance of corn in supplying the human food directly and indirectly, it is one of the most important plants among crops. One of the major problems in corn production systems, is competition with weeds that reduce corn yield significantly. Weeds not only reduce crop yields but also decrease the commercial quality and the feeding palatability of main crops. They enhance the soil seed bank of weeds, which may cause continuous weed infestation of field crops as well. Herbicide application is a reliable and highly effective method for weed control. However, demand for safe food products that have been produced with a minimum application of chemical inputs is increasing. Therefore, farmers interested in weed management have to rely on other control approaches. An alternative weed control method is the use of cover crops, which can suppress the growth of weeds by preventing them from light and by producing allelopathic compounds. Cover crops successfully have been integrated into conservational agriculture systems in many areas of the world. Legumes are used as cover crop because of their rapid growth, in addition their potential to provide further nitrogen, along with high ability to compete with weeds.

Materials and Methods: In order to study the effect of cover crops (soybean and wheat) and different fertilizers sources on yield of corn and weed control, a field experiment was conducted in randomized complete block design with three replications in 2012. Treatments included two cover crop (wheat and soybean) and three fertilizer (no fertilizer, chemical fertilizer and compost). Fertilizer treatments was used according to soil analysis and requirement of corn (as a main plant). Weed-infestation and weed-free plots were used as controls. Study cultivars of corn, wheat and soybean were NS-640, Milan and Sari, respectively. Planting of corn was in June and cover crop was planted with corn simultaneously and between corn rows. The dominant weed species were velvetleaf (*Abutilon theophrasti* Medic.), johnson grass (*Sorghum halepense* (L.) Pers.), wild melon (*Cucumis melo* var. *agrestis*) and giant foxtail (*Setaria glauca* L.) in the field. In order to determine the dry weight and density of weeds, three-stage sampling was performed from the middle rows of corn. Corn yield was also measured by mechanical harvesting in middle rows and adjusting to 14% moisture. A week before the final harvest, ten plants of corn were selected randomly from the three middle rows of each plot and yield components including the number of rows in corn, number of kernels per row, weight of 1000 grains was measured.

Results and Discussion: The results showed that soybean cover crop reduced weed density compared to control in weed infested plots, but wheat was not successful in suppressing weeds and reducing their density due to poor biomass and dying at the end of growing season. So, the lowest dry matter of velvetleaf, wild melon and other weeds were related to planted corn with soybean and compost and the maximum was related to monoculture of corn with weed infestation. Results of analysis of variance indicated treatments had significant effect on grain yield. The lowest yield of corn ($2733.3 \text{ Kg ha}^{-1}$) was in weed-infestation control while the highest one (12124.0 and $8351.3 \text{ Kg ha}^{-1}$ respectively) was in weed-free control and soybean cover crop plus compost. For both cover crops between fertilizer treatments, compost and chemical fertilizer had more corn biological yield than no fertilizer and differences between this two fertilizer treatments wasn't significant. Reduction of yield under no fertilizer treatments was due to competition for nutrients, light between weed and corn. The maximum and minimum number of rows in corn and number of kernels per row respectively was obtained with monoculture of corn in weed free and weed infestation, while the maximum weight of 1000 grains was observed in soybean with applying compost. Generally, the living mulch as cover crops can reduce competition between weeds and the main crop.

Conclusion: As cover crops reduce weeds growth, and there was no significant difference between chemical and organic fertilizer in corn yield, so we can use these two options for producing optimum yield in sustainable agriculture. Results showed application of soybean as cover crop, especially integrated with organic fertilizers,

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can be an alternative approach for herbicides and are more effective than the others.

Keywords: Chemical Fertilizer, Competition, Compost, Cover Crops

Evaluation the Effect of Weed Management Strategies on Weed Seed Bank and Spinach Yield (*Spinacia oleracea* L.)

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Introduction: Spinach (*Spinacia oleracea* L.) is a quick-maturing, cool-season, hardy vegetable crop that is produced for both fresh markets (field packed in bunches or lightly processed in cellophane bags) and processing markets (frozen products). A fresh-market spinach can be grown in 30 to 50 days, while the same variety grown for freezer processing takes 70 to 120 days. Spinach require weed control from nearly the moment a gardener decides to plant them, extending throughout the growing season. Weeds are often the most serious threat for organic vegetable production. Therefore weed control is especially important at the early growth of them. Two recent trends in agriculture have encouraged to use alternative weed control methods instead of chemical control (Cordill and Grift, 2011). Soil solarization is a nonchemical method to control soilborne pests in which high temperatures produced by capturing the sun radiant energy. The method involves heating soil by covering it with a clear plastic tarp during a hot period of year when soil will receive the most direct sunlight. Another method is use of cover cops. Cover crop help to maintain soil organic matter, improve soil fertility, prevent erosion and assist in nutrient management. They can also contribute to weed management, increase water infiltration, and maintain populations of beneficial fungi. Therefore, this research was conducted to evaluate the effect of different weed management methods on the weed seed bank and growth characteristics and fresh weight of spinach based on principles of sustainable agriculture.

