Mycopath (2019) 17(2): 69-78 **Research Article**

Infection mechanism of *Aspergillus* and *Fusarium* species against *Bemisia tabaci*

Farman Ahmad[1], *Waheed Anwar[2], Muhammad Asim Javed[2], Ramsha Basit[2], Adnan Akhter[2], Sajid Ali[2], Hafiz Azhar Ali Khan[2], Huma Amin[2] and Muhammad Saleem Haider[2]

[1]School of Food and Nutrition, Minhaj University Lahore
[2]Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan, 54590
[*]Corresponding author's email: waheedanwar.iags@pu.edu.pk

Abstract

Entomopathogenic fungi are emerging as an effective management method as compared to potential snags created by chemical insecticides that includes environmental pollution and the development of resistance. Many entomopathogenic fungi are in use as a bio-control agent while different insect associated fungi are isolated from insects. The goal of this study was to check the infection mechanism of *Fusarium* and *Aspergillus* species on 4th instar of *Bemisia tabaci* isolated from insects previously. Both genera showed the efficient results against 4th instar of *B. tabaci* with growth retardation and severe infection in insect body. Directly spores of *Fusarium* and *Aspergillus* species were applied on 4th instar of *B. tabaci* and data regarding duration of spore germination, penetration, colonization and infection was determined under control conditions. It was observed that spores of *Aspergillus* species germinate quickly as compare to *Fusarium* species. There was no significant difference in spore germination on species level. Penetration time of *Aspergillus* and *Fusarium* species vary and colonization and infection duration of *Aspergillus* species was less as compared to *Fusarium* species used in this study. In this study, it was observed that *Aspergillus* is more aggressive as compare to *Fusarium* and also it depends on the isolates of insects.

Keywords: Aggressiveness, *Bemisia tabaci,* Entomopathogenic fungi, Infection, Mechanism.

Introduction

The whitefly *Bemisia tabaci* Gennadius (Hemiptera: *Aleyrodidae*) is considered as a chief pest of economically key crops (Xu *et al*., 2012*) B. tabaci* as a polyphagous insect has a wide hosts range and it can affect more than 500 species and 74 families of the plants (Greathead, 1986), both in field and in greenhouses (Agro *et al.,* 2007; Anwar *et al.,* 2019).It is a complex species which is composed of more than 35 morphologically indistinguishable cryptic species which exhibit a wide range of genetic variations and many species of this complex show a specific pattern of geographic distribution (Liu *et al*., 2012; Tay *et al*., 2012). As vicious pests, they are involved to damage the quality and quantity of agricultural crops. They usually damage plants by feeding and sucking sap from plants, secreting honey dews and transmission of more deadly plant viruses. Over 111 different plant viruses, primarily begomoviruses (family Gemniviridae) have been transferred by *B. tabaci* (Fortes *et al*., 2016).

Considering the management strategies to control whitefly, many control measures as, use of predators and chemical insecticides are in practice from the last few decades (Palumbo *et al*., 2001). The overuse of insecticides has enhanced the selection of pressure and also generate resistance in most of insects. Owing to the broad host ranges and resistance towards many pesticides, there is a huge need to use an alternative method to control whitefly. For the efficient control of insect's pest; bio-control is considered as an efficient tool as it has no environmental effect. Taking in account the feeding strategies of white fly, the only pathogen that can penetrate the cuticle is considered as viable potential bio-control agent and fungi have been reported as one of them (Agro *et al* ., 2007). From the last few years, entomopathogenic fungi have been demonstrated to control various type of insects including whitefly, aphids, mites and weevils (Scholte *et al.,* 2004; Zhang *et al.,* 2018). Almost 20 different species of entomopathogenic fungi have been reported yet that can infect *B. tabaci* (Lacey *et al*., 1995; Steenberg and Humber, 1999). Several studies have demonstrated that many of entomopathogenic fungi as *Paecilomyces fumosoroseus, Lecanicillium muscarium, Beauveria bassiana, Metarhizium anisopliae* have been widely used to infect many of economic insect pests (Poprawski *et al*., 2000, Scholte *et al.,* 2003a, Cuthbertson *et al*., 2012).

