Control managements of *Aspergillus flavus* a main aflatoxin producers and soil borne fungi on pistachio in Kerman

Saremi Hossein*[1], Mahmood Okhovvat[2], Hanieh Saremi[3]

(Received: August.5-2007; Accepted: September. 7-2007)

Abstract

The pistachio gardens in Iran rival other countries with 280000 hectares and rank first in the world in terms of annual production 300,000 MTs. Ecological factors for Pistachio planting favor the growth of *Aspergillus flavus* which produce aflatoxin as secondary metabolites which reduces Pistachio exports. It is important to control *A. flavus* and its aflatoxin production to increase Pistachio exportation in the country. Soil borne fungi such as *Fusarium spp., Phytophthora spp.* and *verticilium spp.* makes also root rot on pistachio trees which resulted yield reduction. It was necessary to find various ways for control management of these fungi to control wilting and inhibit toxic production on pistachio. Soil solarization and biological control application were two best methods for controlling soil borne fungi and *A. flavus* which are used in many countries. We used soil solarization method for controlling soil borne fungal pathogens in Kerman Application of this method reduced population density of the pathogen from 1100 CFU –g/soil to 300 after 2 weeks and reduced to 100 CFU –g/soil after 4 weeks. This method was simple, effective, no side effect and economic which can be used in nearly warm areas.

Keyword: Control, Soil solarization, *Aspergillus flavus*, Pistachio

Introduction

Pistachio as a commercial output has a special importance in the agricultural production of some countries especially in Iran and contains large portion of non-petroleum exportation. Generally Iran ranks first in the world in terms of pistachio production and harvested area but it does not benefit from high position in global marketing due to exporting challenges. So, it is necessary that Iran observe international standards and make investments in marketing and exporting processing industries and find new target markets for this product. Normally, existing position and challenges of major producing and exporting countries with especial reference to Iran and USA are considered and a number of strategies are recommended for an effective competition in global markets. We have to solve all the problems in nuts quality and yield production particularly management of fungal diseases.

In fact, many agricultural products attack by a group of fungi that are able to produce toxic metabolites called mycotoxins. Among various mycotoxins, aflatoxins have assumed significance due to their deleterious effects on human beings (7, 17). The aflatoxin problem in pistachio can reduce its marketing all over the world especially in Iran (2, 5). The cause of the disease was shown due to toxins in pistachio nuts infected with *Aspergillus flavus* which it is necessary to be controlled. Analysis of *A. flavus* population in pistachio orchard will provide information on biocontrol

∗Corresponding Author Email: Saremih@znu.ac.ir
1-Associate Professor, Department of plant pathology, Faculty of Agriculture. Zanjan University, Iran. Email: Saremih@znu.ac.ir
2- Professor, Department of plant pathology and Entomology College of agriculture University of Tehran, Karaj, Iran
3-MSc. Student, Department of soil sciences, College of agriculture University of Tehran, Karaj, Iran

strategies to reduce aflatoxin in the edible nuts to meet the mandatory levels of 2-4ppb (4).

Aflatoxins in dry state are very stable to heat up to the melting point. However, in the presence of moisture and at elevated temperatures there is destruction of aflatoxin over a period of time. Such destruction can occur either with aflatoxin in oilseed meals, aflatoxin in roasted peanuts or aflatoxin in aqueous solution at pH 7. Although the reaction products have not been examined in detail it seems likely that such treatment leads to opening of the lactone ring with the possibility of decarboxylation at elevated temperatures (17).

There are other sever diseases in pistachio in Iran particularly wilting of trees by soil born fungi. Wilting on pistachio was caused by soil born fungi such as *Fusarium oxysporum*, *Phytophthora sp*. and *verticillium dahlia* as occurred on other trees. These soil born fungi have been isolated from infected trees in Kerman province. We tried to find best way for controlling these fungal pathogens to increase yield production. Using soil solarization method for controlling soil borne fungi on some crops and trees is carried out in at several countries (12). Generally, soil solarization, as a hydrothermal process in moist soil was 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.