Materials and Methods: A field experiment was performed at the Agricultural Research Station Field of Ferdowsi University of Mashhad in growing season 2012-2013. Seven treatments including solarization using by dark and transparent sheets, three crop residues, sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.) and garlic (*Allium sativum* L.), hand weeding and weed-infest control were arranged based on a randomized complete block design with three replications. Before the start of experiment, soil seed bank was sampled from the soil depth of 0-30 cm. Seed bank samples were conducted at two stages including before land preparation and after harvest times. Weed seed were pulled out from the soil depth of 0-20 cm and recognized by stereomicroscope. Yield of spinach was determined at the end of the growing season. Plant height, leaf area index (LAI), as well as dry and fresh weight were measured in five randomly selected plants. Data were subjected to the analysis of variance (ANOVA) using MSTAT-C. Significant difference among means were identified by Least Significant Difference (LSD) at the 0.05 level of significance.

Results and Discussion: In this experiment, the weed community of seed bank was comprised for 7 species. The results showed that solarization and use of cover crops significantly affected seed number of weeds on the seed bank ($p \leq 0.01$). The minimum and maximum relative density ranges were observed for amaranth (*Amaranthus* sp.) and barnyard grass (*Echinochloa crus-galli* L.) with 5.26-25.00 and 22.00-70.00%, respectively. Although decreasing of identified weed seed number affected by non-chemical treatments compared to control, but the highest rate of decrease (93%) was recorded in solarization with dark polyethylene. The impact of non-chemical management treatments was significant ($p \leq 0.01$) on growth characteristics and yield of spinach. The highest and the lowest fresh weight of spinach were observed for solarization with dark polyethylene (1921.3 g.m^{-2}) and control plot (413.3 g.m^{-2}), respectively. Application of garlic, barley and sunflower residues improved the fresh yield of spinach up to 41, 55 and 63% compared to control, respectively. These improvements were 75% and 78% for solarization with transparent polyethylene and hand weeding, respectively. Dark polyethylene enhanced growth characteristics and yield of spinach due to controlling of weeds, temperature regulating and improving moisture content of soil. Impact of solarization with dark sheets on seed bank, growth characteristics and yield of spinach was higher than transparent sheets. Crop residue application enhanced growth characteristics of spinach due to their positive effects on soil properties. So, based on the results of the present study solarization with dark and transparent sheets is an effective approach for weed control and improving growth characteristics of spinach.

Keywords: Crop residues, Hand weeding, Organic vegetable production, Solarization

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Identification and Prevalence of *Potato Virus M* and *Potato Virus S* in Some Provinces of Iran

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Introduction: *Potato virus M* (PVM) and *Potato virus S* (PVS) are members of the genus *Carlavirus* in the family *Betaflexiviridae*. They have worldwide distribution and potato is a major host of the viruses. They have flexuous filamentous virions with a linear, single-stranded, positive-sense RNA genome of about 8.5 kb. The RNA genome contains a 5'-cap structure, six open reading frames (ORFs), and a poly-A tail at the 3'-terminus. Potato virus M was first isolated from *Solanum tuberosum* in 1923 in the United States by Schulz *et al.* It has since been found in all potato production areas worldwide, alone or in mixed infection with PVS. Despite evolutionary adaptation to broad geographical niches, PVM and PVS have a narrow host range limited to solanaceous species. In naturally infected plants, PVM causes mottling, mosaic, crinkling, leaflet deformation and stunting of shoots. The symptoms of PVM on potato vary from latency to severe and are similar to those caused by some other potato-infecting viruses, including potato virus S (PVS, *Carlavirus*), potato virus X (PVX, *Potexvirus*) and the common strain of potato virus Y (PVY-O, *Potyvirus*). PVS has a severe symptom of mosaic and yellowing on potato plants. Most *carlaviruses*, including PVM and PVS, are naturally transmitted by aphids in a non-persistent manner. PVM and PVS are also transmissible experimentally by mechanical inoculation, however, the common route for the spread of PVM and PVS within the crop is through activities such as seed cutting operations and machinery movements. All of the potato plants grown in a field may become infected, depending on the potato cultivar and environmental conditions.

Materials and Methods: In the present study the prevalence of PVM and PVS were surveyed in potato fields from different fields of major potato producing area in Hamedan, Northern Khorasan, Khorasanrazavi, Esfahan, Kerman provinces of Iran. Specific antibody against PVM and PVS (DSMZ, Germany) were used to detect the viruses in fresh leaf crude extracts by DAS-ELISA. Antigen samples were prepared by grinding 500 milligrams of leaf tissue in 200 mMTris-HCl, pH 8.2, 140 mMNaCl, 2 % polyvinylpyrrolidone 40, and 0.05 % Tween 20. Leaf samples from healthy potatoes were also included as negative controls. DAS-ELISA was performed as described by Clark and Adams, according to the instructions of the antibody supplier. The rate of substrate hydrolysis was measured at 405 nm using a microplate reader. A number of samples were also tested by mechanical inoculation on test plants *Solanum tuberosum*, *Lycopersicum escolentum* and *Nicotiana glutinosa* by using 0.1M sodium phosphate buffer. The presence of infection was confirmed about two weeks later by the symptoms and DAS-ELISA results. Total RNA was extracted from fresh young leaf tissue by Accuzol, total RNA extraction kit (Bioneer). Polymerase Chain Reaction (PCR) was carried out using specific primers, corresponding to a complete coat protein gene of PVM and PVS. Amplified fragments were ligated in pTZ57R/T plasmid and cloned in DH5 α isolate of *Escherichia coli* using InsT/A clone PCR product cloning kit (Fermentas) as manufacturer's protocol. Recombinant plasmids extracted using Prime prep Plasmid DNA isolation Kit (Genetbio) then sequenced bidirectional using pUC-M13 universal primers with an ABI PRISM system (Bioneer company, Korea). Consensus sequences were compared with those deposited in the GenBank using BLAST program from the National Center for Biotechnology Information (NCBI).