Entomopathogenic fungi usually infect their host through a special pathogenicity mechanism that involved the successful adhesion, germination, differentiation and penetration of fungal hyphae. The success of infection usually depends upon adhesion of spore. Mostly, the entomopathogenic fungi secrete many enzymes as chitinases, lipases and proteases that hydrolyze the epidermis of insect (Boucias *et al.,* 1998). The successful adhesion and penetration of fungi in the insect cuticle usually cause the death of

insects by limiting the nutrient resources, hydrolyzing the insect cuticle and secretion of toxin in insect's body (Smith *et al*., 1981). Till the crisis of *B. tabaci* from last few years, *Fusarium* is found as saprophytes on dead insects (Sun, 2008). Different experimental studies have been conducted on *Fusarium* and it is well documented that the toxins of penetrating hyphae can kill many insect pests (Gupta *et al*., 1991). Many of the *Fusarium spp* has been found an effective agent against glasshouse whitefly (*Trialeurodes vaporariorum),* spruce budworm (*Choristoneura fumiferana*) and wheat stem sawfly (*Cephus cinctus*) (Eleonora- Rojas *et al*., 2003; Strunz and Strongman, 1988; Sun, 2008). Besides *Fusarium, Aspergillus* is considered as one of important entomopathogenic fungi due to its potential to secrete various enzymes and toxins that can hydrolyzed the insect cuticle and lead to death of insects (Moraes *et al*., 2001b). The objective of this paper was to emphasize and find out the on bio-control mechanism of different species of entomopathogenic fungi *Aspergillus* and *Fusarium* against whitefly.

Materials and Methods

Insect rearing

The counted *B. tabaci* was inoculated on cotton plants for rearing in cages and took observations on daily basis (Fig. 1A and B). The life cycle of *B. tabaci* from egg to adult was completed within 2 to 3 weeks in warm weather (Butler *et al*., 1983). The rate of reproduction of *B. tabaci* varies with the host plant and weather condition (Arnal *et al*., 1993). The females deposited their eggs on the underside of leaves which were clustered. The number of eggs deposition per female was 50–400 eggs (average 160 eggs). The eggs were small, 0.2 mm long and 0.1 mm in width, elliptical in shape, elongated toward the unattached end. Newly laid eggs were smooth and whitish-yellow in color but near hatching turned brown as in literature (Butler *et al*., 1983). After hatching, first instar or crawler was flattened, oval in shape, attached underside of leaf near empty egg shell. It spent three more molts there. At third & fourth instar, the prominent eye spots were started to develop and referred as red-eyed instar. These four immature instar stages were thin, flat, elliptical, and greenish-yellow in color. The nymphal stages can be distinguished by several morphological characters and easiest by size (Lopez-Avila, 1986). After larval stages *B. tabaci* entered into pupal stage having prominent eye spots, it was yellow in color, 0.7 mm long, oval in shape with round external margin (Butler *et al*., 1983). When the adult *B. tabaci* was emerged from the pupa, it left a distinctive T-shaped dorsal split in the pupal case. The adult of *B. tabaci* adult had a small size with 0.8 mm body length and white wings as a roof over its pale yellow body. It inhibits and feeds the plant sap by sucking the lower surface of the host plant leaves with piercing- sucking mouthparts. The insect's snow-white color was attributed to the secretion of waxy powder on its body and wings (Hoelmer *et al*., 1991). The adult life span was ranged from 6 to 55 days depending on temperature. Reproduction may occur with or without copulation. Unmated females can reproduce by parthenogenesis in which the female produce only male progeny (Butler *et al*., 1983).

Isolation of fungal strains

The fungal isolates originally isolated from insects were selected and obtained from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agriculture Sciences (IAGS) Punjab University Lahore (Table 1). These cultures were preserved at 4 ^{o}C in FCBP.

Revival of fungal strains

Already preserved cultures were revived on Sabouraud dextrose agar + yeast extract (SDAY/4) media plates. For the preparation of media, 10 g dextrose, 2.5 g neopeptone, 2.5 g yeast extract and 15 g agar were added in 1.0 L distilled water. The pH was adjusted to 6.5 before autoclaving the media. After autoclaving, streptomycin injections were added in media as antibacterial before pouring into sterilized petriplates. The cool media was poured in the sterilized petriplates in the laminar flow under the sterilized conditions and kept it for hardening in petriplates. After that fungal cultures were inoculated by heat sterilized needle on SDAY/4 media plates in the laminar flow under the sterilized condition. After labeling, the inoculated media plates were incubated at 28 ^{o}C ± 2 for a week for the proper growth of fungi.

Spore suspensions of fungal strains

After seven days of incubation, pure and proper growth of fungus cultures was obtained (Fig. 2 A and B). For spore suspension, spores were harvested from fungal plates and shifted into eppendorf tubes of 1.5 mL volume having 1.0 mL solution of autoclaved distilled water and tween 80 (Fig. 2 C and D). Eppendorf tubes were labeled and vortex to homogenize the spores.

