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Isolation and Identification of The Fungal Pathogen *Aspergillus aflatoxiformans* Strain and Its Role in Controlling The Two-Spotted Spider Mite *Tetranychus urticae* (Koch)

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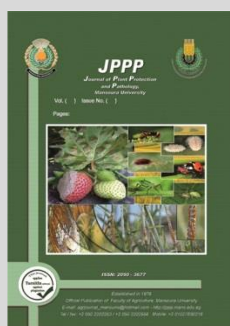

ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is the major mite pest infesting many cultivated crops. The fungus, *Aspergillus aflatoxiformans* Frisvad, Ezekiel, Samson & Houbraken produces aflatoxins where it considered as secondary polyketide metabolites, which cause the death of pests. Therefore, this work aimed to isolate and identify this fungus by amplify the internal transcribed spacer (ITS) region of the rDNA, as well as to evaluate the efficiency of this fungus to control *T. urticae*. Results showed 300bp DNA product corresponding to the *omt-A* gene. The existence of *omt-A* gene in *A. aflatoxiformans* reflected its ability to produce aflatoxin, which may be responsible of mite control. The efficacy of *A. aflatoxiformans* against *T. urticae* recorded the highest reduction percentage of eggs hatch at concentration of $1x10^8$ conidia/ml within the 5th and 6th day after exposure. These percentages were 99.42 and 100%, respectively. The highest mortality percentage was recorded on the 4th, 5th and 6th day at higher concentration of $1x10^8$. No eggs and larvae of *T. urticae* were observed within the 5th and 6th day after treatment at $1x10^8$. There were not observed nymphs at all tested concentrations. Microelectronic photos showed fungal conidia with cluster shaped which were developed at all *T. urticae* body surface 6 days after treatments at $1x10^7$ and $1x10^8$**.** Results also confirmed that *A. aflatoxiformans* could be used successfully to control *T. urticae* eggs, immature stages and adults. Therefore, this fungus could be involved in any IPM programs of the two-spotted mite.

Keywords: Biological control**,** *Tetranychus urticae, Aspergillus aflatoxiformans*, molecular identification, fungal PCR.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is considered as a mite pest, which infesting many plant crops. Due to environmental and health risks caused by chemical pesticides and their impacts on other non-targeted organisms (Horikoshi *et al*. 2017), one of the proposed methods is the biological control of *T. urticae* by fungal agents (Chandler *et al*. 2000). Biological control can be applied by using fungi and their mycotoxins such as aflatoxin B and trichothecenes as chemical defenses to different pests including insects and mites (Srivastava *et al*., 2009). *Beauveria bassiana* (Balsamo) Vuillemin is used for the control of whiteflies, aphids and leafhoppers. (Faria and Wraight, 2001, Feng *et al*., 2004). It was also reported that the fungal efficacy against *T. urticae* as well as the control application of *B. bassiana* together with low release rate of the predatory phytoseiid mite, *Phytoseiulus persimilis* successfully controlled *T. urticae* (Irigaray *et al*. 2003, Chandler *et al*. 2005, Ullah and Lim 2017). Some strains of the entomopathogenic fungi have also proven to control the spider mites. Using spray technique of *B. bassiana* and *Metarhizium anisopliae* against *T. urticae*, infesting eggplants, caused high significant mortality (Batta, 2003). *Beauvaria bassiana, M. anisopliae* and *Paecilomyces fumosoroseus* were high infective to immature stages and adult females of *T. cinnabarinus* (Shi and Feng, 2004, Shi *et al*., 2008), while fecundity of *T. evansi* was reduced when treated with *B. bassiana* or *M. anisopliae* (Wekesa *et al*., 2006). *Aspergillus flavus* and *A. parasiticus* fungi produce aflatoxins, which considered secondary polyketide metabolites, that cause the death of pests. Among the aflatoxins, that has been identified as aflatoxin B1 (AFB1), the most prevalent form to control the pests (Diaz *et al*. 2010). Zhang *et al*. (2014) found that the entomopathogenic fungus, *B. bassiana* can be used as a biocontrol against *T. urticae* and affected the egg viability which depend on the protraction of fungal hyphae to absorb egg nutrition, resulting in embryo disturbance.

So, the present study aimed to isolate, identify and evaluate the effetivness of different concentrations of the entomopathogenic fungus *A. aflatoxiformans* against *T. urticae* under laboratory conditions.

