Research Article

Effects of entomopathogenic *Aspergillus flavus* on tomato plant (*Solanum lycopersicum*) endophytic activity under agro-climatic condition of Lahore, Punjab-Pakistan

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Citation

Amina Abrar, Zarafshan Ali, Tahira Aziz Mughal, Kausar Malik, Samina Sarwar, Muhammad Oneeb, Moneeza Abbas, Husnain Qamar and Rida Nasir. Effects of entomopathogenic *Aspergillus flavus* on tomato plant (*Solanum lycopersicum*) endophytic activity under agro-climatic condition of Lahore, Punjab-Pakistan. Pure and Applied Biology. Vol. 9, Issue 1, pp517-527. <http://dx.doi.org/10.19045/bspab.2020.90057>

Received: 31/08/2019

Revised: 25/10/2019

Accepted: 12/11/2019

Online First: 05/12/2019

Abstract

Entomopathogenic fungi can Control population of various insects. A number of entomopathogenic fungi also have ability to endophytically colonize many plant species. The Endophytic colonization by these fungi provides a source of indirect interactions between fungi and insects. This endophytic activity of entomopathogens may contribute in regulation of insect populations but also can cause diseases and damage to the plants. The entomopathogenic *Aspergillus flavus* is able to colonize different plant species by producing aflatoxins which is more favored in warm temperatures (32-38°C). The present study is aimed to assess the effects of *Aspergillus flavus* on tomato plant (*Solanum lycopersicum*) and to determine its endophytic activity. *Aspergillus flavus* was isolated from soil using insect bait method. The fungal suspension was prepared and applied on tomato plant leaves by spraying. After 7 days significant disease symptoms related to gray mold disease, late blight and leaf mold were observed when compared to plants of untreated control group. Microscopic observations showed 100% colonization of *Aspergillus flavus* on treated leaves. It is evident that the *Aspergillus flavus* can endophytically colonize tomato plant leaves and cause various diseases. So the utilization of entomopathogenic *Aspergillus flavus* to control insect population needs further research investigation for its environmental safety to non-target organisms.

Keywords: Aflatoxins; Endophytic; Entomopathogen; Non-target

Introduction

The number of entomopathogenic fungi forms a total of approximately 750 species from 85 genera that are found in all classes

of fungi. In almost every order of insects and taxa of arthropods these pathogens can cause mycoses. These fungi are commonly found in terrestrial habitats. However, there

are minimal effects of entomopathogenic fungi on non-target organisms and these fungi provide safer alternative to chemical insecticides [1]. Entomopathogenic fungi differ from other microorganisms in causing insect diseases because they infect the insects by penetration through their cuticle, insects do not need to ingest them and have a great potential for controlling the sucking insects such as aphids [2]. The mechanism of pathogenesis of these fungi starts particularly in the areas of formation of an infection structure, entry into the host and toxin-mediated host death [3].

Aspergillus flavus have worldwide distribution and can infest a wide range of agricultural products. *Aspergillus flavus* produces aflatoxins. The growth of *Aspergillus flavus* and synthesis of aflatoxins depends on substrate, pH, temperature, moisture, aeration and competing micro-flora [4]. *Aspergillus flavus* produces airborne conidia, which easily disperse by air movement or by insects. *Aspergillus flavus* grows better with water activity and the optimum temperature for the growth of *Aspergillus flavus* ranges from 12°C to 48°C [5]. Although most of the entomopathogenic fungi have no adverse effects on non-target organisms but some strains of *Aspergillus flavus* are found to be an unspecialized saprophyte along with its pathogenic potential against various insects [6, 7]. *Aspergillus* species are considered as opportunistic pathogens that take advantage of an opportunity not normally available, such as a host with a weakened immune system, an altered micro-biota for colonization [8]. Fungal entomopathogens are capable of naturally controlling the populations of various insects. A wide range of insects pests in the agricultural crops are efficiently biologically controlled by entomopathogenic fungi [9]. Entomopathogenic fungi infect insect in all stages of life cycle from egg to adult [10, 11]. The production of secondary metabolites varies among the different

strains of same species of entomopathogenic fungus and affect differently against various target and non-target organisms [12]. *Aspergillus flavus* has been reported entomopathogenic but due to its non-specificity of host it can also affect various non-target organisms [13].

The main objective of the study was to evaluate the safety of entomopathogenic *Aspergillus flavus* isolated by employing insect bait method, against tomato plant (*Solanum lycopersicum*) to explore its potential as safe alternative to chemical insecticides.