Spore counting

The spore counting was done under the microscope by using Hemocytometer. Mass per volume concentration equation which was used for the calculation of the concentration of spores was given below:

$$\text{Concentration (spores per mL)} = \frac{\text{No. of spores}}{\text{Volume in mL}}$$

The Dilution equation which used for the preparation of the different spore concentrations was given below:

Spore Concentration = (Total number of spores in concentration) × (Required concentration of spores) × (Required volume of spores) / (Total volume of

spore concentration)

Different strains of *Fusarium* and *Aspergillus* fungus species had different numbers of spores.

Bioassays

For the infectivity of different spore concentrations of different fungal isolates, 4th instar nymphal life stage of *B. tabaci* were selected. For nymphal stage, the *B. tabaci* infected cotton leaves were cut from the cotton plant, washed the upper surface of leaves by autoclaved distilled water for removing the *B. tabaci* sugary secretions which cause sooty molds. The under surface of cotton leaves were observed under the stereomicroscope of 150X in Insectary laboratory of Institute of Agricultural Sciences, Punjab University Lahore. The eggs, instars, pupa and adult of *B. tabaci* were present on the under surface of leaves. Except 4th instar stage, everything was removed from the leaf with the help of camel hair brush under the stereomicroscope of 150X. The leaves were cut into pieces by a sterilized scissor; each piece contained 5 nymphs at 4th instar life stage. The autoclaved filter papers were fitted in the Petri plates and were moistened by autoclaved distilled water. The small pieces of leaf containing 4th instar nymphs of *B. tabaci* were placed in the Petri plate on moist filter paper. Different leaf pieces were inoculated by different fungal culture spore suspensions in Petri plates. Each fungal culture spore suspension had three replicates. The spore concentrations 2.5×10^2 of each fungal culture were applied to the replicates. The Petri plates were labeled according to fungal spore suspension and their replicates. Three replicates of control were also applied for comparison. In control distilled water along with tween 80 (5%) were applied. The filter papers were moistened to keep the pieces of leaves fresh by applying autoclaved distilled water (Fig. 3).

Determination of infection steps of *Fusarium* and *Aspergillus*

To study the infection process of *Fusarium* and *Aspergillus* isolates, viable spores of different isolates of *Fusarium* and *Aspergillus* after viability test were applied on nymphal stage of *B. tabaci*. The adhesion or attachment was the first and most important part of infection process and necessary for successful infection. Duration of germination time of spores, duration of hyphal invasion through the body parts of the nymphs, duration of colonization of spores inside or outside of the nymph body and the duration of infection in nymphs were calculated after observing constantly the treated nymphs and adults of *B. tabaci* under stereomicroscope of 150X. Whole mechanism from adhesion to infection of *Fusarium* and *Aspergillus* isolates on nymphs of *B. tabaci* were repeated thrice to avoid error chances (replicates). Total infection steps and time duration for these steps in hours were measured and calculated.

Data analysis

Duncan Multiple Range Test (DMRT) was applied through Statistix 8.1 software to analyze the data.

Results and Discussion

Duration of spore germination

When spores of different isolates of fungi were applied on nymphal stage of *B. tabaci*, they took different time to germinate on body of insect. Duration of *Aspergillus* species was different from *Fusarium* species. The *Aspergillus fumigatus* (FCBP. 1383), *A. fumigatus* (FCBP. 1386), *A. fumigatus* (FCBP. 1395), *A. fumigatus* (FCBP. 1398), *A. nidulans* (FCBP. 1384), *A. flavus* (FCBP. 1388) and *Fusarium* sp. (FCBP. 1413) spores were germinated after 48 h of inoculum application. In the case of *A. fumigatus* (FCBP. 1392), *Fusarium* sp. (FCBP. 1407), *Fusarium* sp. (FCBP. 1376), *Fusarium incarnatum* (FCBP. 1301), *F. oxysporum* (FCBP. 1304), *F. eqiuseti* (FCBP. 1302), *F. solani* (FCBP. 1401), *F. eqiuseti* (FCBP. 1389) and *F. eqiuseti* (FCBP. 1299) spores were germinated after 72 h of inoculum application. The spore germination rate of *Aspergillus* species were rapid as compared to *Fusarium* species at 25 ºC temperature and 55% humidity (Fig. 4).