Aspergillus flavus is an economically important fungus which produces carcinogenic compounds known as aflatoxins in agricultural crops and tree nuts. The fungus has no known sexual stage; consequently, most studies on its genetic variability have been evaluated mainly by characterizing isolates based on vegetative compatibility. A better understanding of the population structures of *A. flavus* will facilitate the development of effective biocontrol strategies. We tried to fallow different ways in management all the fungi which make diseases in pistachio in Iran especially in Kerman province.

While conditions favor mould growth and aflatoxin production in pistachio nuts and how can this be prevented. Many *Aspergillus* species infect nuts and cause decay of the kernels before harvest. For example, it was reported that up to 13 species were isolated from pistachio kernels from orchards in Iran and 14 species from orchards in the United States (California) (Doster and Michailides, 1994a). Awareness that undesirable levels of aflatoxin in food and feed may have serious consequences for human and animal health is increasing. Aflatoxins, probably the most studied and widely known mycotoxins, were first noted in the early 1960s. They are among the most potent mutagenic substances known; there is extensive experimental and epidemiological evidence that they induce liver cancer (WHO, 1998). The term aflatoxin refers to a class of chemical compounds of related structure; among this aflatoxin B_1 is considered to be the most potent carcinogen.

Materials and Methods

Soil disinfestations by soil solarization method was carried for the relatively control the pathogen in Kerman province as the nearly warmer climate in studied areas (Fig. 1b). The technique was used in a location which pistachio trees had been infected by soil borne fungi such as *Phytophthora sp.* or *Fusarium oxysporum* (Fig. 1b). This method facilitates solar energy to raise the temperature of moistened soil which can result the control of soil borne pathogen. By this way the infested soil was covered with transparent polyethylene to raise soil temperatures high enough for controlling the pathogen. The plastic edges were buried in the trench to ensure that the plastic is held in place to prevent heat.

The experiments also demonstrated that the yeast, *Pichia anomala* can modulate spore production of fungi including *A. flavus* on the pistachio nut-fruits. Field spraying of these effective yeasts to pistachio trees decreased the population of *A. flavus* in the orchards and thus lowers the number of nuts infected by this fungus. The outcome may be a reduction of aflatoxin contamination in the edible nuts. Monitoring *A. flavus* and *A.*

parasiticus on pistachio buds provided the growers a way to evaluate the population of the aflatoxin-producing fungi in orchards and help them make decision on control strategy.

a **b**

Figure 1: Application the soil solarizasion method by covering plastic on infected soil in pistachio orchards in Kerman (a) and increased soil temperature after covering soil (b).

Existing literatures indicate that wounds in plant tissues provide the entry to A. flavus. Two experiments were conducted in a commercial orchard in the summer of 2005. Nut-fruits of pistachio were individually wounded with a dissecting needle. Another way which can be helpful is working in harvesting stage tree nuts, including pistachios, should be harvested as soon as possible after maturation to avoid quality loss and to minimize problems involving fungal attack and infestation with insects, especially the navel orange worm.

Pistachios hulled and dried soon after harvest to minimize shell staining and decay and to ensure safety. Temporary storage of fresh pistachios at the dehydration plant was done and be cooled and held before hulling at 0°C and relative humidity lower than 70 percent. Pistachios were sorted before cold storage to remove defective nuts, leaves, twigs and other foreign materials. Fumigation with methyl bromide used to control insects in stored nuts. The hulled nuts were first dried for about three hours in a column dryer or a rotating drum dryer to reach kernel moisture of 12 to 13 percent. Dried nuts were usually stored in bins, silos or other bulk storage containers for a few weeks or several months before final processing and preparation for market. Optimum storage conditions of 10°C or lower and 65 to 70 percent relative humidity must be maintained to minimize deterioration, including the growth of *Aspergillus flavus*.

