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Full Length Research Paper

Fusarium diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in Iran

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The most frequently soil-borne fungal pathogens on plants are *Fusarium* species that make high economical damages in various agricultural locations in Iran. Our studies show that *Fusarium* species cause significant yield losses in main crops especially potato, pea, bean, wheat, corn and rice in several parts of the country. The diseases resulted in yield losses to the extent of 30 to 70% in the fields and made economical problems for growers. Infected plants were collected and cultured on common medium; potato dextrose agar (PDA) and selective media [peptone pentachloronitrobenzene (PCNB) agar (PPA), and carnation leaf agar (CLA)] for *Fusarium* species, and then isolated species were identified. The dominant species were *F. solani, F. oxysporum, F. pseudograminearum, F. moniliforme* and *F. sambucinum* in the area studied. Soil solarization method was carried at the summer season in three soil infested locations to assess the control management of the pathogens. Application of this method reduced population density of the pathogen from 1833 to 500, 266, and 100 CFU g $^{-1}$/soil after 2, 4, and 6 weeks. The method was simple, effective, non negative side and applicable in diverse farming areas at warm season.

Key words: *Fusarium*, diseases, yield losses, soil solarization.

INTRODUCTION

Fusarium species the main soil borne are the best known plant pathogens in terms of economical damage in agricultural productions all over the world (Saremi, 2000; Bentley et al., 2006; Bockus et al*.,* 2007). Regarding to *Fusarium*-induced diseases in agricultural plants, yield reduction in different crops is a major problem in Iran. Dry rot on potato, wilting and decline on bean or pea, crown rot and head blight on wheat, bakanae disease on rice caused by *Fusarium* species result to yield losses in most crops fields (Saremi et al., 2007a). Several *Fusarium* pathogens cause essentially similar symptoms on different crops such as cortical decay of roots, root rot, wilting, yellowing, rosette and premature death on infected plants. On the other hand, *Fusarium* involves several species that produce mycotoxins that associate with serious animal diseases like feed refusal syndromes, moldy sweet potato toxicity, and bean hulls poisoning (Summerell et al., 2001; Saremi 2995).

Fusarium dry rot of seed tubers can reduce crop establishment by killing developing potato that crop losses can be up to 25%, while more than 60% of tubers can be infected in storage (Wharton et al., 2006). Generally, fungus pustules contain mycelia which often grow on the depressed surfaces and rotted tubers shrivel and become mummified. Tubers of potato cultivars differ in susceptibility to *Fusarium* species and none certain tolerant cultivars were introduced. *Fusarium* dry rot disease develops most rapidly in high relative humidity and at 14 to 21°C and then continues at the coldest

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Abbreviations: GMA,

temperatures as Zanjan province environment. Some varieties were tolerant to infection when harvested but develop susceptibility during storage. This disease occurs in many parts of the country, but was most common in the northwest. However, it has been reported that *Fusarium* dry rot is a major problem in the potato growing areas all over the world (Wharton et al., 2006). Tuber potatoes become infected through wounds during storage or preparation for planting by *Fusarium* dry rot diseases all over the world (Hooker, 1990).

The major legumes, such as bean, pea and lentil also suffer from heavy yield losses due to *Fusarium* diseases including root rot, decline and wilting in the Northwest Iran. Root rot caused by *Fusarium* is detected in the reddish lesions on the taproot which later turn brown. The common root rot and decline or damping off of bean is spread in different countries where this crop is cultivated (Schwartz and Yuen 2005; Hall, 1991). Since legumes are the main agricultural production in Zanjan province, the yields reduction of legume crops by *Fusarium* species create main troubles for growers (Saremi et al., 2007b; Naseri, 2008).

The crown rot disease of wheat caused by *Fusarium* species can results in white heads producing either shriveled or no grain (Bentley et al., 2006; Bockus et al., 2007; Wiese, 1998). The disease could result in grain losses from 20 to 35% in some growing locations in Zanjan Province (Saremi et al., 2007a). Scab or head blight disease of wheat is caused also by another *Fusarium* species which occurs in some humid regions of the area under study. The disease is frequently severe in the wheat crop raised in a field saturated with residue from a previous host crop like wheat or corn. The infected crop produces immature heads in which one or more spikelets or the entire head appears prematurely dried. The grain from head-blighted fields is unsuitable for feeding farm animals as it may contain mycotoxins that induce vomiting in animals. Bread made from scabby wheat has been reported to be intoxicating (Saremi 2005).