Penetration point and duration of Penetration

The penetration point and duration of penetration were different for different fungus cultures of *Aspergillus* and *Fusarium*. The *A. fumigatus* (FCBP. 1383) had rapid penetration. The hyphae of *A. fumigatus* (FCBP. 1383) were penetrated by the outer body wall of *B. tabaci* after 60 h of inoculum application. The hyphae of *A. fumigatus* (FCBP. 1386) and *A. fumigatus* (FCBP. 1395) were penetrated from mouth and outer body wall of *B. tabaci* after 72 h of inoculum application. The hyphae of *A. fumigatus* (FCBP. 1398) and *Aspergillus flavus* (FCBP. 1388) were penetrated by the outer body wall of *B. tabaci* after 72 hours of inoculum application. The hyphae of *F. eqiuseti* (FCBP. 1302) were penetrated by body wall after 84 hours of inoculum application. The hyphae of *A. fumigatus* (FCBP. 1392), *Fusarium* sp. (FCBP. 1407), *Fusarium* sp. (FCBP. 1413), *F. incarnatum* (FCBP. 1301) and *F. solani* (FCBP. 1401), were penetrated by the outer body wall after 96 h of inoculum application. The hyphae of *Aspergillus nidulans* (FCBP. 1384), *Fusarium* sp. (FCBP. 1376), *F. oxysporum*, *F. eqiuseti* (FCBP. 1389) and *F. eqiuseti* (FCBP. 1299) were not penetrated. The penetration point and duration of penetration were done by fungus at 25 ºC and 55 % humidity (Fig. 5).

Duration of colonization

In colonization, the fungus colonizes or multiplies into the body cavity of insects. The fungus

cultures of *Aspergillus* and *Fusarium* had different duration of colonization. The hyphae of *Aspergillus nidulans* (FCBP. 1384) were colonized outside the body wall of *B. tabaci* after 72 hours of inoculum application. The hyphae of *A. fumigatus* (FCBP. 1383) were colonized inside the body wall of *B. tabaci* after 72 hours of inoculum application. The hyphae of *A. nidulans* (FCBP. 1384) and *A. fumigatus* (FCBP. 1383) were colonized rapidly. The hyphae of *A. fumigatus* (FCBP. 1386) were colonized inside and outside of body wall specifically on the mouth part also after 96 hours of inoculum application. The hyphae of *Aspergillus fumigatus* (FCBP. 1395), *Aspergillus fumigatus* (FCBP. 1398), *Aspergillus flavus* (FCBP. 1388) and *Fusarium eqiuseti* (FCBP. 1302) were colonized inside the body wall after 96 hours of inoculum application. The hyphae of *Fusarium oxysporum* (FCBP. 1304), *F. eqiuseti* (FCBP. 1389) and *Fusarium eqiuseti* (FCBP. 1299) were colonized outside the body wall after 96 hours of inoculum application. The hyphae of *A. fumigatus* (FCBP. 1392) were colonized inside the body wall of *B. tabaci* after 120 hours of inoculum application. The hyphae of *Fusarium sp.* (FCBP. 1376) were colonized outside the body wall of *B. tabaci* after 120 hours of inoculum application. The hyphae of *Fusarium sp.* (FCBP. 1407), *Fusarium* sp. (FCBP. 1413), *F. incarnatum* (FCBP. 1301) and *F. solani* (FCBP. 1401), were colonized inside and outside of the body wall of *B. tabaci* after 120 h of inoculum application (Fig. 6).

Duration of infection

The fungus cultures of *Aspergillus* and *Fusarium* had different duration of infection. The hyphae of *A. fumigatus* (FCBP. 1383) were caused infection as endo-parasite, inside the body of *B. tabaci* after 96 hours of inoculum application. The hyphae of *A. nidulans* (FCBP. 1384) were caused infection as endo-parasite, inside the body of *B. tabaci* after 96 hours of inoculum application. The hyphae of *A. fumigatus* (FCBP. 1383) and *Aspergillus nidulans* (FCBP. 1384) were caused infection rapidly. The hyphae of *A. fumigatus* (FCBP. 1386), *A. fumigatus* (FCBP. 1395), *A. fumigatus* (FCBP. 1398), *A. flavus* (FCBP. 1388) and *F. eqiuseti* (FCBP. 1302) were caused infection as endo-parasite, inside the body of *B. tabaci* after 120 hours of inoculum application. The hyphae of *F. oxysporum* (FCBP. 1304), *F. eqiuseti* (FCBP. 1389) and *F. eqiuseti* (FCBP. 1299) were caused infection as ecto-parasite, outside the body of *B. tabaci* after 120 hours of inoculum application. The hyphae of *A. fumigatus* (FCBP. 1392) were caused infection as endo-parasite, inside the body of *B. tabaci* after 144 hours of inoculum application. The hyphae of *Fusarium sp.* (FCBP. 1376) were caused infection as ecto-parasite, outside the body of *B. tabaci* after 144 hours of inoculum application. The hyphae of *Fusarium sp.* (FCBP. 1407), *Fusarium* sp. (FCBP. 1413), *F. incarnatum* (FCBP. 1301) and *Fusarium solani* (FCBP. 1401) were caused infection as both ectoparasite and endo-parasite, outside and inside of the body of *B. tabaci* after 144 h of inoculum application (Fig. 7). The infection mechanism of both *Aspergillus* and *Aspergillus* has shown (Fig. 8 and 9).