Results and discussion

Normally, soil borne fungi caused root rot in pistachio trees in Iran as it occurred all over the world (15). Root rot and crown root diseases on trees resulted wilting on branches and fruits as happen in pistachio trees in Kerman (Fig. 2). Obviously, wilting on pistachio trees provides a situation for infection of *A. flavous* and aflatoxin production. So any activities for protection of wilting disease can reduce other fungal infection and yield production. On the whole, controlling 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 at several countries (1,8,9,12).

Generally, soil solarization, as a hydrothermal process in moist soil was 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 Soil solarization and amendments were analyzed as a control measure against soil born fungi and nematodes and resulted in about 12°C increase in the soil temperature in other places (15). This increase in soil temperature caused a reduction of about 70 to 80% in the fungal population and about 99% in nematode population at various depths.

Figure 2: Wilting of pistachio branch and fruits because of root rot of tree by soil borne fungi in gardens.

The results were proved synergistic for solarization and also improved the properties of soil in the benefit of crop plants. It was found that even after 40 days the solarized plots contain significantly less number of fungi and nematodes as compared to the nonsolarized plots, which confirmed the durability of this process (15). By the way, amount of *Fusarium oxysporum* in the upper 15 cm of a naturally infested soil was reduced by soil solarization (9). During the 9 months following treatment, the *F. oxysporum* population stabilized at a low level in soil solarized for 2 months, but fluctuated in soil solarized for 1 month. Actually, the soil heating could also lead to the control of other nematodes such as *Heterodera radicola* the causal agent of sugar beet in Iran as occurred in Hawaii.

This study showed population density or colony forming unit (CFU g^{-1} soil) of *Fusarium oxysporum* a main soil borne fungal pathogen was reduced by using the soil solarization method in Kerman (Fig.3). Actually, some soil borne pathogens can be highly sensitive to soil solarization method (9). The population density of was *F. oxysporum* decreased quickly after application two weeks soil solarization in studied areas. Results showed that the population density reduced from 100 CFU g^{-1} soil to 300 CFU g^{-1} soil after two weeks. Population density of the pathogen was also decreased to 100 CFU g^{-1} soil after 4 weeks soil solarization technique (Fig. 3).

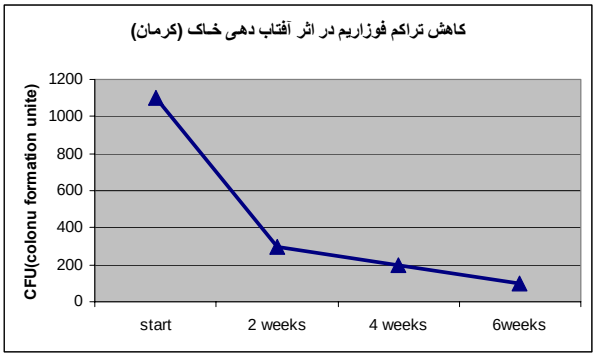

Figure 3: Population density of *Fusarium oxysporum* after 2, 4 and 6 weeks soil solarization method in Kerman province nearly south, Iran

Pre-harvest aflatoxin contamination can also be reduced by applying Integrated Phytosanitary management which seeks to minimize the mould spore count in the orchard and minimize the chances of insect attack (3). Removal or burial of tree litter has been suggested as a measure that would significantly reduce spore count. Post-harvest aflatoxin contamination can occur as a result of harvesting by shaking the tree. This can cause tearing of the hull which can let in spores and allow aflatoxin to be produced. Nuts which are allowed to fall to the ground naturally may also become moldy if they are left on the ground for an extended period. Pistachios are then either harvested by hand or natural fallers are collected daily. Immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *A. parasiticus* on variety of food products (16).