Corn and rice as other major crops in the area studied were also contaminated by *Fusarium* species which showed sever damages. These two main crops are the key food for humans and animals so low production due to disease effect have economical problem for producers. Therefore it is necessary to recognize the pathogen and find the best managing way for their control in farming areas. In fact, it is essential to develop an effective control method to ensure good production and yield stability. Many methods such as chemical, cultural and biological techniques have been developed for the control of plant disease by soil borne pathogens (Singleton et al. 1992). By the way, there is no a general perfect method to be used in all instance of soil borne pathogens control. Thus, any new method of control, even if restricted in its use, is of value because it adds to our rather limited arsenal of control methods.

So, soil solarization or solar heating which is the relatively new approach can be a good way for controlling soil borne pathogens (Pullman et al., 1981; Pinkas et al., 1984; Annesi and Motta, 1994; Ashrafi et al., 2010; Saremi et al., 2010). The aim of soil solarization is to harness solar energy to raise the temperature of moistened soil which can result to the control of soil borne pathogens especially *Fusariuim species*. Soil solarization is obtained by covering soil with plastic films, a useful practice able to reduce soil pathogen populations. Actually, light plastic films (LPFs) are nowadays widely used especially in open and greenhouse vegetable crop cultivations in some countries as they are able to raise soil temperature more than 20°C above air temperature (Gonzalez et al., 1993; Kumar et al., 2002). In general, solarization appears to be an effective practice able to control nematodes, even though it may cause stress on the soil microbial biomass. In addition, it is demonstrated that the organic amendments exert a protective role keeping soil microbial biomass and enzymatic activities protected from the detrimental effect of heating.

Actually, we tried to use this method in northwest Iran at the warm season which is climatically marginal weather for this method. However, this study has been carried out to study the main crops disease in Iran and improve the level of their control managements. This study was carried out to assess the *Fusarium* diseases on crops which cause high damage in Iran and manage them by soil solarization method which is simple, effective, has no negative side effect, an economic technique and can be used in nearly different areas.

MATERIALS AND METHODS

Sample collections

Samples of infected plants as well as root, crown, tuber, stem, head and leave were collected from farms and stores located in northwest Iran. Collected samples of potato, pea, bean, wheat, corn, and rice were cultured on media after surface sterilization with sodium hypochlorite. Since *Fusarium* species can be isolated directly from soil around the roots, the dilution plate technique was used as the useful recommended method (Saremi 2005; Burgess et al., 1994). 1 g of the soil from the vicinity of the infected plant roots was added to 0.05% water agar (WA) and used to prepare soil suspensions of different dilutions. One ml of soil suspension was uniformly spread over a selective medium such as PPA (Peptone PCNB Agar).

Media and identification

All samples were transferred to the *Fusarium* Laboratory in Agriculture Faculty of the Zanjan University and were cultured on common potato dextrose agar (PDA) and peptone PCNB agar (PPA) and carnation leaf agar (CLA) media as selective media. The *Fusarium* colonies were sub-cultured from PPA on CLA plates by transferring small mycelia plugs from the colony margins. The plates were incubated in an illuminated room under alternating

Figure 1. Covering the infested soil with a clear plastic to trap the sun's heat to raise soil temperatures.

a b c

Figure 2. Dry rot disease on tuber potato (a) and wilting of bean (b) caused by *Fusarium solani* and its conidia (c) isolated from infected plants.

temperature regime for two weeks to allow the growth of colonies and make sporodochia. All isolated cultures were examined after incubation and identified to species level using the methods described by researchers (Nelson et al., 1981; Burgess et al., 1994; Saremi 2005).