Infection process of entomopathogenic fungi, *Aspergillus* and *Fusarium* were observed at nymphal stage of *B. tabaci*. In this study, it was found that there are four steps during infection mechanism. These steps are duration of spore germination, penetration point and duration of penetration, duration of colonization and duration of infection. Similar observations regarding the infection steps of entomopathogenic fungus like spore germination in the presence of suitable condition, the penetration point and duration of penetration of hyphae, duration of colonization and duration of infection in different insect pests were represented by (Hassan *et al.*, 1989). In this study, the duration of spore germination, penetration, colonization and infection of *Aspergillus* (entomopathogenic fungus) are 48 to 144 hours at 55% humidity. Similar observation regarding the duration of spore germination, penetration, colonization and infection were represented by Cloyd (2003), according to Cloyd 24 to 72 hours are required for the production of infection symptoms in insects by *Aspergillus* (entomopathogenic fungus) at 85 % humidity. The difference in time duration of fungus action in our studies is due to the percentage of humidity. In this study, the mycelia growth observed after 48 to 72 hours, the mycelia of *Aspergillus* species infected the thoracic and side limbs of *B. tabaci* body. But after fifth to sixth day of inoculum application, the mycelia cover the whole body by causing infection. In this study, it was observed that the entomopathogenic *Fusarium* species cause moderate infection and in some cases severe infection against the *B. tabaci*. Similar observations regarding the *Fusarium* effect were represented by Teetor-Barash and Roberts, 1983. In this study, the *Fusarium* hyphae penetrated the insect body wall and produced toxins to cause infection in *B. tabaci*. Similar observations regarding the infection process of entomopathogenic *Fusarium* were represented by Gupta *et al*. (1991).

Table 1: List of cultures to check the pathogenicity.

Sr. No	FCBP accession No.	Name of fungi	Source of isolation	Code	FCBP DNA No./ Gene Bank No.	Preservation conditions
01	FCBP-EPF-1299	*Fusarium eqiuseti*	Aphid of Cotton [Field, IAGS], (Waheed Anwar, IAGS, PU, LHR)	Sample K	G. Bank. LN827599	Media: ¼ SDAY 4 °C Temperature
02	FCBP-EPF-1301	*Fusarium incarnatum*	*Bemisia tabaci*, [IAGS field] (Waheed Anwar, IAGS, PU, LHR)	W 05	G. Bank. LN827601	Media: ¼ SDAY 4 °C Temperature
03	FCBP-EPF-1302	*Fusarium eqiuseti*	Mealy bug, [IAGS field] (Waheed Anwar, IAGS, PU, LHR)	W 08	G. Bank. LN827600	Media: ¼ SDAY 4 °C Temperature
04	FCBP-EPF-1304	*Fusarium oxysporum*	Aphid, cotton field, [Bahawalpur] (Waheed Anwar, IAGS, PU , LHR)	W 06	N/A	Media: ¼ SDAY 4 °C Temperature
05	FCBP-EPF-1376	*Fusarium sp.*	Mealy bug of cotton field, IAGS, [Lahore] (Waheed Anwar, IAGS, PU, LHR)	W 03	G. Bank. LN827602	Media: ¼ SDAY 4 °C Temperature
06	FCBP-EPF-1389	*Fusarium equiseti*	Aphid of cotton Field, IAGS (Waheed Anwar, IAGS, PU, LHR)	Sample J	G. Bank. LN827603	Media: ¼ SDAY 4 °C Temperature
07	FCBP-EPF-1407	*Fusarium sp.*	*Bemisia tabaci* of cotton field, [Layyah] (Waheed Anwar, IAGS, PU, LHR)	W 01	N/A	Media: ¼ SDAY 4° C Temperature
08	FCBP-EPF-1413	*Fusarium sp.*	*Bemisia tabaci* of cotton field, [Sahiwal] (Waheed Anwar, IAGS, PU, LHR)	WA	N/A	Media: ¼ SDAY 4 °C Temperature
09	FCBP-EPF-1401	*Fusarium solani*	*Bemisia tabaci* of cotton field, [Rajanpur] (Waheed Anwar, IAGS, PU, LHR)	W 12	N/A	Media: ¼ SDAY 4 °C Temperature
10	FCBP-EPF-1383	*Aspergillus fumigatus*	*Bemisia tabaci* of cotton Field, [Layyah] (Waheed Anwar, IAGS, PU, LHR)	Sample A	G. Bank. LN849889	Media: ¼ SDAY 4 °C Temperature
11	FCBP-EPF-1386	*Aspergillus fumigatus*	Aphid of cotton Field, [Sahiwal] (Waheed Anwar, IAGS, PU, LHR)	Sample B	G. Bank. LN849890	Media: ¼ SDAY 4 °C Temperature
12	FCBP-EPF-1392	*Aspergillus fumigatus*	Mealy bug of cotton Field, [Bahawalpur] (Waheed Anwar, IAGS, PU, LHR)	Sample C	G. Bank. LN849891	Media: ¼ SDAY 4 °C Temperature
13	FCBP-EPF-1398	*Aspergillus fumigatus*	Aphid of cotton Field, [Lahore] (Waheed Anwar, IAGS, PU, LHR)	Sample U	G. Bank. LN849892	Media: ¼ SDAY 4 °C Temperature
14	FCBP-EPF-1395	*Aspergillus fumigatus*	Mealy bug of cotton Field, [Lahore] (Waheed Anwar, IAGS, PU, LHR)	Sample L	G. Bank. LN849893	Media: ¼ SDAY 4 °C Temperature
15	FCBP-EPF-1384	*Aspergillus nidulans*	*Bemisia tabaci* of cotton Field, [Layyah] (Waheed Anwar, IAGS, PU, LHR)	Tn-7	G. Bank. LN849894	Media: ¼ SDAY 4 °C Temperature
16	FCBP-EPF-1388	*Aspergillus flavus*	*Bemisia tabaci* of cotton Field, [Bahawalpur] (Waheed Anwar, IAGS, PU, LHR)	Tn-17	G. Bank. LN849895	Media: ¼ SDAY 4 °C Temperature