The biocontrol activity of one particular strain of yeast against *A. flavus* was nearly evaluated (13). The effect of this yeast on the growth of *A. flavus* on pistachio flowers and almond leaves was monitored over a period of 2 weeks. Spore production of *A. flavus* was reduced by 60-80% on plant samples sprayed with this particular yeast. The experiments demonstrated that certain strain of saprophytic yeast could modulate *Aspergillus flavus* spore production in leaves and flowers. Field spraying of this effective yeast to almond and pistachio trees may decrease the population of *A. flavus* in the orchards and thus lower the number of nuts infected by this fungus. Growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound. *Aspergillus flavus* is also the second leading cause of aspergillosis in humans. Patients infected with *A. flavus* often have reduced or compromised immune systems.

The epidemiology of *Aspergillus flavus* differs depending on the host species (6,10,11). The fungus overwinters either as mycelium or as resistant structures known as sclerotia. The sclerotia either germinate to produce additional hyphae or they produce conidia (asexual spores), which can be dispersed in the soil and air. Unlike most fungi, *Aspergillus flavus* is favored by hot dry conditions. The optimum temperature for growth is 37°C. But the fungus readily grows between the temperatures of 25-42°C and will grow at temperatures from 12-48°C. Such a high temperature optimum contributes to its pathogenicity on humans.

On the whole, we should emphasis pistachio growers to control infection of *Aspergillus flavus* to be convinced on probability of aflatoxin production on nuts. Of course, it can increase pistachio production and nuts marketing all over the world.

References

1. Al-Ahmad, M. 1993. The solar chamber, an innovative technique for controlling Verticillium wilt of olive. EPPO Bull. 23: 531-535.
2. Bhatnagar, D., K. C. Ehrlich, and T. E. Cleveland. 2003. Molecular genetic analysis and regulation of aflatoxin biosynthesis. Applied Microbiology and Biotechnology, 61 (2):83-93.
3. Campbell, B. C., R. J. Molyneux, and T. F. Schatzki. 2003. Current research on reducing pre- and post-harvest aflatoxin contamination of US almond, pistachio, and walnut. *Journal* of Toxicology-Toxin Reviews, 22 (2-3):225-266.

4. Cleveland, T. E., J. J. Yu, D. Bhatnagar, Z. Y. Chen, R. L. Brown, P. K. Chang, and J. W. Cary. 2004. Progress in elucidating the molecular basis of the host plant - Aspergillus flavus interaction, a basis for devising strategies to reduce aflatoxin contamination in crops. Journal of Toxicology-Toxin Reviews, 23 (2-3):345-380.
5. Danesh, D., H. Mojtahedi, R. Barnett, & A. Campbell. 1979. Correlation between climatic data and aflatoxin contamination of Iranian pistachio nuts. *Phytopathology*, 69(7):
6. Doster, M.A. & T.J. Michailides, 1995. The relationship between date of hull splitting and decay of pistachio nuts by *Aspergillus* species. *Plant Dis.*, 79(8): 766-769.
7. Gilbert, J., and E. A. Vargas. 2003. Advances in sampling and analysis for aflatoxins in food and animal feed. Journal of Toxicology-Toxin Reviews, 22 (2-3):381-422.
8. Ghini, R., W. Betiol, C.A. Spadotto., G. J. de, Moraes, L. C. Paraiba, J. L.de. Mineiro. 1993. Soil solarization for the control of tomato and eggplant Verticillium wilt and its effect on weed and minor arthropod communities. Summa Phytopathol. 19:183-189**.**
9. Gonzalez-Torres, R., J. M. Melero-Vara., J. Gomez-Vazquez. and R. M. Jimenez Diaz. 1993. The effects of soil solarization and soil fumigation on Fusarium wilt of watermelon grown in plastic house in south-eastern Spain Plant Pathology 42 (6), 858–864.
10. Horn, B. W. 2003. Ecology and population biology of aflatoxigenic fungi in soil. Journal of Toxicology-Toxin Reviews, 22 (2-3):351-379.
11. Hua, S.T., C.E. Mcalpin.and S.B. Ly, 2006. Population of *Aspergillus flavus* on pistachio buds and flowers. In: Mendez-Vilas, A., editor. Modern Multidisciplinary Applied Microbiology: Exploiting Microbes and Their Interactions. Weinheim, Germany, Wiley-VCH Verlag. P.440-445.
12. Kumar, V. K. K., Narjrppa and Rama Chandrappa B. K. 2002a. Growth yield and economics of weed control as influence by soil solarization in tomato. Karratak J. Agric. Sci. 15: 682-684.
13. Mishra, H. N., and C. Das. 2003. A review on biological control and metabolism of aflatoxin. *Critical Reviews in Food Science and Nutrition* 43 (3):245-264.
14. Mojtahedi, H., D. Danesh, B. Haghighi, & R. Barnett, 1978. Postharvest pathology and mycotoxin contamination of Iranian pistachio nuts. *Phytopathology*, 68(12): 1800-1804.
15. Nafees B., Najma A., Yasmin A., Muhammad A. and Abdul R. 2007 Soil Solarization: A safe, affective and practicable technique for the control of soil born fungi and nematodes. Pakistan Journal of Biological Sciences 10 (1): 57-64.
16. Scheidegger, K. A. and G. A. Payne. 2003. Unlocking the secrets behind secondary metabolism: A review of *Aspergillus flavus* from pathogenicity to functional genomics. Journal of Toxicology-Toxin Reviews. 22(2-3): 423-459.
17. Singhal, K. K., and H. Kaur. 2005. Aflatoxins in livestock and poultry: A review. *Indian Journal Of Animal Sciences* 75 (1):113-120