MATERIALS AND METHODS

Isolation and preparation method of *Aspergillus aflatoxiformans*

The infected larvae of silkworm, *Bombyx mori* L. were collected, cut into bits, placed in Petri dishes (10 cm in diameter), provided with Potato Dextrose Agar medium (PDA) and incubated at 28± 1°C for 7-days. After the appearance of mycelial growth, they were transferred to a fresh PDA slant. For purification of the isolated pathogen, a

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single hyphal tip method was used. The process was conducted aseptically, in an inoculation hood/UV laminar flow chamber Shanmugam and Seethapathy (2017).

The fungus, *A. aflatoxiformans* was sub-cultured on several PDA slants and incubated at room temperature and relative humidity to the requirements of inoculation. Conidia were harvested by scraping the surface of culture using a sterile camel hair brush by shaking thoroughly with 3 ml of sterile water to each slant for about 3-5 minutes. Spore suspension from each slant was pooled to obtain required quantity of spore suspension required for inoculation. The concentrated spore suspension was transferred to a conical flask containing distilled water with a drop of tween-80 to keep the conidia dispersed and thoroughly shaken for 10 minutes. The suspension was then filtered through sterilized double-layered cheesecloth. All these steps were carried out in an inoculation chamber to avoid any kind of contamination. The stock inoculum suspension was quantified by the standard procedure of 'Neubauer' improved double ruled hemocytometer and expressed as number of spores per ml Dutta et al. (2003). The count of conidia was recorded according to Ashley et al. (2016). The required concentration of A. aflatoxiformans inoculum (1×10^5 to 1×10^8 conidia/ml) was prepared by suitable diluting the stock inoculum with sterilized distilled water.

Growth conditions to fungal isolation

The tested fungal strain was grown in potato dextrose agar (PDA) and yeast extract sucrose (YES) liquid growth mediums at 25 °C for one week.

Molecular identification of the fungal isolate

The DNA was extracted from 25 mg of the harvested mycelia of the isolate, which was previously frozen in liquid N2 and ground by a mortar, according to the protocol recommended for the DNA tissue purification mini kit (Qiagen, Maryland, USA). The genomic DNA was checked by 1.5 % agarose gel electrophoresis. Primers (ITS4 and ITS5) were used to amplify ribosomal internal transcribed spacer (ITS) according to Sohail and Bayan (2018). These primers amplify the entire ITS region (Table 1).

Table 1. Primers of ITS

Primer name	Primer sequence (5'-3')
ITS5-F	GTGAATCATCGAATCTTTGAA
ITS4-R	TCCTCCGCTTATTGATATGC

Detection of AFB1 gene (*omt-A* gene)

Primers were selected according to the sequence of the *omt-A* gene of *A. aflatoxiformans* from GenBank database (http://www.ncbi.nlm.nih.gov/) (Table 2). Aflatoxin B1 (AFB1) standard was purchased from Sigma, Chemical Co. (St. Louis, MO, U.S.A).

Table 2. Primers for amplification of AFB1 gene

Primer	5'-3' nucleotide sequence
omt-F	GACCAATACGCCCACACAG
omt-R	CTTTGGTAGCTGTTTCTCGC

Aflatoxin B1 (AFB1) gene (*omt-A*) PCR was carried out in a total reaction volume of 20µl, containing (10 µl of 2 X Go Taq master mix (Promega Corporation, Madison, WI), 10 pmol of each primer and 50 ng template DNA. Amplification was performed in a T100-Bio-Rad Gradient Thermal cycler. The following programmer was used to amplify the DNA: 3 min at 94°C (1 cycle); 0.30s at 94°C, 0.30s at 59°C, and 0.30s at 72°C (35 cycles); and 10 min at 72°C. A 10µl aliquot of PCR products were separated on a 1.5% agarose gel stained with ethidium bromide (0.1 mg/l) and photographed under Gel Doc™ XR+ Gel Documentation System. Thermo Scientific GeneRuler 100 bp DNA Ladder was used as a size standard.

Mite Rearing

One newly matted mite adult female was transferred by a fine camel hair brush to a sweet leaf disc (1mm, in diameter), preserved on a humid cotton wool pad in a Petri dish and left for 24-48 h to allow it to lay eggs. The deposited eggs were preserved under laboratory conditions at 27±2 °C, 60±5 % R.H. and 16 L: 8 D photoperiod until hatching. The newly hatched larvae were transferred singly to fresh sweet potato leaves to follow their development (Pritam and Clare 1993).