Results and Discussion: Results showed that 10.5% and 17.1% of the total 970 samples were infected with the PVM and PVS, respectively. The highest abundance of infection was among Kerman and then Esfahan samples. PVS prevalence as greater than PVM; however, most of the samples had mixed infection. The PVM-inoculated test plants developed mottling and leaf deformation symptoms, whereas mosaic, leaf necrosis and leaf malformation were the characteristic symptoms induced by PVS. Mechanical inoculation of PVS on *Lycopersicum escolentum* resulted no symptoms but in PVM produced mosaic and yellowing. However, a number of symptomless samples were found to be infected by the viruses. The symptoms on test plants and the results of their ELISA confirmed the previous ELISA results. RT-PCR was amplified a 915 and 1118 bp DNA fragment from the ELISA positive samples of PVM and PVS, respectively. However rarely amplification was carried out in ELISA negative samples, indicated DAS-ELISA has low sensitivity to identify PVM infection in the samples. Blast search showed that 915 and 1118 bp fragments amplified by RT-PCR were corresponding to full length sequence of coat protein gene of PVM and PVS, respectively. The coat protein gene of PVM and

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PVS isolates analyzed in this study had 73.3-100 and 70-96 percent nucleotide identity with the corresponding sequences in GenBank, respectively.

Keywords: *Potato virus M*, *Potato virus S*, ELISA, PCR, Identity

The Alfalfa Root Coccid, *Neomargarodes* sp. nr. *festucae* (Archangelskaya) (Hemiptera: Coccoidea: Margarodidae)

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Introduction: Margarodidae, which are the largest adult females of the Coccoidea, are found both above and under ground parts of the host plants. All species typically found above ground have been removed from margarodids (Gaint scale) by Jakubski and includes only the cyst form of margarodids, which is divided to four sub-families, Neomargarodinae, Porphyrophorinae, Eumargarodinae and Margarodinae. Recently, Margarodidae *sensu stricto*, include 10 hypogean genera. Genus *Neomargarodes* is one of several hypogean genera, which consists of 26 recognized species. They are occurring worldwide, with most of the species in central Asia. *Neomargarodes* spp generally occur on roots of a wide variety of plant from annual grasses to perennial shrubs and perhaps even trees. The characters of major taxonomic importance of adult female of *Neomargarodes* genus are fossorial prothoracic legs, prothoracic claw with a prominent heel, 7-8 pairs abdominal spiracles, multilocular disc-pores and long hair-like setae throughout body, thoracic and abdominal spiracles with pores in atrium, perispiracular sensillia, absence of mouthparts, antennal apical segment with flagellate setae and fleshy setae, simple anal tube, genital opening surrounded by a radially folded membranous area. They are living underground on roots or crown of hosts and having a cyst-form second instar nymph. The common name “ground pearls” is derived from pearl-like cyst stage. The *Neomargarodes* genus differs from all other related genera, mainly in having prothoracic claw with a prominent heel. Here, we present the results of a survey on a root species coccid collected from *Medicago sativa* L., a perennial herbaceous legume, with superior forage quality in Iran. Morphological studies on the collected specimens were conducted to determine its species name in order to decide on integrated management of pest and its control by using the results of this study. The objectives of this work are root coccid recognition and morphological description of the adult female.

Materials and Methods: During 2010, in Songhor, Kermanshah, the specimens were collected by cutting the infested roots of alfalfa, *Medicago sativa*, when the second instar were in the mature cyst stage and then the samples were kept in the black plastic carry bag in the normal lab condition, till adult emergence. The material preserved in 75% alcohol and than microscope slides were prepared. The morphological characters are described, illustrated and take photographs based on preparation, by using light microscopy. with mounted digital camera.

Results and Discussion: This species is identified as *Neomargarodes* sp. nr. *festucae*. belongs to ground pearls, family Margarodidae *sensu stricto*. Based on observation, the 1st and 2nd nymphal instar stages in large numbers feed on the root of lucerne, *Medicago sativa*. Morphological study shows the adult female with 6 segmented antennae; thoracic spiracles with 6-8 multilocular disc-pores, narrow biforked spiracular apodeme; 4 spiracular sensilla, 5-8 abdominal spiracles, powerful and fossorial prothoracic legs, short femur, length of tibia+tarsus < femur and claw fused with the tarsus, basal bulbous protrusion of the claw is very extended, genital opening surrounded by a radially folded membranous area. *Neomargarodes* sp. nr. *festucae* is new record for Iran. Addition record, cultivated alfalfa, *Medicago sativa* (Leguminose family) have not been reported as host for *Neomargarodes* spp from the other parts of the world,. It is not possible to recognize this species using a single character for various reasons. Most characters are relatively variable, more than one of the characters is almost always obscured by other structures and presence of characters which are shared by many other species.