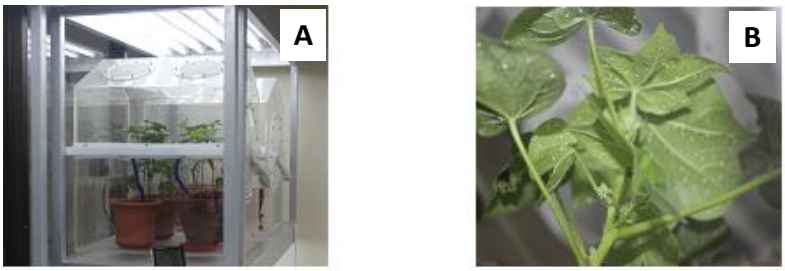

Fig. 1: Glass cages for *B. tabaci* rearing on cotton plants and *B. tabaci* reared on cotton plant in glass cages.

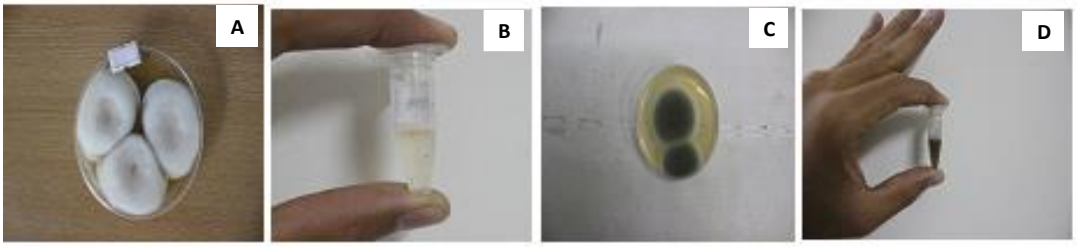

Fig. 2 (A-D): Pure *Fusarium* culture, spore suspension of pure *Fusarium* culture, Pure *Aspergillus* culture and spore suspension of pure *Aspergillus* culture

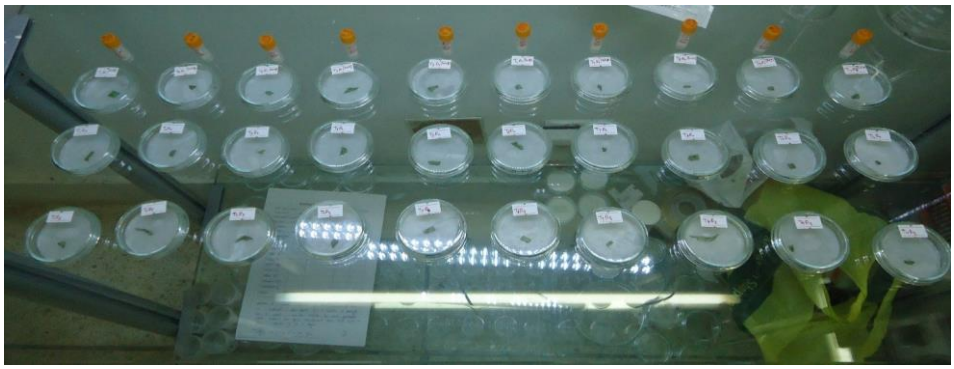

Fig. 3: Small pieces of cotton leaves containing 4th instar stage of *B. tabaci* in petri plate with different fungal spores inoculum.