Materials and methods

Plant material

The experiment was conducted at Lahore College for Women University (LCWU), Lahore, Pakistan after approval of university Board of Studies. The plants were acquired by local nursery in Lahore, Pakistan to assess the safety of *Aspergillus flavus* against tomato plant.

Isolation of entomopathogenic fungi from soil

The collection of soil samples was done from Jallo Park, Lahore from 10 inches depth. For the isolation of *Aspergillus flavus* the insect bait method was used [14]. Baiting of soil sample was done using *Culex pipiens* to isolate indigenous species of entomopathogenic fungi. *Aspergillus flavus* species was cultivated using Potato Dextrose Agar (PDA) which ensures its mycelial growth and maintains the other growth factors. The *Aspergillus flavus* colonies were fully grown after 5-6 days. All procedures were carried out at room temperature i.e. 25 to 30°C. *Aspergillus* species grown on PDA media were observed (with naked eye) for identification of morphological characteristics. Initially the colony growth was low and mycelium acquired white color. The mycelium acquired green to olive green color after 3-5 days of incubation and also the colony growth was increased. The colony was plain flat at the edges but elevated in the centers [15]. Scanning electron microscopy of conidia

was also performed (Fig. 1). The conidial heads observed under microscope typically radiate longitudinally, conidiospores are uncolored, coarsely roughened, approximately less than 1mm long. The samples were further identified for macroscopic and microscopic morphological characteristics by the experts. The *Aspergillus flavus* conidia are hydrophobic in nature. To prepare a conidial suspension, 0.01% tween80 solution was added to ensure its uniform mixing in water.

The conidial suspension of *Aspergillus flavus* was prepared to count the no. of conidia per ml on hemocytometer (MARIENFELD SUPE-RIOR, Germany) using the microscope (NIKON DS-L2, Japan) and for further dilutions needed for the experimentation. The conidia of *Aspergillus flavus* were counted on the 25 grid chamber. Three dilutions of 10^5 , 10^6 and 10^7 were prepared from the conidial suspensions as per the requirement of experiment.

Application of *Aspergillus*

***flavus* suspension on tomato plant**

24 tomato plants were obtained out of which 18 plants were allocated to experimental group. Three experimental groups with 6 plants in each group and 6 plants as the control group were used for the study. External contamination and fungal suspension runoff was prevented by covering the base of plant by aluminum foil and plastic cover. Four treatments were used in the test, 0.01% tween80 solution for control group and three different concentration of conidial suspension 10^5 , 10^6 and 10^7 . 3ml from each suspension solution was taken in the sprayer and applied onto the leaves of plants one by one. The humidity of plants was maintained. After 7 days observation the leaves were randomly selected from each plant. Each leaf from experiment group and control group plant was disinfected. Six leaves per treatment were disinfected by first washing them with running tap water and then submersing them in 70% ethanol

for 15 sec, followed by 20 min in 5% sodium hypochlorite and rinsing 10 times in sterile distilled water. Leaves were cut to form small leaf sections (3 sections of each leave) of 1 x 1 cm. Each leaf section was placed on PDA plate and kept in fume hood. The data was expressed as colonization frequencies.

Colonization frequency = 100 x No. of plant sections colonized/ Total No. of plant sections

The morphological characteristics of conidia grown on treated leaves were microscopically examined by experts to identify the incidence of *Aspergillus flavus* grown on treated tomato plant leaves.

Results

After 1 day of exposure no significant changes were observed in plants. *Aspergillus flavus* did not cause any physical change in the plant. After 4 days of exposure slight changes were observed in plants. Leaf curling and formation of small greenish-yellow spots near the margins of leaves were observed in 10^7 treatment plant leaves which showed that *Aspergillus flavus* has started its endophytic activity. After seven days significant changes were observed on the leaves of all treatment plants using checklist.

Morphological change

After 7 days of the exposure of plants by fungus (*Aspergillus flavus*) various morphological changes were observed. Dark brown blotches on the upper leaves surface turned black because of mold growth on them (Fig. 2) showed the prevalence of gray mold disease. After that, all leaves of plant wilted and died. Appearance of dark brown spots with slightly green edges which resulted in the dark brown dry foliage (Fig. 3) showed the presence of late blight disease. Greenish-yellow spots which turned brown at the margins or tip of the leaves were observed which indicates the presence of leaf mold disease, a specific fungal disease (Fig. 4).