Conclusion: The basic structure of the adult female of *Neomargarodes* sp. nr. *festucae* appears very similar to that of *Neomargarodes festucae* apart from host plant *Medicago sativa* (Leguminose family) and a number of unstable morphological character, such as prominent basal heel with a dentical and thoracic spiracular apodeme with a biforked shap. So, based on the unstable futures character and polyphagus *Neomargarodes* species with broad spectrum of host plants, can not say that this species is a new species. Because two species, *N. borchsenii* (Hadzibejli) and *N. setosus* (Borchsenius) on roots of *Festuca* and Compositae in Georgia, have been become synonym with *Neomargarodes festucae* (Archangelskaya). Although, the rest of recorded species of *Neomargarodes* from the Middle East have not recorded off *Medicago* spp., but, other species *N. niger* (Green)

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from China, India and Pakistan have been recorded off Leguminose, such as *Alhagi camellorum* and *Arachis hypogaea* which are close related to *Medicago* spp.. Moreover, *N. niger* (Green) recorded off Gramineae: such as *Cynodon dactilon*, and undetermined perennial grass.

As with *Porphyrophora* species for example *Porphyrophora medicagenis* from USSR and *Porphyrophora* sp from Songhor, Kermanshah were found on roots of cultivated alfalfa, as same as *Neomargarodes* sp. nr. *festucae*. Additionally in Songhor, Kermanshah, Western parts of Iran. *P. tritici* infests wheat and barley and Vahedi (1999) found some effective farming practices on its population and suggest an on time ploughing to reduce *P. tritici* population. Contrary to, irrigation experiment has no negative effect on its population. It is here recommended that the manipulation of ploughing times can be effective for population reduction of *Neomargarodes* sp. nr. *festucae* in the infested field. In the end, considered that all root coccids, for example *Porphyrophora* spp and *Neomargarodes* spp no need chemical control at all.

Keywords: Forage crop, Root coccids, Morphology, Margarodidae and *Neomargarodes*

Molecular Identification of Weed hosts of *Tomato yellow leaf curl virus* in southeast of Kerman Province

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Introduction *Tomato yellow leaf curl virus*, TYLCV belongs to the family Geminiviridae and Begomovirus genus (27). In recent years, extensive damage to tomatoes and cucurbits plants in the south and the southeast of Iran has arrived (23). This virus family have circular, and single-stranded DNA genome and are widespread in tropical and subtropical areas (30). They are infected several plant species with economic importance. *Begomoviruses* are dicot-infecting, whitefly-transmitted viruses with a genome comprised of one or two molecules DNA (5). Up to now, studies have been performed to evaluate the status of distribution, and identification of natural host and assess the genetic diversity, but there is not a comprehensive review about its weed hosts yet.

Materials and Methods In this research, The weeds from margins and inside greenhouses and farms of tomato and cucurbit in severely infected areas including Manoojan, Kahnooj, Faryab, Anbrabad and Jiroft to identify weed hosts of the virus in nature, were collected. Identification of collected samples were conducted by botanical specialists. Total DNAs were extracted from leaves according to the method of Zhang *et al.* (1998) and stored at -20 °C. Identification of infected samples were carried out by PCR using degenerate primer pairs PCRv181/Bc that direct the amplification of 550 bp fragment of mono – and bipartite begomoviruses genome comprising the C-terminal portion of the intergenic region (IR) N-terminal portion of the CP gene. PCR were performed in 25 µl reaction volumes containing 1 µl of template DNA, 0.5 µl of *Taq* DNA polymerase Sinaclon (IRAN), 1.2 µl MgCl₂, 0.5 µl dNTPs, 1 µM of each forward and reverse primers, 4.3 µl of 10× reaction buffer and 15.5 distilled water. The amplification were performed using a peqSTAR 96x Thermal Cycler (Peqlab, Germany). PCR conditions consisted of initial denaturing 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 50s, annealing at 55 °C for 60s, and extension at 72 °C for 1 min followed by 1 cycle at 72 °C for 10 min. Electrophoresis of polymerase chain reaction products on 1% agarose gel was performed and stained with DNA safe stain (Sinaclon-IRAN). The polymerase chain reaction product were sequenced using automatic sequencer AB13730XL (Macrogen, Korea). The resulting sequences were looking similarity and after obtaining a degree of homology, 550 bp fragment of the coat protein gene of four isolates were ordinated by Bio Edit software. Looking for similar sequences were obtained and then achieved the 550 bp fragments of the coat protein gene homology four software isolates Bio edit (21) were ordination. To study the phylogenetic relationship of study strains, the phylogenetic tree was drawn with maximum likelihood way in the MEGA 5 software. Then percentage of similarity at the nucleotide and amino acid sequence with a genetic distance matrix was determined by using the software CLC Main work bench.