Managements of the pathogen

Soil disinfestations by soil solarization method at warm season was carried out for the relative control of *Fusarium* pathogens, the main crops cultivated areas, in northwest Iran. In soil solarization method, infested soil was thoroughly plowed to destroy all large clods and remove any existing plant materials. The soil was irrigated deeply since it was a very important step in the process for increasing transmission of heat through the soil. Then, the moistened soil was covered with transparent polyethylene sheet (Figure1) to raise soil temperatures high enough; more than 10°C above air temperature (Kumar et al., 2002). The plastic edges were buried in the trench to ensure that the plastic is held in place to stabilize the heat. The process was facilitated to raise solar energy and to raise the temperature of moistened soil which can result in the control of soil borne pathogen. The plastics were leaved in treated soil for six weeks during the hottest part of the summer. The method came out to be a successful practice to control soil borne fungi, as well as *Fusarium* species (Pinkas et al., 1984).

Soil dilution

The dilution plate technique was used by adding 1 g of the collected soil to 0.05% water agar (WA) prepared by mixing 0.5 g agar in 1 L water. Then, 100 ml of the solution was poured in a special glass bottle and autoclaved and used to prepare soil suspensions of different dilutions (Burgess et al., 1994). 1 ml of the soil suspension was uniformly spread over a selective medium such as PPA (peptone PCNB agar) to produce 10 to 25 colonies per plate. The plate was then held with a slight slope away from the suspension and gently shaken at right angles to the slope. The suspension slowly spread across the plate with a uniform wetting front (Burgess et al.1994, Saremi 2005).

RESULTS

A range of *Fusarium* species were isolated from potato tubers, bean, peas, wheat, rice, corn and other crops. Recovery showed that most pathogenic *Fusarium* species were *F. solani* (Mart.) Apple and Wollenw. W. C. Snyder, *F. oxysporum* Schltdl, *F. moniliforme* Sheldon and *F. pseudograminearum* (Aoki) in the regions studied. In fact *F. solani* was a facultative parasite that caused root rot and crown rot on many plants in most locations. There were serious cases of dry rot on potato on diverse areas particularly in Zanjan province as one of the largest location of potato production in Iran (Figure 2). The fungus is a cosmopolitan soil saprophyte and facultative parasite that causes root rots, stem cankers and storage rots of a variety of host plants in Iran and other countries

a b c

Figure 3. Macroconidia and microconidia of *Fusarium oxysporum* (a) infected chickpea (b) and pea (c) by *F. oxysporum* in northwest, Iran.

(Saremi, 2000; 2006; Hooker, 1990). Generally, the fungus can be root rot pathogen on many economical plants all over the world especially in northwest the county which various plants were grown. Mainly, the most fungal pathogen isolated from different plant was *F. solani* and caused yield reduction.

Fusarium disease on potato

Normally, potato is the most important agricultural production in northwest Iran especially in Hamedan and Zanjan provinces; the nearly cool temperate regions. Recently, potato suffers from root rot and lesions on leaves resulted to depressed necrotic tissue and early blight, which caused yield losses (Figure. 2). There have been reports that several *Fusarium* species can cause disease and make sever damage on potato in Iran and other countries (Wharton et at., 2006; Saremi, 2005; Saremi and Amiri, 2010). However, researchers supposed that *F. solani* is the main fungal pathogen isolated from potato tubers and leaves all over the world (Hooker, 1990). The fungus produces the sexual form called *Necteria haeamatococca* Berk & Br. which produce asci and ascospores in culture.