Acaricidal activity *of Aspergillus aflatoxiformans* against *Tetranychus urticae* under laboratory conditions

Five concentrations of *A. aflatoxiformans* ($1x10^4$, $1x10^5$, $1\ x10^6$, $1\ x10^7$ and $1x10^8$ conidia/ml) as well as an untreated control group were prepared under laboratory conditions 25±3°C and 72±2% R.H and evaluated against egg stage and adult females of *T. urticae*. Twenty eggs were counted, and twenty healthy of adult females per four replicates for each concentration, were transferred to the treated leaf discs $4cm^2$ in area placed upside down on moist cotton wool in a Petri dish using a fine camel hair brush (5 individuals of female mite/leaf disc). Two ml suspension of each concentration of a single dose was sprayed using hand spray atomizer at distance of 25–30 cm on the surface of leaf disc and the replicates, which served as a control, were sprayed with water. After applications, numbers of live and dead individuals were counted by using a dissecting microscope at 6 days intervals. The average number of stages resulting from the live treated females (eggs, larvae and nymphs) at each concentration was calculated. The mortality percentage of mites was calculated and corrected according to Abbott's formula (Abbott, 1925). Also, reduction percentage of hatchability was calculated according to Kapil *et al.* (2017).

Scanning Electron Microscope Procedure (SEM)

Infected adult females of *T. urticae* were examined by SEM. This work was carried out in the electronic microscope unite, Faculty of Agriculture, Cairo University, Giza, Egypt. The adult females of spider mite were fixed in glutaraldehyde 3% for three hours at room temperature (25±3°C). Subsequently, the samples were dehydrated in 25 %, 50%, 75%, and 95% ethanol graded series. Then the specimens were mounted on scanning electron microscopy. A thin layer of gold-coated samples was prepared by ion sputtering. Samples were examined using (Jeol-JSM-5200) Scanning electron microscope (Tokyo, Japan)

Statistical analysis

Data of mortality percentage of mites were analyzed according to Steel and Torrie (1984). The means were compared by Duncan's Multiple Range Test (DMRT) at 5% clarifying by LSD test (Duncan, 1955).

RESULTS AND DISCUSSION

Molecular identification of the fungal isolate

Fungus, which isolated from the larvae of silkworm, *Bombyx mori*, was identified based on sequencing the ITS

region of the rDNA, that was accurate and reliable. Its sequencing yielded an estimate of 600-bp DNA fragment product of the ITS region that was obtained purified and sequenced (Figure 1) as found by Njambere *et al*. 2008. The sequence comparisons were carried out using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST). ITS region of the rDNA gene sequence of bacterial isolate scored 99% similarity with *Aspergillus aflatoxiformans* strain DTO 228-G2. This sequence was recorded at GenBank data base as *Aspergillus aflatoxiformans* strain SMA_NRC1 under accession number ***MZ314277***.

Detection of aflatoxin biosynthesis (*omt-A*) gene

The existence of aflatoxin AFB1 biosynthesis in *A. aflatoxiformans* was toxic to the immune system of larvae of silkworm, *Bombyx mori* hence the death of larvae. This gene was studied in *A. aflatoxiformans* strain SMA_NRC1 using PCR technique by using specific primers for the AFB1 biosynthesis, O-methyltransferase gene (*omt-A*). Three hundred base pairs amplicon was found in *A. aflatoxiformans* strain SMA_NRC1 which corresponded (*omt-A*) gene showed that in Figure 2.

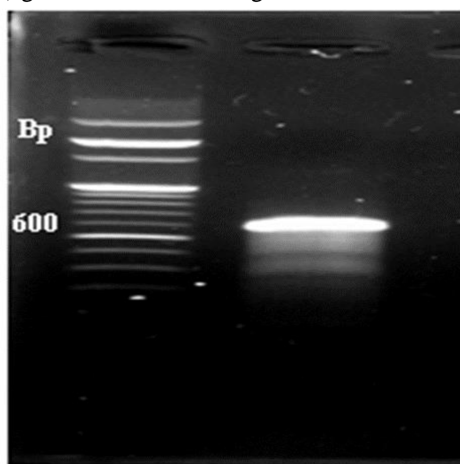

Fig. 1. Fragment banding patterns of amplified DNA of isolate with primer pairs ITS 4 and ITS 5, DNA amplified with the same primer pairs is shown in the intervening lanes. 100-bp DNA ladder are also shown.