Results and Discussion: The results showed that four weeds including *Chrozophora tinctoria*, *Heliotropium annuum*, *Malva neglecta* and *Chenopodium murale* were infected with TYLCV. To compare the TYLCV isolates in infected weeds, 550 bp fragment of the coat protein gene in four different strains of the virus was sequenced. Assessment of the genetic similarity between study isolates and strains in the Gene Bank revealed that study isolates with isolates from Gene Bank have similarity in the range of 93/24-99/98% at the nucleotide level and in the range of 87/42-98/15% at the amino acid level. Sixty-six mutations at the nucleotide level in compared sequences in this study was also found. Drawn Phylogenetic tree was confirmed the results of the genetic distance matrix. The results showed that the virus has a wide host range, and identification of weed hosts to remove the maintenance of virus plays an important role in the epidemiology of the disease. and also it's the management of this disease. This is the first report from *Malva neglecta* and *Chenopodium murale* as weed host of TYLCV in Iran. TYLCV causing major damage to the cultivation of tomatoes. Infection caused by it in the South and South East farms in Iran is usually very widespread. Because of the subtropical climate in these areas that is essential for the activity and proliferation of whitefly vector, this virus can cause a high damage in these areas (34). High genetic variability in begomovirus populations infecting different non-cultivated hosts has been

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reported (28, 35, 38). Conversely, begomovirus populations infecting cultivated hosts seem to have lower variability (6,14,41,43). Wild/non-cultivated plants from different botanical families can sustain a high species diversity of begomoviruses and can play an important epidemiological role serving as alternate/reservoir hosts, preventing local extinctions of the virus when the cultivated host is absent (1, 14). In these cases, whiteflies transmitting begomoviruses between cultivated and non-cultivated hosts contribute to virus evolution and disease epidemics (1, 34). Weeds and plant host of indigenous origin and imported ones can be by a large number of plant viruses including Begomoviruses infected which are as reservoirs host of the virus, the primary focus for infection of crops, and play a pivotal role in the emergence of new viral strains (35). Identification and control of the weeds have an important role in disease management. Relatively high incidence of the virus in the weeds with a high density of whitefly population throughout the year, requires new management guidelines.

Conclusion: The results showed that the virus has a wide host range, and identification of weed hosts to remove the maintenance of virus that play an important role in the epidemiology of the disease, in the management of this disease is important. This is the first report from *Malva neglecta* and *Chenopodium murale* as weed host of TYLCV in Iran.

Keywords: *Begomovirus*, Weed, Whitefly, Polymerase Chain Reaction

Effects of Temperature, Salinity, and Planting Depth on Seed Germination and Emergence of Tall Morningglory (*Ipomoea* spp.)

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Introduction: Tall morning-glory is an annual broadleaf weed and a problem in many crops globally. Tall morning-glory belongs to the family Convolvulaceae. It is a summer climbing vine with distinctive, heart shaped, alternating leaves and large, showy, white to pink to dark purple flowers. It is a very competitive weed in annual crops. This plant was introduced as an invasive plant in soybean fields of Golestan province. Because of the lack of these weed management recommendations, every year large quantities of crops have been lost. Germination is one of the most critical stages in weed establishment. A better understanding of the germination ecology of tall morning-glory would facilitate the development of more effective management strategies for its control.

Materials and Methods: To study the effect of some environmental factors on seed germination of tall morning-glory (*Ipomoea* spp.), two experiments were conducted in Plant Protection Laboratory of Agriculture Jihad Organization of Golestan in 2013. In the first experiment, effect of six planting depths (1, 3, 5, 7, 10 and 13 cm) and two seed sizes (small and large) on emergence of morning glory at 30 °C as factorial block design in four replications were performed. In the second experiment, effect of salinity and temperature on the germination of seeds of morning glory were studied a completely randomized design with four replication. Temperature and Salinity stress treatments were in 7 levels (0, -0.2, -0.4, -0.6, -0.8 and 1.2 MPa) and 7 levels (15, 20, 25, 30, 35, 38 and 40 °C) respectively. Germination was monitored daily until germination ceased and the number of the germinated seeds was recorded. Seeds were observed twice daily and considered germinated when the radical was approximately ≥ 2 mm long. To quantify the response of germination rate to temperature and to determine cardinal temperatures for germination, beta, and beta changed, segmented and dent models were used (Soltani et al., 2006). Water potential data showed a sigmoid trend and a three-parameter logistic model was fitted to data (Equation 3).

$$G(\%) = G_{max} / (1 + (x/x_{50})^n)^{Grate} \quad \text{Equation (3)}$$

where G is the total germination (%) at concentration x , G_{max} is the maximum germination (%), x_{50} is the osmotic potential required for 50% inhibition of the maximum germination and $Grate$ indicates the slope of the curve in x_{50} . Statistical Analysis System (SAS) was used for analyzing data.