Endophytic effect on plant growth

Endophytes significantly reduced the growth of plants. It did not cause direct

mortality of plants rather than prevalence of different diseases in plants were observed which resulted in causing mild to severe infections in plants demonstrating the pathogenicity of *Aspergillus flavus* (Table 1). Differences in endophytic

fungal effect in different treatment group tomato plants were observed (weakened or damaged leaves were affected at first), compared with the plants of control group which has shown no endophytic effects (Fig. 5).

Figure 1. Scanning Electron Microscopy (SEM) of isolated *Aspergillus flavus*

Figure 2. Symptoms observed related to gray mold disease in treated tomato plants

Figure 3. Symptoms observed related to late blight disease in treated tomato plants

Figure4.Symptoms observed related to leaf mold disease in treated tomato plants

Table 1. Symptoms observed in treated plants related to diseases caused by *Aspergillus flavus*

Diseases	Symptoms observed in plants
Gray mold	Irregular brown blotches on the leaves which turns into black and plant died
Late blight	Dark brown spots with slight green edges resulting in dark brown dry foliage
Leaf mold	Greenish-yellowish spots on the upper side of leaves at the margins and turn brown

Figure5. Comparison of treated plants with control group plants(A: Control group plant physical conditionB: Treated Plant physical condition)

Colonization of tomato plant leaves with *Aspergillus flavus*

There was no fungal (*Aspergillus flavus*) growth on leaf sections of all treatment plants placed in cultural plates after 1 day. After 5 days 100% colonization frequency was observed on leaf sections of all treatment plants placed in cultural plates. They were colonized by *Aspergillus flavus*. The growth rate of conidia on exposed leaves was different and it was observed further. After 10 days there was no

substantial further growth of *Aspergillus flavus* colonies.

Morphological and microscopic examination of recovered *Aspergillus flavus* colonies

The slide containing the fungal conidia grown by taking exposed leaves on PDA plates was microscopically observed and round shaped conidia were seen. It clearly indicated the presence of *Aspergillus flavus* conidia on exposed plant leaves. The conidial heads observed under microscope

typically radiate longitudinally, conidiospores are un-colored, coarsely roughened, and approximately less than 1mm long. The samples were further identified for macroscopic and microscopic morphological characteristics by the experts. The severity level varied among

different treatment plants. Moreover, this disease was more prevalent than late blight in all treatment plants. Severity level of gray mold, late blight and leaf mold disease in 10^7 , 10^6 , and 10^5 treatment plants is graphically presented (Fig. 6, 7, 8).

Figure 6. Severity level of gray mold, late blight and leaf mold disease in 10^7 treatment plants

Figure 7. Severity level of gray mold, late blight and leaf mold disease in 10^6 treatment plant

Figure 8. Severity level of gray mold, late blight and leaf mold disease in 10^5 treatment plant

Discussion

The safety of *Aspergillus flavus* was evaluated against tomato plant (*Solanum lycopersicum*). Endophytic effects of *Aspergillus flavus* on tomato plant leaves were evaluated and different symptoms related to fungal plant diseases were observed. Tomato plant is cultivated worldwide as an important vegetable crop. Tomato contains minerals and vitamins. Tomato production is declining due to environmental stresses, declining soil fertility, poor crop management and low quality seeds. The yields are also reduced because of the increasing pests and disease pressure [16].

During the present study, it was observed that beside entomopathogenic nature of *Aspergillus flavus*, it is capable of affecting the non-target organisms including plants, when it was inoculated in tomato plant leaves. As it has been reported, *Aspergillus* species are known to cause disease in plants, insects, and other animals. For example, *Aspergillus flavus* causes disease of economically important crops, such as corn and peanuts. However, strains of *Aspergillus flavus* are not host specific [8]. After the exposure of tomato plants by *Aspergillus flavus* suspension, it started its endophytic effect by causing some physical changes in tomato plant. The number of possible pathways for natural colonization of plants by entomopathogens are through roots, stem, seeds and leaves. In present study, the leaf spraying technique for inoculation of entomopathogens in tomato plant was selected. When inoculated tomato plant leaves were placed in PDA plates the highest rate of colonization was noticed just after 5 to 7 days. A previous research study demonstrated that *Beauveria bassiana*, when inoculated either by leaf spraying, root dipping or seed immersion, was effectively established as an endophyte in tomato plants and was reisolated from leaves 7, 14 and 28 days after its inoculation. Leaf spraying was the most effective inoculation technique and the

highest percentage of colonization was recorded 7 days after inoculation. The endophytic colonization, estimated by the percentage of recovery of *Beauveria bassiana* after inoculation, decreased over time [11].