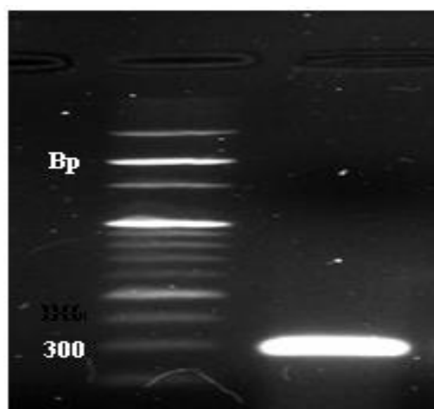

Fig. 2. Amplification omt-A gene of A.*aflatoxiformans*. Left lane Thermo Scientific Gene Ruler 100bp DNA. Ladder.

Acaricidal activity of *A. aflatoxiformans* against *T. urticae*

The present results showed the efficacy of entomopathogenic fungus, *A. aflatoxiformans*, using different concentrations against eggs and female adults of *T. urticae*. Also, the average numbers of stages resulting from the live treated females (eggs, larvae and nymphs) were recorded at each concentration. Additionally (SEM) was used to observe the shape of fungal conidia and the infection process of *A. aflatoxiformans* against adult females of *T. urticae*.

Results in Table (3) showed that the reduction percentages of egg hatchability have not been recorded within the 1st and 2nd day after exposure at all concentrations in addition to the 3rd day at 1x10^4, 1x10^5 and 1x10^6. At the same time, reduction percentage was observed at the 3rd day at concentrations of 1x10^7 and 1x10^8 (conidia/ml) where it recorded 47.55±0.67 and 59.11±0.61% respectively. Highly significant differences were recorded within 4th, 5th and 6th day among concentrations. The highest reduction percentage of egg hatchability was recorded at high concentration 1x10^8 (conidia/ml) within the 5th and 6th day being 99.42±0.31 and 100±0.73%, respectively. While the lowest reduction percentage was observed within the 4th day at concentration of 1x10^4 being 74.13±0.57%.

Table 3. Reduction % in egg hatchability of *Tetranychus urticae* as affected by different concentrations of *A. aflatoxiformans*.

Conc. (conidia/ml)	Reduction % in egg hatchability ±SE after detected days					
	1st	2nd	3rd	4th	5th	6th
1 x 10 4	0.00	0.00	0.00 ^{c}	74.13±0.57 ^{e}	86.45±0.61 ^{e}	92.31±0.52 ^{e}
1 x10 5	0.00	0.00	0.00 ^{c}	84.26 ± 0.91 ^{d}	91.35 ±0.26 ^{d}	94.62±0.50 ^{d}
1 x10 6	0.00	0.00	0.00 ^{c}	87.06±0.55 ^{c}	93.94 ±0.29 ^{c}	95.38±0.75 ^{c}
1 x10 7	0.00	0.00	47.55±0.67 ^{b}	93.36±0.35 ^{b}	95.96± 0.50 ^{b}	98.72 ±0.44 ^{b}
1 x10 8	0.00	0.00	59.11±0.61 ^{a}	98.25± 0.50 ^{a}	99.42±0.31 ^{a}	100.00±0.73 ^{a}
F. Value			0.95	0.02	0.95	0.07
P. Value			0.36**	0.88**	0.35**	0.80**
L.S. D			0.02	0.01	0.03	0.04

Means with the same letter within each column are not significant (*P*≤ 0.05; using Duncan's Multiple Ranges test at 5% clarifying by LSD test).

Results in Table (4) showed that, the lowest mortality percentage was recorded within the 1st day of treatment (5.00±0.78%) at concentrations of 1x10^4 and 1x10^5 conidia/ml, and no significant differences between them were recorded. The mortality percentage increased gradually within the 2nd day at concentrations of 1x10^7 and 1x10^8 being 60.00±0.45 and 65.00±0.46%, respectively. Within the 5th and 6th day at concentration of 1x10^7, the same mortality percentages (95.00±0.23%) was recorded. Meanwhile, the highest mortality percentage was recorded in the 4th, 5th and 6th day intervals at the high concentration of 1x10^8 conidia/ml compared to control treatment, where the symptoms was appeared at the end of the 6th day.

Table 4. Mortality percentages of the adult females of *T. urticae* as affected by different concentrations of *A. aflatoxiformans*.