Results and Discussion: Depth and interaction between seed size and depth showed significant effect on seeds emergence rate and percentage. Result indicated that higher planting depth than 5 cm, reduced the percentage of seedling emergence. The highest and lowest emergence observed for 1 and 13 cm depth with 62% and 34% respectively. The interaction between seed size and planting depth, indicated that for the depth of 1 cm, the germination of large seeds were more than small seeds, but just at the depth of 13 cm the significant difference between two seed size was observed. A linear trend in reduced germination rate was observed with increasing planting depth. The lowest and the highest emergence rate with 0.013 and 0.007 seedling per hour observed for 1 and 3 cm depth. Results showed that the temperature, salinity and interaction between them had significant effects on germination percentage and rate, and the time required to reach 5% (D05), 10% (D10), 50% (D50), 90% (D90) and 95% (D95) germination. Germination of tall morning-glory occurred over a wide range of salt concentrations. According to three-parameter logistic model, the salt concentration required for 50% reduction in the tall morning-glory germination rate and percentage were -0.59 MPa and -0.73 MPa, respectively. These results indicated the germination rate is more sensitive than germination percentage to salinity stress. To describe the rate of germination to temperature and water potential, the original beta, beta changed, segmented and teeth models were evaluated. The results showed that the segmented model compared to other models better described speed germination response to temperature and water potential. Thus segmented model used to determine the cardinal temperature of tall morning glory. Based on this model, base, optimum and ceiling temperatures of the plant in control treatment were 9, 30, 42 °C respectively. The result indicated that by

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increasing the salt concentration, the base temperature and biological hour for germination increased and optimum and ceiling temperature decreased. For a unit reduction of water potential, base temperature and the numbers of biological hours increased 6 °C and 31 hours respectively. However the reduction per unit of water potential reduced the optimum and ceiling temperature respectively 8 and 4°C.

Conclusion: These results suggest that the tall morning-glory can tolerate some level of salt stress and a proportion of tall morning-glory seed may still germinate even at salinity levels up to 1 Mps. This could be an important parameter for successful adaptation in the saline areas. Based on the segmented model, base, optimum and ceiling temperatures in control treatment were 9, 30, 42°C, respectively. The results of this research showed that environmental factors affected the germination of tall morning-glory, and this information could help to predict the spread of tall morning-glory in new areas.

Keywords: Cardinal temperatures, Soybean, Environmental factors, Golestan province. Regression models



Identification and Population Estimation of Epiphytic Ice Nucleation Active Bacteria Isolated from Stone Fruit Trees in Khorasan-Razavi Province

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Introduction: Epiphytic bacteria are common inhabitants of plant leaflet surfaces and can have important influences on the plants on which they live. Accurate estimates of epiphytic bacterial population sizes are needed to detect their roles in several important biological phenomena. For example, reliable disease forecasts and anticipate of frost damage depend on the exact estimation of bacterial population in their habitat. Frostbite is one of the major agricultural problems in Iran and is one of the most important environmental stresses which influences plant growth and crops productivity. Chilling and freezing limit the geographical distribution and growing season of many crops and cause significant crops losses. Apical meristem and upper leaves show the first symptoms of frostbite. Without bacteria involved, freezing damage on actively growing shoots is usually limited to the tip or outer branches, and the plant will recover. Freezing is not the sole reason of severe damage to such plants and if such damage or dieback occurs there must be synergistic effects between frost and bacterial infection. Ice nucleation epiphytic bacteria are one of the casual agents of frost and canker in stone fruit trees.

Materials and Methods: In order to identify ice nucleation active (INA) bacteria, 248 samples from twigs of sour cherry, sweet cherry, apricot, prune, peach, nectarine, almond and plum from the main areas of stone fruit tree orchards in Khorasan-Razavi province were collected in spring of 2013. In each area, 2 orchards and in each orchard 4 trees were randomly selected. The samples were placed in paper bags and were kept in a refrigerator until use. The weight of tissues was measured and bacteria were washed from the surface of plant parts in distilled water treated with 0.2 percent. The samples were homogenized for 1 hour at 100 rpm rotary shaker. One milliliter of this suspension was serially diluted to 10^{-9} , 10^{-8} , 10^{-7} and 10^{-6} cfu/ml. Twenty-five micro liter of each dilution was cultured on King's medium B. Among bacterial colonies grown after 48 h, 820 isolates were randomly selected (10 colony of each types that observed on petri dishes). Bacterial population in different areas and hosts, were estimated by counting of the bacterial colonies in each gram of fresh weight. To discover INA bacteria, bacterial suspensions in distilled water were incubated at -5 to -7 °C. After 30 min frozen bacterial

Cells

suspensions were selected. The population of INA bacteria were estimated by the formula
$$\frac{\text{Total}}{\text{INA} + 1} \times \text{g (fresh weight)}$$

The ice nucleation active bacteria were purified and identified by different tests including Gram reaction, catalase, fluorescent pigment production on KB or casamino acid medium, levan, oxidase, arginine dihydrolase, potato rot and hypersensitive reaction on tobacco. The growth with 5% and 7% NaCl, anaerobic condition, at 37 °C, were also tested. The yellow bacteria were cultured on YDC, and the production of xanthomonadin was tested.