The current study showed that the *Aspergillus flavus* can grow and start its endophytic activity at warm temperatures 30 to 40^o C. At first, it attacked the weakened or damaged leaves. As it has been reported earlier that hot, dry conditions cause extensive colonization of peanut fruit by the *Aspergillus flavus* group, a prerequisite for aflatoxin production, when most associated microorganisms failed to grow (because temperature or water activity or both became limiting) or grew only weakly [17]. In North Carolina, 73% of kernels were infected due to silk inoculation of corn grown at 32 to 38°C in the greenhouse, whereas 2.5% of the kernels were infected in corn grown at 21 to 26°C [18].

It was observed in the present study that humidity factor was much important for the endophytic activity of *Aspergillus flavus* thus we maintained that. The diseases were showing more severe physical changes due to humidity. Following studies has reported that during the rainy season higher levels of aflatoxins were observed than in winter, which may be due to favorable environmental conditions in rainy season, i.e. suitable moisture and temperature [19]. The peanuts were not artificially inoculated, but a natural seed infection of 2-3% by *Aspergillus sp.* was present in almost all genotype of the *Aspergillus flavus*. After 9-10 days of storage in 87-95% of relative humidity all genotypes had appreciable levels of aflatoxins [20].

In present study, brown wilted leaves after 5 to 7 days of fungal inoculation showed dark brown blotches which turned black over time. Damaged or old leaves were found to be more affected. The leaves died after 7 days. These observed symptoms were similar to the symptoms of gray mold

disease. Identification of the factors behind this effect was not clear. It has been reported that gray mold causes brown, water soaked spots or decay on leaves or petals. Tiny, almost translucent spots appear as the first symptoms. Infected terminals and stems may girdle and rot. Wilting of diseased tissues and death of infected seedlings occurs. Active, healthy tissues except petals are seldom infected directly. Most crops are attacked on the weakened (such as cutting stubs and tissue damaged by other pathogens), or injured, old, or dead parts. Primary limiting factor of this disease is moisture. Only after about 6 or more consecutive hours in contact with water, including free moisture from splashing, condensation or where relative humidity is higher than about 90%, Gray mold spores germinate and produce new infections. The optimum temperature for gray mold development is 16⁰C to 25⁰C [21].

It has also been reported in another study that characteristics circular or oval spots from yellowish to reddish brown in color is caused by infection of leaves. The spots dry out in dry weather and fungus stops spreading. When the weather is moist, the spots run together so that the whole leaf may be blighted. Stems also are attacked and when disease has progressed sufficiently the stem may break over at the point of infection [22].

After 7 to 10 days of leaf spraying some changes were observed in physical health of plant. Brown spots on leaves with slightly greenish boundary were present which was similar to the symptoms of late blight disease. The effect increased in high humidity. As it has been reported in recent study that late blight is serious fungal disease. The disease is detected by the first shown symptoms that are often present on lower leaves. They consist of small, pale to dark green spots that change into brown or black lesions, depending on the humidity of the air. Lesions start frequently at the leaf tips and margins. The dead and healthy tissues are separated by a pale green or

yellow border, a few millimeters wide. Sporulation may be visible at lower surface of leaves as a white mildew surrounding the lesions. Leaves may drop off [23]. It has been illustrated in a book that due to late blight disease in tomato plant dark spots will appear on stems or leaves. Death occurs within 2 or 3 days after their appearance. On mature plants blight starts with dark, water-soaked leaf spots and large dark brown spots on fruits, with most of the leaves soon hanging lifeless and fruit falling on the ground and rotting [24].

It was observed in present study that after 7 to 10 days of exposure greenish-yellowish spots at the tips of the leaves were enlarged over time. The severity levels among different treatment plants were different. In previous studies similar physical changes in plants were observed due to fungal infection and those changes were reported as symptoms of leaf mold disease. Disease symptoms are usually limited to the foliage and appear on the upper surfaces of leaves as pale green or yellow spots with indefinite margins. Patches of velvety, olive-brown fungus growth can be seen on the lower surfaces. As the disease progresses, the leaf spots turn yellowish brown. The leaves curl, wither, and drop prematurely. Defoliation starts at the bottom of the plant and progresses upward [25]. According to another investigation similar symptoms related to leaf mold disease were observed just after 5 to 7 days of fungal inoculation as noticed in our study. Necrotic lesions were produced at the site of inoculation within 5 to 7 days. From the lesions, the development of discontinuous streaks of necrotic tissue on stem and uppermost leaves of the plants became necrotic. The foliar symptoms included epinasty of the petiole with inward rolling and angular interveinal necrotic areas that developed on one or both sides of the midvein [26].