Conc. (conidia/ml)	Mortality % of *T. urticae* after detected days					
	1st	2nd	3rd	4th	5th	6th
1 x 10 4	5.00±0.78 d	15.00 ± 0.50 e	30.00± 0.47 e	40.00±0.46 e	60.00±0.44 e	65.00±0.35 e
1 x 10 5	5.00±0.78 d	20.00± 0.39 d	35.00±0.26 d	50.00±0.42 d	65.00±0.31 d	75.00± 0.51 d
1 x 10 6	10.00± 0.35 c	25.00± 0.44 c	40.00±0.63 c	60.00±0.37 c	70.00±0.37 c	80.00±0.40 c
1 x 10 7	20.00±0.37 b	60.00± 0.45 b	75.00±0.41 b	85.00±0.20 b	95.00±0.23 b	95.00±0.23 b
1 x 10 8	40.00±0.35a	65.00± 0.46 a	80.00±0.37 a	100.00±0.35 a	100.00±0.47 a	100.00±0.76 a
Control	0.00 e	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
F. Value	0.06*	3.33**	2.64**	3.58**	6.9**	5.71**
P. Value	0.81	0.08	0.12	0.07	0.01	0.02
L.S.D	1.23	0.82	0.67	0.63	0.59	0.63

Means with the same letter within each column are not significant ($P \leq 0.05$;using Duncan's Multiple Ranges test at 5% clarifying by LSD test).

Data in Table (5) showed the highest average number of *T. urticae* eggs was recorded (61.25±0.85 individuals) within the 1st day at concentration of $1x10^4$ conidia/ml compared to control treatment. At concentration of $1x10^6$, average number of stages resulting from the live treated females of *T. urticae* was started to decrease sharply among the detected days after treatment, where they were observed clearly on the 4th, 5th and 6th day with an average number of 13.50±0.78, 9.00±0.28 and 7.75±0.35 individuals, respectively. While the lowest average number was recorded at $1x10^8$ at all detected days within the 3rd and 4th day (2.50±0.37 and 1.25±0.45 individuals, respectively). Also, the 5th and 6th day with the same concentration, results proved that there were no eggs were recorded compared to control treatment. There were highly significant differences between all concentrations of detected days.

Also, it was noted a highly significant differences between all concentrations of larvae. Generally, average numbers of larvae were decreased at all concentrations and was clearly observed at concentration of $1x10^7$ at the 5th and 6th day with an average of 1.75±0.48 and 1.25±0.37 individual. Followed by concentration of $1x10^8$ where they was recorded the lowest average numbers of larvae at the 1st, 2nd, 3rd and 4th day compared to control treatment. In addition, at the 5th and 6th day no number of larvae were recorded. Also, no numbers of nymphs were recorded at all concentrations. Thus, the life cycle was stopped during the larval stage. It means that, the high concentrations of fungus, *A. aflatoxiformans* was very effective on individuals, resulting from the live treated females of *T. urticae* as a result of treatment with entomopathogenic fungus which produced aflatoxine that cause the death of *T. urticae* individuals.

Table 5. Average number of mites resulting from the live treated females of *T. urticae* as affected by different concentrations of *A. aflatoxiformans*