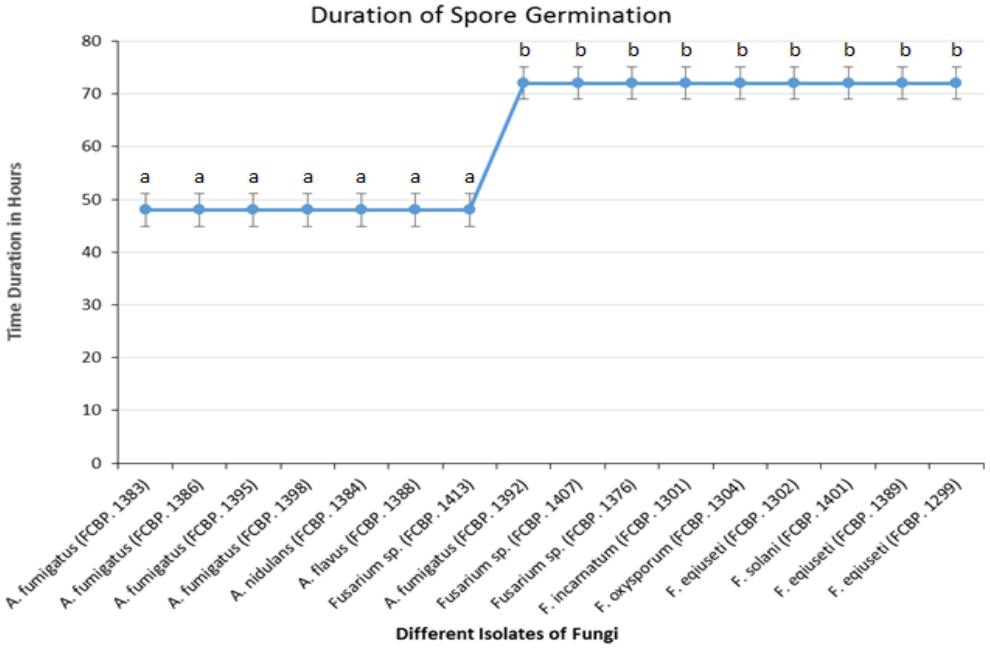

Fig. 4: Duration of spore germination of *Fusarium* and *Aspergillus* strains at nymphal stage of *B. tabaci*

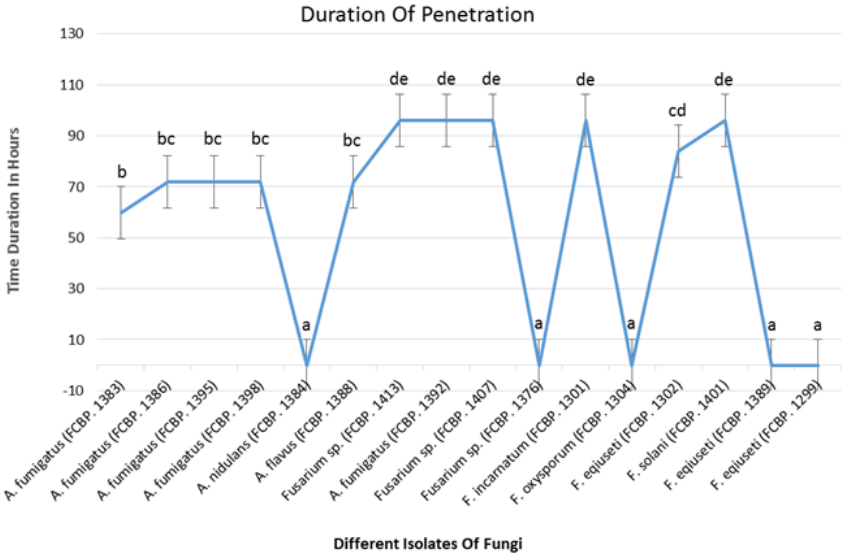

Fig. 5: Duration of penetration of *Fusarium* and *Aspergillus* strains at nymphal stage of *B. tabaci*