The minor pathogen of corn, peanuts and cotton is *Aspergillus flavus*. In corn, *Aspergillus flavus* causes an ear rot. In peanuts, it causes a seedling disease known as yellow mold of seedlings or aflaroot. The

symptoms comprise of necrotic lesions, chlorosis on above-ground parts and lack of development of secondary roots, 'aflaroot' [27]. The root effect may be due to aflatoxin toxicity as it has been shown to inhibit root hair development in tobacco. *Aspergillus flavus* may also cause a mature peanuts rot in the soil. In cotton, cotton quality is affected by *Aspergillus flavus* by causing boll rot [28]. Species of *Aspergillus* in the *Aspergillus flavus* group frequently causes mold of peanuts, *Arachis hypogaea* L., and contamination with aflatoxins. When peanuts are stored under high humidity then it favors the growth of the fungus and this increases the potential for aflatoxins production [20]. Inoculation of exposed silks of two field-grown commercial dent cultivators 0, 1, 2, and 4 week after silk emergence resulted in infection and aflatoxins production [18].

In present study, *Aspergillus flavus* was found to colonize the tomato plant. Although colonization was greatest in 10^7 treatment plants. The low rate of colonization was seen in 10^5 treatment plants. A significant change was observed in plant physical health when compared to control plant although no colonization was observed in control plants. As it has been reported that three entomopathogens *Beauveria bassiana*, *Lecanicillium lecanii*, and *Aspergillus parasiticus* were found to endophytically colonize a range of crop plants and could be isolated from inoculated leaves of cotton, bean, corn, tomato, pumpkin, and wheat. Although colonization of leaves was generally greatest in the most recently inoculated leaves, it consistently declined thereafter [29].

Conclusion

In agro-ecosystem Entomopathogens are one of the natural enemies of insect pests. *Aspergillus spp.* causes disease in a wide range of organisms, in plants, insects, and other animals. Our results show that *Aspergillus flavus* has parasitic attributes that is not specialized to a particular host. This study revealed significant data of the

interaction between *Aspergillus flavus* and plants through which *Aspergillus flavus* hinders plant growth and they cause impact on plant physiology. The symptoms related to late blight, leaf mold and gray mold disease were observed. This present work showed that *Aspergillus flavus* could infect tomato plant by contacting directly with conidia. A higher mortality rate was shown because of direct contact by leaf spraying. As it has been reported entomopathogenic; but the endophytic colonization of tomato plant leaves and tissues by *Aspergillus flavus* and its capacity to infect provides the basis for further investigation, on the virulence of the endophytic *Aspergillus flavus*.

Authors' contributions

Conceived and designed the experiments: A Abrar, Z Ali & T Mughal, Performed the experiments: Z Ali, H Qamar & R Nasir, Analyzed the data: S Sarwar, M Oneeb & A Abrar, Contributed materials/ analysis/ tools: K Malik, T Mughal & M Abbas, Wrote the paper: Z Ali & A Abrar.

References

1. Shahid AA, Rao QA, Bakhsh A & Husnain T (2012). Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. *Arch Biol Sci* 64(1): 21-42.
2. Pedrini N, Crespo R & Juarez MP (2007). Biochemistry of insect epicuticle degradation by entomopathogenic fungi. *Comp Biochem Physiol C Toxicol Pharmacol* 146(1-2): 124-137.
3. Clarkson JM & Charnley AK (1996). New insights into the mechanisms of fungal pathogenesis in insects. *Trends Biotechnol* 4(5): 197-203.
4. Gourama H & Bullerman LB (1995). *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds: A review. *J Food Prot* 58(12): 1395-1404.
5. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P & Denning DW (2007). *Aspergillus flavus*: human pathogen,