Stages	Conc. (conidia/ml)	Average numbers of *T. urticae* stages after detected days					
		1st	2nd	3rd	4th	5th	6th
No. of Eggs	1 x 10 4	61.25±0.85 b	55.50±0.87 b	53.25±0.75 b	40.50±0.66 b	34.75±0.39 b	23.75±0.37 b
	1 x 10 5	52.75±0.75 c	49.50±0.68 c	42.25±0.60 c	27.25±0.83 c	18.75±0.57 c	15.75±0.79 c
	1 x 10 6	29.25±0.80 d	27.00±0.68 d	20.00±0.50 d	13.50±0.78 d	9.00±0.28 d	7.75±0.35 d
	1 x 10 7	18.75±0.20 e	14.25±0.35 e	9.50±0.42 e	7.75±0.16 e	4.50±0.20 e	3.25±0.35 e
	1 x 10 8	7.75±0.45 f	5.75±0.57 f	2.50±0.37 f	1.25±0.45 f	0.00 f	0.00 f
	Control	60.50±0.79 a	63.50±0.81 a	71.50±0.54 a	75.50±0.69 a	78.25±0.79 a	87.25±0.67 a
	F. Value	0.13**	0.28**	0.01**	0.01**	0.81**	0.96**
	P. Value	0.72	0.60	0.98	0.91	0.38	0.34
	L.S. D	0.04	0.28	0.13	0.22	0.20	0.22
No. of Larvae	1 x 10 4	14.50±0.26 b	12.75±0.65 b	10.75±0.45 b	10.50±0.33 b	10.25±0.20 b	7.50±0.23 b
	1 x 10 5	11.75±0.20 c	10.50±0.20 c	9.50±0.21c	7.50±0.40 c	7.25±0.47 c	6.50±0.33 c
	1 x 10 6	9.25±0.50 d	8.50±0.31 d	6.25±0.20 d	5.50±0.81 d	5.00±0.35 d	3.50±0.16 d
	1 x 10 7	3.50±0.20 e	3.25±0.28 e	2.75±0.35 e	2.25±0.57 e	1.75±0.48 e	1.25±0.37 e
	1 x 10 8	1.50±0.42 f	1.25±0.35 f	1.00±0.42 f	0.75±0.33 f	0.00 f	0.00 f
	Control	48.50±0.31 a	55.75±0.24 a	61.50±0.93 a	67.25±0.79 a	73.50±0.20 a	84.25±0.20 a
	F. Value	0.59**	0.53**	0.94**	0.43**	0.11**	0.22**
	P. Value	0.44	0.47	0.34	0.51	0.74	0.64
	L.S. D	0.03	0.11	0.23	0.17	0.22	0.16
No. of Nymphs	1 x 10 4	0.00	0.00	0.00	0.00	0.00	0.00
	1 x 10 5	0.00	0.00	0.00	0.00	0.00	0.00
	1 x 10 6	0.00	0.00	0.00	0.00	0.00	0.00
	1 x 10 7	0.00	0.00	0.00	0.00	0.00	0.00
	1 x 10 8	0.00	0.00	0.00	0.00	0.00	0.00
	Control	28.50±0.86	38.25±0.35	40.50±0.81	53.25±0.35	66.50±0.42	78.50±1.06

Means with the same letter within each column are not significant ($P \leq 0.05$;using Duncan's Multiple Ranges test at 5% clarifying by LSD test).

Scanning Electron Microscope (SEM) on infected *T. urticae*

After 6 days of treatments with high concentrations, $1x10^7$ and $1x10^8$ showed some symptoms. Moving stages of *T. urticae* were generally decreased in activity and feeding. Then the death occurred. Microelectronic photos showed fungal conidia with cluster shaped were developed in different degrees and covered all the body surface of *T. urticae* (Figures 3, 4 and 5).

The fore-mentioned results are in harmony with Singh and Pathak 2010. Under laboratory conditions, *Aspergillus fumigatus* Fresenius caused a gradual decrease in the adult stage of *Dysdercus similis* (Heteroptera: Pyrrhocoridae). Females lived up to 92 to 99 h. while, males

died after 75 h. of treatment. Sultana *et al*. 2017 reported that *Aspergillus* acridid species is microbial insecticide that did not cause any injury to non-target organisms in the field. Also, it caused a reduction in feeding which may affected body fat accumulation at sexual maturity and thus, the reproductive potential of insects was decreased (Kumar 2007). The entomophthogenic fungi, *Neozygites floridana*, *Acremollium*, *Aspergillus*, *Fusarium*, *Lecallicillium*, *Paecilomyces* and *Pellicillium* caused the death of tetranychids mites by way of pathogenicity under greenhouse and laboratory. (Nguya *et al*. 2008). Entomopathogenic fungus, *Neozygites* spp. caused high mortality in spider mite populations. These fungi strategy is being carried out to develop as mycoacaricides, to replace the artificial acaricides used or as an agent of integrated mite management.

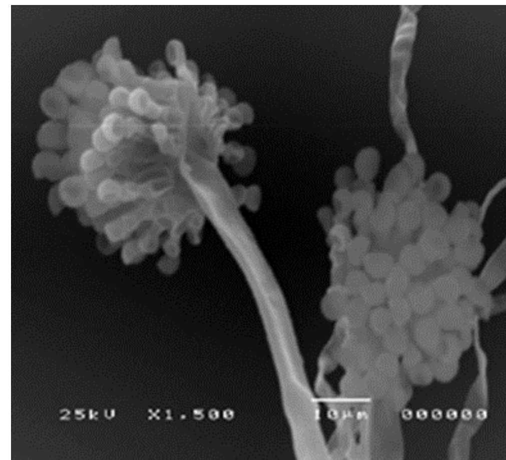

Fig. 3. Fungal conidia of *A. aflatoxiformans* using SEM.

Fig. 4. (A, B, C, D, E, F) Development of infection process caused by *A. aflatoxiformans* on *T. urticae* after 6 days of treatment.