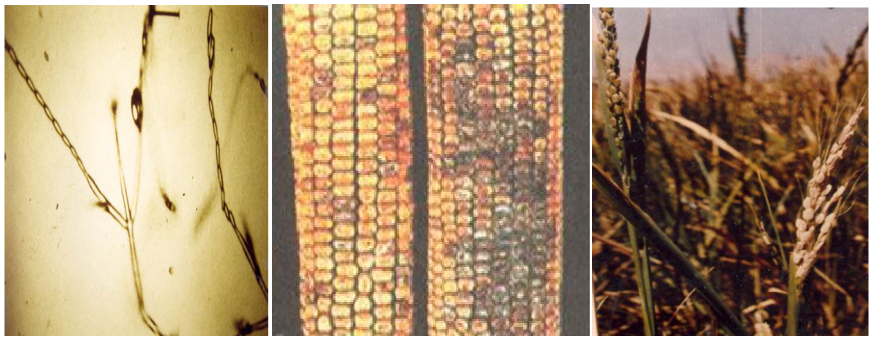
Fusarium disease on peas and bean

Another *Fusarium* species that cause vascular wilt and damping off diseases on crops was *F. oxysporum* Schlecht emend Snyd. & Hans. The fungus was identified as a pathogen and was isolated all over the country as a soil saprophyte (Figure 3). Normally, *F. oxysporum* forms several pathogenic strains that are specific to different hosts called forma specials. For instance *F. oxysporum* f. sp. *medicaginis* (Weimer) only infects alfalfa and *F. oxysporum* f. sp. *pisi* only causes the pea plants. Study show that *F. oxysporum* f. sp. *pisi* caused root rot and wilting of the pea crop by means of sever damage (Figure 2) in Zanjan province (Saremi et al., 2007b). This species may produce mycotoxine on infected plants and cause disease in animal fed with the infected plants. It has been concluded that feed naturally contaminated with *Fusarium* mycotoxins can affect the metabolic parameters and immunity of dairy cows and that GMA can prevent some of these effects (Korosteleva et al., 2006).

They reported the effect of feed borne *Fusarium* mycotoxins on the performance, metabolism, and immunity of dairy cows which is severe in milk production. Further, mycotoxin producing species such as *F. sambucinum* Fuckel and *F. culmorum* (W.G. Smith) Sacc. were also isolated from many crops in northwest Iran. *F. sambucinum* was a dominant fungus in the legume field, which caused root rot and stem rot in the bean plants. Actually, this *Fusarium* species occurs in cool area such Zanjan province. Researchers reported that *F. sambucinum* can occur only on temperate areas and can be pathogen over there on different growing plants (Saremi, 2000; Saremi et al., 1999; Burgess et al.1994).

Fusarium disease on corn and rice

Other common *Fusarium* species isolated from corn and rice in the area studied was *F. moniliforme* Sheldon. Many *F. moniliforme* cultures were isolated from corn and rice as human food and animal feeds. Microconidia of this species were formed in a chain and the sexual stage was *Gibberrella moniliforme* (Sheld.) Syn. Commonly, *Fusarium moniliforme* was mostly associated with moldy corn in the country and it was isolated from rice, corn and other cereal grains (Figure 4). This soil borne *Fusarium* isolate had the masses yield reduction on main crops especially corn and rice all over the northwest Iran. By the way, our findings on *F. moniliforme* confirm the observations of other researchers that reported the extract of an isolate of this species from moldy corn that was toxic to chick embryos (Voss et al., 1996). Wheat was also infected by some *Fusarium* species especially *F.*



(a) (b) (c)

Figure 4. Microconidia of *Fusarium moniliforme* in chain (a) and contaminated corn (b) and rice (c) by the fungus on the field.

a b c

Figure 5. Conidia of *Fusarium pseudograminearum* (a), root rots and crown rot (b) and wilting disease (c) on wheat, northwest Iran.

Graminearum in north Iran as reported recently (Saremi, 2007).

Fusarium crown rot disease on wheat

Generally, various fungal species produced different symptoms on the infected wheat plants. However, the main symptoms of common root rot and crown rot in the areas studied were yellowing, growth reduction, pink coloration around the crown, and wilting caused by *F. pseudograminearum.* The diseased plants were mostly stunted and the symptoms were most striking near or below the surface (Figure 5). They included brown spots, blotches and rotting on the crown, roots and sub crown internodes (Figure 5). Some of the infected plants had stunted growth. In such cases, the rootlets were necrotic or rotten and engulfed in a mass of white fungal growth. The disease resulted in yield reduction in wheat growing areas in the regions studied.

Effects of soil solarization on *Fusarium* population in soil

Soil solarization greatly reduced the population densities of *Fusarium* species as the major soil borne fungal pathogens in this research. Population density of colony forming unit (CFU g^{-1} soil) of *Fusarium* species was decreased quickly after application of two weeks soil solarization method in the studied areas. Results show that the mean of population density reduced from 1833 CFU g^{-1} soil to 900 CFU g^{-1}/soil after two weeks and then reduced to 500 CFU g^{-1} after four weeks. The population of the *Fusarium* species was also reduced to 100 CFU g^{-1}/soil after six weeks solarization process (Table 1).