- allergen and mycotoxin producer. *Microbiol* 153(6): 1677-1692.
6. Diener UL, Cole RJ, Sanders TH, Payne GA, Lee LS & Klich MA (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annu. Rev. Phytopathol* 25(1): 249-270.
7. Mellon JE, Cotty PJ & Dowd MK (2007). *Aspergillus flavus* hydrolases: their roles in pathogenesis and substrate utilization. *Appl Microbiol Biotechnol* 77(3): 497-504.
8. Leger RJS, Screen SE & Shams-Pirzadeh B (2000). Lack of Host Specialization in *Aspergillus flavus*. *Appl Environ Microbiol* 66(1): 320-324.
9. Shoukat RF, Freed S & Kanwar WA (2018). Assessment of binary mixtures of entomopathogenic fungi and chemical insecticides on biological parameters of *Culex pipiens* (Diptera: Culicidae) under laboratory and field conditions. *Pak J Zool* 50(1).
10. Gindin G Samish, M Zangi G, Mishoutchenko A & Glazer I (2002). The susceptibility of different species and stages of ticks to entomopathogenic fungi. *Exp Appl Acarol* 28(1-4): 283-288.
11. Allegrucci N, Velazquez MS, Russo ML, Perez E & Scorsetti, AC (2017). Endophytic colonisation of tomato by the entomopathogenic fungus *Beauveria bassiana*: the use of different inoculation techniques and their effects on the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). *J Plant Prot Res* 57(4): 206.
12. Greene-McDowelle, DM, Ingber B, Wright MS, Zeringue Jr HJ, Bhatnagar D & Cleveland TE (1999). The effects of selected cotton-leaf volatiles on growth, development and aflatoxin production of *Aspergillus parasiticus*. *Toxicon* 37(6): 883-893.
13. Kumar S, Riffat S & Wagan MS (2013). Pathogenic Application of *Aspergillus* species for the control of agricultural important grasshoppers. *JBES* 3(12): 223-229.
14. Meyling NV & Eilenberg J (2007). Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. *Biol Control* 43(2): 145-155.
15. Thatana MG, Murage H, Abia ALK & Pillay M (2017). Morphological Characterization and Determination of Aflatoxins-Production Potentials of *Aspergillus flavus* Isolated from Maize and Soil in Kenya. *Agric* 7(10):80.
16. Omukoko CA, Turoop L & Wekesa VW (2017). Laboratory assessment of red spider mites (*Tetranychusevansi*) damage on tomato plants enhanced with *Beauveria bassiana*. *AJHS* 12: 8-15.
17. Hill RA, Blankenship PD, Cole RJ & Sanders TH (1983). Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. *Appl Environ Microbiol* 45(2): 628-633.
18. Jones RK, Duncan HE, Payne GA & Leonard KJ (1980). Factors influencing infection by *Aspergillus flavus* in silk-inoculated corn. *Plant Dis* 64(9): 859-863.
19. Raghavender CR, Reddy BN & Shobharani G (2007). Aflatoxin contamination of pearl millet during field and storage conditions with reference to stage of grain maturation and insect damage. *Mycotoxin Res* 23(4): 199-209.
20. Wilson DM, Mixon AC & Troeger JM (1977). Aflatoxin contamination of peanuts resistant to seed invasion by *Aspergillus flavus*. *Postharvest Pathol and Mycotoxins* 67(7): 922-924.
21. Dreistadt SH (2012). Integrated Pest Management for Citrus. 3rd Ed. Agriculture and Natural Resources

- Publications 3303; University of California (California), pp 61.
22. Pirone PP (1978). *Diseases and pests of ornamental plants*, 5th Ed. John Wiley & Sons, New York. pp 342.
 23. Henfling JW (1987). *Late blight of potato* (Vol 4), International Potato Center, Peru. pp 6.
 24. Horst RK (2013). *Westcott's Plant Disease Handbook*, 5th ed. Springer Science& Business Media, New York. pp 140.
 25. Iida Y, Iwadata Y, Kubota M & Terami F (2010). Occurrence of a new race 2.9 of leaf mold of tomato in Japan. *J Gen Plant Pathol* 76(1): 84-86.
 26. Gilchrist DG & Grogan RG (1976). Production and nature of a host-specific toxin from *Alternaria alternata* f. sp. *lycopersici*. *Phytopathol* 66(2): 165-171.
 27. Stack JP & Pettit RE (1984). Germination of *Aspergillus flavus* Sclerotia in Soil. In *Phytopathology. Amer Phytopathological Soc* 74(7): 799-799.
 28. Klich MA (2007). *Aspergillus flavus*: the major producer of aflatoxin. *Mol Plant Pathol* 8(6): 713-722.
 29. Gurulingappa, P Sword, GA Murdoch G & McGee PA (2010). Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. *Biol Control* 55(1): 34-41.