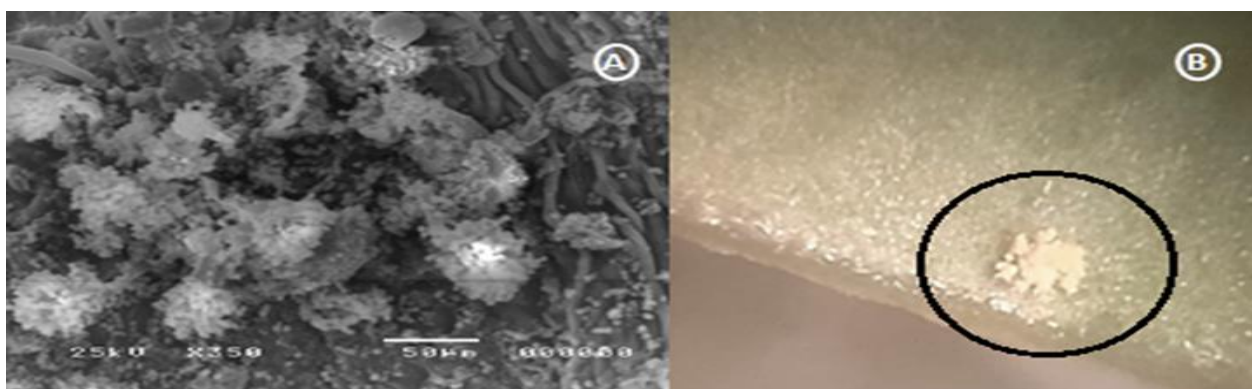

Fig. 5. (A) Symptoms of fungal infection with *A. aflatoxiformans* were observed on *T. urticae* after 6 days post-treatment using SEM, (B) Symptoms of fungal infection using the dissecting microscope.

Under laboratory conditions, Afifi *et al*. 2007 found that the fungus of *Trichoderma harzianum* and *Cladosporium herbarium* gave high mortality percentages than a single one for all stages of *Tetranychus urticae* using spraying techniques. Whereas the mortality percentage reached 46.8% and 59.8% after 3 and 7 days, respectively. while at 35ºC, it increased to 65.1% and 83.0% after the detected periods, respectively. Shengyong *et al*. 2020

observed that, fecundity of female mites of *Tetranychus urticae* was significantly reduced after fungal treatment by using spraying techniques under laboratory conditions. Also, corrected mortalities were recorded 2.7-3.8, 17.5–25.8 and 63.2-71.2%, respectively, after 7days of treatment with *Beauveria bassiana* fungus. Scanning electronic microscope observed that after fungal spray on mites, mycelia grew prolifically from the adult mite after 60 h of treatment, though no symptoms exhibited of fungal infection in the most immature stages of mites.

Chuanwen *et al*. 2019 recorded highly effective of the entomopathogenic fungus, *Aspergillus oryzae* against the poultry red mite, *Dermanyssus gallinae*. It showed highly mortality % in adult mites, up to 24.83 ± 2.25% compared to control which were 15.17 ± 2.75. Generally, *Aspergillus* sp. has pathogenic effects on the adult stage of *D. gallinae*, as a bio-control agent. Mohamed *et al.* 2019 showed that, efficacy of four fungal strains *Aspergillus melleus, Aspergillus terreus, Emericella nidulans,* and *Chaetomium globosum* against *Tetranychus urticae* under Laboratory conditions. Also, observed LC50 values were 10.27, 33.05, 14.68, and 22.40 mg/ml on *Tetranychus urticae* adult females, respectively. In addition, LC50 values recorded on eggs were 8.81, 23.17, 11.66, and 11.05 mg/ml. Therefore, these fungal strains are used in biocontrol of *T. urticae*.

Moreover, Zhang *et al.* 2018 observed that the entomopathogenic fungus *Isaria cateniannulata* caused high mortality to *T. urticae* (100%) using dipping method while it recorded 92% in spraying method and didn't cause any effect on its predator *E. nicholsi* females, or any apparent effect on their fertility and vitality. Therefore, *I. cateniannulata* is safe to *E. nicholsi* when applied in the biocontrol programs of *T. urticae.* Emer *et al*. 2004 showed an infection with *Aspergillus* conidia and indicate a critical role for gliotoxin production rather than growth rate or enzymatic activity in vitro and in vivo by Reversed Phase-High Performance Liquid Chromatography, being produced by *A. fumigatus* ATCC 26933 (350 ng/mg hyphae) that *Galleria mellonella* is more susceptible to fungal infection by *A. fumigatus* ATCC 26933.