Normally, solarization procedure heats the covered soil through repeated daily cycle and the temperatures in solarized soil were 5 to 12°C higher than those in comparable non-solarized ones. Soil solarization effectively reduced the population of *Fusarium* pathogen in the infected soil (Figure 6). Disease incidence in the

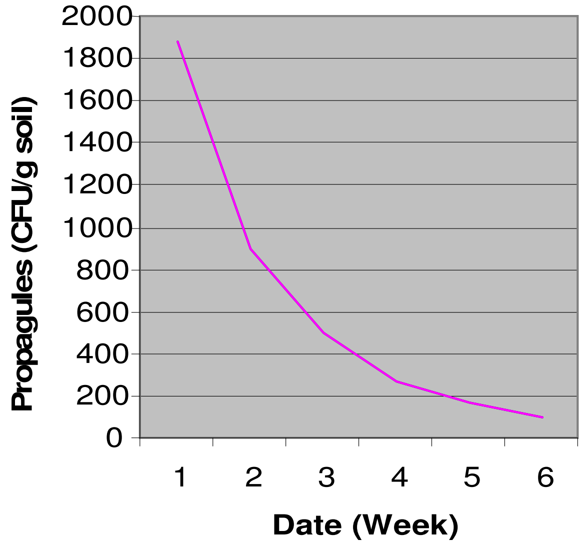


Figure 6. Reduction of colony forming propagule on *Fusarium* species after six weeks of application of soil solarization in the soil field in northwest, Iran.

untreated plots was significantly and negatively correlated with the yield. Soil solarization depressed *Fusarium* disease and increased crop yields after treatment.

DISCUSSION

Studies have confirmed many economical losses in many crops for the infection of several *Fusarium* species in Iran. The damage of potato on farm and storage by *F. solani* was reported from different parts of Iran and other countries (Hooker, 1990; Saremi, 2000; Saremi and Amiri, 2010). Recently, high incidence of root rot and crown rot of wheat caused by *F. pseudograminearum,* which made a lots of damages in the various locations in northwest Iran (Saremi et al,. 2007a). Crown rot disease of rice caused by *F. moniliforme* were also noticed in the Zanjan and Gillan provinces recently (Saremi and Okhovvat, 2004). There have been reports that several *Fusarium* species in particular *F. solani* cause disease and make sever injure on potato in Iran (Saremi, 2005). Diseases on other crops including corn are also significant in different parts of the country mainly in northwest Iran. Involvement of *Fusarium* species on crops infection and yield reductions along with mycotoxin production have been reported all over the world (Summerell et al., 2001; Korosteleva et al., 2006; Bockus et al., 2007; Saremi and Amiri, 2010).

On the whole, control of the soil borne pathogens, especially *Fusarium* species has changed over the last few decades. Application of soil solarization for controlling *Fusarium* and *Verticillium* wilt on some crops is carried out in several countries (Ghini et al., 1993; Gonzalez-Torres et al., 1993; Kumar et al., 2002; Saremi and Amiri 2010). Generally, soil solarization, as a hydrothermal process in moist soil is now very common in controlling soil borne plant pathogens all over the world. Solarization had been carried out in many countries. This method as natural solar heating would be beneficial for the control of soil borne fungi, bacteria, nematodes and weeds in the fields. Solarization method can become more effective in controlling the soil borne causal agents since high temperatures in soil can be generated and kill pathogens (Kumar et al., 2002).

Solarization causes physical, chemical, and biological changes in the soil by raising soil temperatures from 6 to15°C above the temperatures of the untreated soil (Stapleton and DeVay, 1984; Chen and Katan, 1980). It has been reported that with solarization during July and August, the temperature achieved at levels between 5 and 20 cm below the soil surface, were 45 to 55 C° and 39 to 45°C, respectively in California (Katan 1981). In Florida, the temperatures at depths of 5, 15, and 25 cm were recorded as 49.5, 46.0, and 41.5°C respectively in solarized soil (Chellemi 1994).