Results and Discussion: Among these 820 isolates that analyzed, 110 isolates were determined as INA. These bacteria were identified by phenotypic and biochemical tests. According to our data 56, 27, 8, 6 and 3 percent isolates were identified as *Pseudomonas syringae*, *P. fluorescens*, *P. viridiflava*, *Pantoea agglomerans* and *Xanthomonas* sp., respectively. The population of INA bacteria in chenaran and Mashhad was higher than other cities. There was no correlation between plant species and the population of INA bacteria. Although our data indicated the positive correlation between canker symptoms and the population of INA bacteria. This study indicated that plant species cause significant differences in bacterial population size. Effects of plant species on epiphytic bacterial population sizes may also reflect physiological differences. It is fascinating that, for some species, different plants of the same genotype grown in the same area and under similar environmental conditions would exhibit significant differences in mean bacterial population size. It is possible that diversity in soil and water content, soil nutrient availability, release nutrient deposition, nutrient leakage from plants, presence of unseen wounds through which leakage occurs, or exposure to immigrants contribute to the generation of significant differences in bacterial population sizes among plants. Perhaps bacterial population sizes on plant species in which resources required for epiphytic colonization are abundant and are less

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susceptible to influenced by exogenous factors, therefore fewer variables among plants. We found that bacterial population sizes were varied in relation to plant host, and the physical environment.

Conclusion: The ice formation and frost damage in plants are normally depend on the logarithm of the ice nuclei number in freezing time. Therefore, population estimation of the epiphytic INA microorganisms is very important for frost forecasting. Our results, for the first time, demonstrated the population dynamics and the presence of different species of ice nucleation active bacteria in Khorasan-Razavi province.

Keywords: Ice nucleation active bacteria, Khorasan-Razavi province, Population, Stone fruit trees

Study the Stability of SSR Repeats of pEA29 Plasmid of the Casual Agent of Fire Blight

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Introduction: Fire blight disease caused by *Erwinia amylovora* infects a wide variety of rosaceous plants. It was first recorded from pear trees in Karaj in 1990. After that, it was monitored on many pear and apple orchards of the country. *E. amylovora* isolates differed slightly in virulence, symptoms and host range that can be related to different plasmid content. The presence of universal plasmid, pEA29, has been observed in the majority of *E. amylovora* strains. Short-sequence DNA repeat with eight nucleotides were repeated 3 to 15 times in the *Pst*I fragment of the pEA29 plasmid. Here, the stability of SSR units and efficiency of this method to categorize strains was checked. For this reason, two methods including amplification and cloning of whole and a part of *Pst*I fragment was done, along with comparison of the methods. In addition, stability was evaluated based on three treatments including long time propagation, keeping strains in a cold situation and re-isolation of infected tissues.

Materials and Methods: In this study 20 strains were purchased from the Iranian Plant Protection Research Institute. Their typical phenotypic tests were examined for all strains. All of them were checked with lateral flow immune chromatography in different serial dilutions. The pathogenicity was assayed using whole fruit. Direct PCR with A/B primers and nested PCR with AJ75/AJ76 were applied for study strains. Ten representative strains were selected, and part of *Pst*I fragment amplified using RS1/RS2 primers. The PCR products were purified by QIA quick PCR purification kit (Qiagen, USA), cloned in pGEM-T and sequenced (Macrogen Inc., Korea). Five of 10 were chosen for stability tests including long time propagation and subculturing each two weeks for three months, keeping strains in a refrigerator for three months and re-isolation of infected tissues were performed.

Results and Discussion: In phenotypic tests, all study strains were facultative anaerobic growth, oxidase negative, catalase positive and nonfluorescent on King's B. The biochemical tests for reduction of nitrate, were recorded negative. All *E. amylovora* strains induced the hypersensitive reaction (HR) on tobacco and pelargonium leaves. All phenotypic reactions were agreed with standard references. There is no variation in lab experiments.

Pathogenicity assay was checked using immature pear fruit in two separate treatments. Inoculation caused water soaking, tissue necrosis and sometimes necrosis in pear samples. No symptoms were observed in the negative controls. There is no variation in this assay. It seems that in most cases, the pEA29 plasmid can modify synthesis of amylovoran, levansucrase and finally affect pathogenicity in the host plant with respect to the environment. All strains were checked by agri strips (Bioreba, Switzerland, Reinach) in lateral flow immune chromatography to confirm the presence of *E. amylovora*. All strains of *E. amylovora* amplified a fragment of the expected size using primers A and B. The accuracy of the direct PCR was evaluated by nested PCR using primers AJ75/AJ76. The PCR products were visualized after electrophoresis on 1.2% agarose gels. A 3kb DNA ladder (Fermentas, Lithuania) was used as a molecular size marker in all experiments. To characterize SSR units in this study, part of *Pst*I fragment was amplified by RS1/Rs2 primers. The relevant variability found in the length of amplified fragment and the number of copies of SSRs. It is recorded 4 and 8 times in apple and rose plants respectively. Recording the SSRs of 4, 5 and 7 in other hosts including pear, hawthorn and quince may be indicated that under natural conditions, a mixture of *E. amylovora* strains with different SSR numbers could have caused fire blight. Frequent transferring of bacterial isolates to new nutrient agar medium did not change the SSR units after three months. Among three test treatments, keeping in cold weather for at least three months caused variation in repetitive units in IrGh59 isolates. According to SSR stability, some studies indicated that mutation, antibiotic treatment, maintaining in specific situations may change the number of these units. However, our results showed that SSR numbers of several strains remained constant under laboratory conditions.