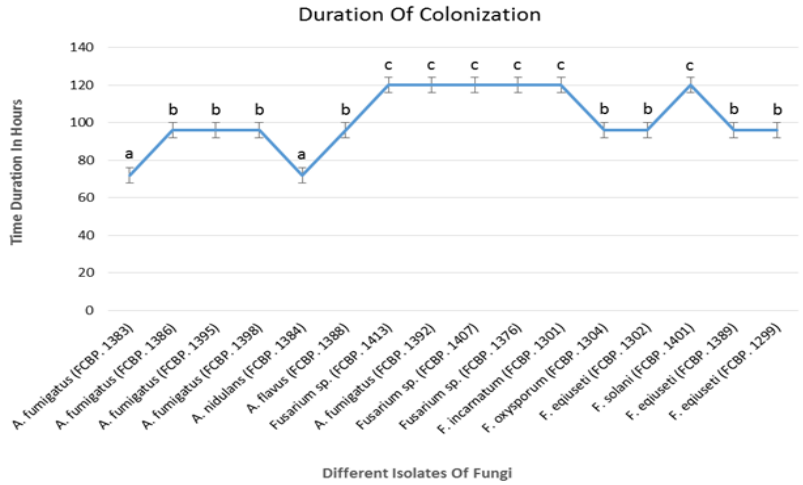

Fig. 6: Duration of colonization of *Fusarium* and *Aspergillus* strains at nymphal stage of *B. tabaci.*

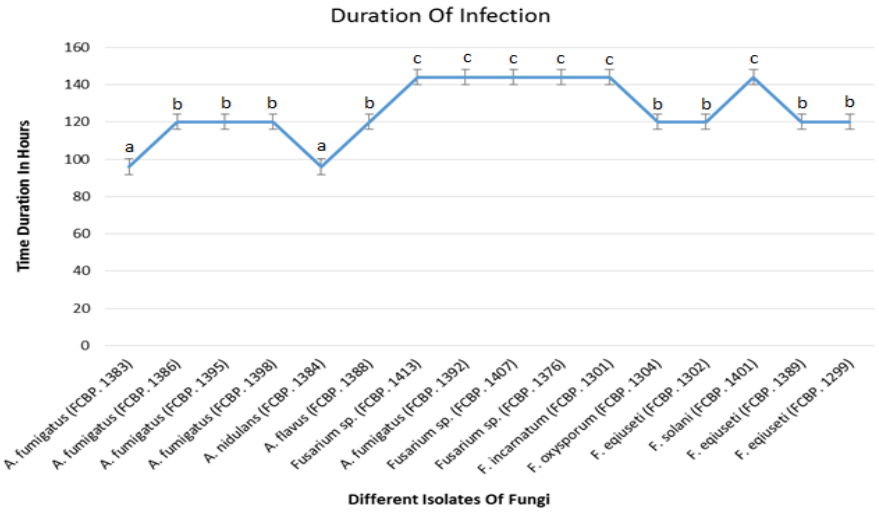

Fig. 7: Duration of infection of *Fusarium* and *Aspergillus* strains at nymphal stage of *B. tabaci.*

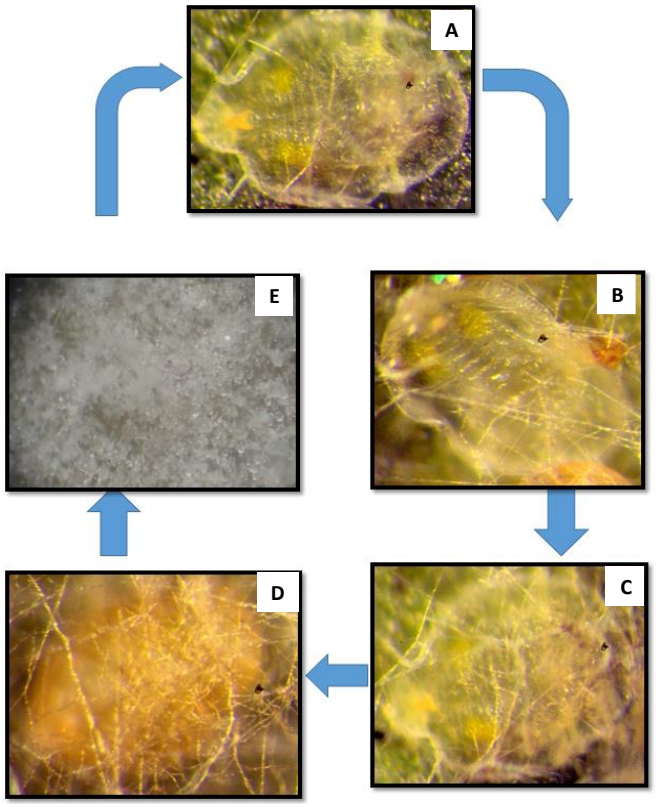

Fig. 8: The Infection Mechanism of *Fusarium* in *B. tabaci* nymph (A: Spore germination), (B: Hyphae penetration), (C: Hyphae Colonization), (D: Infection, covering the body by hyphae) and (E: Hyphal structure of *Fusarium* under the 150X stereomicroscope)

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