The success of soil solarization is based on the fact that most plant pathogens are unable to survive for long periods at temperatures above 37°C. The heat sensitivity of these organisms is related to an upper limit in the fluidity of cell membranes, which lose their ability to function at high temperatures. Other causes of death of organisms at high temperatures involve the sustained inactivation of enzyme systems, especially respiratory enzymes. Pathogens may be killed either directly by the heat or are weakened by sub lethal heat to the extent that they are unable to damage crops (Stapleton and DeVay 1984; Saremi et al., et al., 2010).

Soil solarization is a control measure against soil born fungi and nematodes that result to the increase in soil temperature to about 12°C in other places (Pullman et al., 1981; Duff and Connelly, 1993; Nafees et al., 2007). It has been reported that *Fusarium* species*, Macrophomina phyaseolina* and *Verticillium sp*. of fungi and *Tylenchus sp., Haplolaimus sp., Xiphenema sp.* and almost all of the parasitic nematodes were significantly controlled. On the other hand, it was found that even after 40 days, the solarized plots contained significantly less number of fungi and nematodes as compared to the non solarized plots, which confirmed the durability of this process (Duff and Connelly 1993; Nafees et al., 2007).

By the way, amount of *Fusarium oxysporum* in the upper 15 cm of a naturally infested soil was reduced by soil solarization (Gonzalez-Torres et. al., 1993). During the nine months following treatment, the *F. oxysporum* population stabilized at a low level in soil solarized for two months, but fluctuated in soil solarized for one month. The amount of *Fusarium* wilt on plants into this soil was generally low after treatment. Soil solarization method increased rate of infection of juveniles of nematode *Meloidogyne incognita* for at least ten months (Nico et al., 2003).

Solarization appears to be an effective practice able to control nematodes, even though it may cause serious stress on the soil microbial biomass (Annesi and Motta, 1994; Chellemi, 1994; Pullman, et. al., 1981). In addition, it was demonstrated that the organic amendments exert a protective role keeping soil microbial biomass and enzymatic activities protected from the detrimental effect of heating. Normally, light plastic films (LPFs) are nowadays widely used especially in open and greenhouse vegetable crop cultivations as they are able to raise soil temperature more than 12°C above air temperature (Duff and Connelly, 1993; Saremi et al., 2010).

Generally, plants growing in solarized soil suffered less wilt than plants in untreated soil but the differences were not significant (Saremi and Amiri, 2010). In a soil artificially infested with the highly pathogenic *F. oxysporum* f. sp. *niveum, F. oxysporum* populations were greatly reduced following solarization and fluctuated erratically thereafter. Solarization for two months completely controlled wilt in plants and gave a fruit yield almost five times that of plants in untreated soil. Solarization for one month only slowed disease development slightly but gave a yield more than twice that in untreated soil. Plant performance was significantly better in soil solarized for two months than in uninfected control soil, suggesting beneficial effects of this treatment additional to wilt control (Gonzalez-Torres et al., 1993, Ashrafi et al., 2010).

However, the principles of soil solarization as a hydrothermal process in the moist soil should be followed. Soil mulching should be carried out during the period of high temperatures and intense solar irradiation. The soil should be kept moist to increase the thermal sensitivity of resting structures and to improve heat conduction. The thinnest polyethylene trap (25 to 50 µm thickness) is recommended to be use, since it is both cheaper and more effective than thicker ones. The best time for soil solarization must be determined when climatic conditions are the most favorable (Saremi et al., 2010). Commonly, application of soil solarization not only is a simple and no expensive way but also has no negative side effect which other methods such as chemical management have. The method can be used every where in warm season; use sun heat which is available in most locations. This technique can reduce soil borne pathogens including fungi, bacteria, weed seeds and nematodes even many soil borne larva and insects.

Conclusion

Soil solarization or solar heating of the soil should be introduced in Iran and other countries because it is both widely accepted and concise. In addition, the method implies an active process of solar heating of the soil for controlling soil borne plant pathogens, which cause major diseases in Iran. The process of soil solarization can be used in some agricultural locations where climatic conditions are favorable. Although, it can be applied in different location since the method is used in summer time as a warm conditions.

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