Conclusion: Sequencing the whole *Pst*I fragment may provide better information about SSR units and the flanking regions. The numbers of SSR are stable under experimental conditions and evaluation of *E. amylovora*

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islaes with this method can apply for strain grouping.

Keywords: Grouping, Iran, pEA29 plasmid, Stability of SSR units

Brief report

The investigation (study) of toxicity Imidacloprid, Acetamiprid, Pirimicarb and Diazinon on The Mortality of Rose Aphid, *Macrosiphum rosae* L. (Hem.: Aphididae)

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Introduction: Rose Aphid, *Macrosiphum rosae* L. is one of the most important pests of rose which reducing the quality and quantity of products by attacking and hurting the leaves and branches. Aphids damage plants by sucking sap from plant tissues using highly specialized sucking mouth parts. The usual symptoms of damage are distortion of new leaves and flowers. Aphids damage plants by sucking sap from plant tissues using highly specialized sucking mouth parts. The usual symptoms of damage are distortion of new leaves and flowers. Neonicotinoids insecticides imidacloprid and Acetamiprid is, Which has the effect of sucking insects. The discovery of neonicotinoids as important novel insecticides has represented a milestone in insecticide research over the past three decades. The neonicotinoids represent the fastest-growing class of insecticides introduced to the market since the commercialization of pyrethroids. Diazinon is an organophosphates pesticides to combat pests such as aphids. Pirimicarb of group carbamate insecticides are effective against aphids are active on fruit trees and ornamental plants. Comparison of several insecticides and pesticides and recommend appropriate levels, to combat and prevent outbreaks of pests, can be of great help in pest control product is the increase in market.

Materials and Methods: In this study, different concentrations of Acetamiprid and Imidacloprid with Pirimicarb and Diazinon on rose aphid mortality was compared to find the condition of mortality of *Macrosiphum rosae* as per field conditions in a randomized complete block design during 2011-2012. Each compound was used in 3 different concentration (recommended, higher and lower) including Imidacloprid (200, 600, 400 PPM), Acetamiprid (250, 500, 125 PPM), Pirimicarb (250, 500, 125 PPM) and Diazinon (500, 1000, 250 PPM) and effects checked on aerial organs and compared by sampling of population aphids on the period of "before, 3,5,7 and 14 days after" spraying..

Results and Discussion: results showed that the average mortality of aphids in three days after spraying, There is a significant difference between treatments and blocks, And the average aphid mortality at 5, 7 and 14 days after spraying, only the treatments, showed a significant difference. Recommended concentrations and higher imidacloprid and diazinon were in a group. The results showed that on the third day of treatment, Diazinon (500ppm) 73/46%, Imidacloprid (400 ppm) 88/77%, Acetamiprid (250ppm) 73/95% and pirimicarb (400ppm) with 72/8% had the greatest impact on aphid mortality, respectively. As per result the effects of Insecticide reduced to 14th day. Therefore, because of good performance of the neonicotinoid compounds, it can be effective in rose aphid control. The results indicated the suitability of the recommended concentration is well above the concentration, so no need to use higher concentrations. Low concentrations the recommended concentration decreases gradually as the impact on the fourteenth day and have minimal impact on mortality is low for crop growers. This group had different mode of action as compared to the previous insecticides. Imidacloprid was the first member of this family and was effective against many insects showing resistance to carbamates, organophosphates and pyrethroids. Acetamiprid belongs to the second generation of the nicotinoids. The present study demonstrated rose aphid population with neonicotinoid insecticides was significantly different neonicotinoid insecticides (imidacloprid and acetamiprid) had the most effective on number of *M. rosae*. But the introduction of neonicotinoids in the form of imidacloprid and acetamiprid proved an immediate relief for crop growers. This group had different mode of action as compared to the previous insecticides. Neonicotinoid insecticides, such as imidacloprid, acetamiprid are commonly used against a wide range of herbivorous insect pests such as aphids, mealy bugs and whiteflies in greenhouses or farms. So, parasitoids and predators are

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exposed to these chemicals.

Conclusion: Due to the losses caused by the toxins and the yield obtained in this study, recommended concentrations of insecticides neonicotinoid are best treatment to combat aphids on roses. The use of high aphid densities Imidacloprid and Acetamiprid in areas where spraying is needed more than once is recommended. Our results showed that best effect belongs to recommended dose of Neonicotinoids compounds same as recommended dose for phosphates and carbamate compounds to control the Rose aphid.

Keywords: *Macrosiphum rosae*, Neonicotinoids compounds, Presentation of mortality