راههای کنترل قارچ *آسپرژیلوس فلاووس* مهمترین عامل تولید آفلاتوکسین در پسته به منظور افزایش صادرات این محصول

حسین صارمی*[۱]، سید محمود اخوت[۲]، هانیه صارمی[۳]

چکیده

اصولاً درختان پسته در مناطقی مورد کشت قرار می‌گیرند که از نظر عوامل مهم اکولوژیکی مانند حرارت و رطوبت، مطلوب فعالیت قارچ *آسپرژیلوس فلاووس* می‌باشد. از طرفی میوه پسته حاوی موادی است که نه تنها فعالیت قارچ را بیشتر می‌کند بلکه شرایط را برای تولید متابولیت ثانویه یعنی افلاتوکسین فراهم می‌نماید. با توجه به زیان‌های فراوان افلاتوکسین و تاثیر زیاد آن در کاهش صادرات محصولات پسته جلوگیری از آلودگی پسته به این قارچ ضرورت می‌یابد. طبیعتاً باید از راههای متعددی مانع توسعه این قارچ در باغهای پسته و آلودگی ناشی شد. با اطلاع از بیولوژی و اکولوژی این قارچ و تحقیقات انجام شده راههای زیر کمک زیادی به کاهش جمعیت این قارچ و کنترل نسبی آن در پسته خواهد نمود. استفاده از سموم قارچ‌کش، استفاده از روش کنترل بیولوژیک مانند مخمر *پیشیا آنومالا* برای کنترل *آسپرژیلوس فلاووس*، توسعه کشت ارقام مناسب پسته، از بین بردن بقایای آلوده در زیر درختان پسته و تنظیم روش‌های علمی در برداشت، جمع‌آوری و بسته‌بندی محصول، کمک فراوانی در کاهش آلودگی خواهد کرد.

واژه‌های کلیدی: پسته، کنترل،آسپرژیلوس فلاووس ، افلاتوکسین

۱- دانشیار گروه گیاهپزشکی دانشکده کشاورزی دانشگاه زنجان Saremih@znu.ac.ir

۲- استاد گروه گیاهپزشکی دانشکده کشاورزی دانشگاه تهران

۳- دانشجوی کارشناسی ارشد خاکشناسی دانشکده کشاورزی دانشگاه تهران

* نویسنده مسئول مکاتبات