O'Donnell *et al*. 1998 used the fungal diagnostics by using molecular techniques to prove this strain, classification, external morphology, whereas it is based on the nucleotide sequencing where variation in the DNA sequences is used to identify within individuals or species. Also**,** Duggal *et al*. 1997 reported that the process of PCR amplification of ITS region of the rDNA using the right primer pairs, showed polymorphisms between and within species thus, it can be used for identification of many fungi such as *Fusarium* spp.

Fungi is considered as a pathogen of many insects, where infection leads to apparent symptoms such decrease of feeding followed by slow development and delayed mating success and death at the end (Roberts and St Leger 2004). While Mohamed *et al*. 2018 reported that, the omt-A produced by *A. flavus* caused high reduction at 1.5 mg -100 ml of AgNPs when used in media where it recorded 88.2%, 67.7% and 83.5% reduction by using AgNP HA1N, AgNP HA2N and AgNP EH, respectively. On the other hand, mycelial growth has a significant inhibitory effect.

Insect infestations of plant crops cause an increase in mycotoxin contamination. Such as damage of nut species *Amyelois transitella* Walker is associated with infection by *Aspergillus* species and concomitant production of aflatoxins and ochratoxins (Hedayati *et al*. 2007). Also, fungal isolate of *Aspergillus aflatoxiformans* strain SMA_NRC1 showed high pesticide ability against silkworm, *Bombyx mori* which can be used as one mode of action to create opposite interactions between insect and fungi, where fungi may have both direct and indirect effects on pests.

CONCLUSION

Fungal pathogen *A. aflatoxiformans* was isolated from infested larvae of silkworm, *Bombyx mori* and identified genetically. *A. aflatoxiformans* infected *T. urticae* individuals causing death as a result of producing aflatoxin. High reduction of egg hatch at high concentrations of *A. aflatoxiformans* and high mortality percentage of adult females of *T. urticae* were recorded. Also, high reduction of average numbers of stages resulting from the live treated females of *T. urticae* was found. So, *A. aflatoxiformans* could be effectively used as bio-rational control of spider mites under laboratory conditions.

LIST OF ABBREVIATIONS

(SEM): Scanning Electron Microscope
ITS: Internal transcribed spacer
PCR: Polymerase chain reaction

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عزل وتعريف المسبب المرضي *Aspergillus aflatoxiformans* ودوره في مكافحة أكاروس العنكبوت الأحمر ذي البقعتين *Tetranychus urticae* (Koch)

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يعتبر أكاروس العنكبوت الأحمر ذي البقعتين *Tetranychus urticae* (Koch) من الآفات الرئيسية التي تصيب العديد من المحاصيل المزروعة. ينتج فطر *Aspergillus aflatoxiformans* الأفلاتوكسين و التي تعتبر مركبات أيض ثانوية من البوليكايتد و التي تسبب موت الأفات. لذلك يهدف هذا العمل إلي عزل وتعريف هذا الفطر بواسطة فواصل النسخ الداخلية (ITS) من الحمض النووي الريبوزي وكذلك تقييم كفاءة هذا الفطر في مكافحة *T. urticae*. واظهرت النتائج وجود 300 قاعدة نيتروجينية من الحمض النووي DNA مسئولة عن إنتاج جين omt-A. ووجود جين omt-A في *A. aflatoxiformans* يعكس قدرته على إنتاج الافلاتوكسين المسؤول عن مكافحة الأكاروس. أظهرت النتائج فعالية فطر *A. aflatoxiformans* ضد*T. urticae* حيث تم تسجيل أعلى نسبة انخفاض في فقس البيض عند $1x10^8$(كونيدية / مل) خلال اليوم الخامس والسادس بعد التعرض. كانت هذه النسب 99.42 و 100 ٪ علي التوالي. تم تسجيل أعلى نسبة موت في اليوم الرابع والخامس والسادس عند أعلي تركيز $1x10^8$ ، لم يلاحظ أي من بيض ويرقات *T. urticae* خلال اليوم الخامس والسادس بعد المعاملة بتركيز $1x10^8$. لم تكن هناك حوريات ملحوظة في جميع التركيزات المختبرة. أوضحت الصور الألكترونية الدقيقة الكونيديا الفطرية ذات شكل عنقودي وتنمو علي جميع أجزاء الجسم لـ*T. urticae* بعد 6 أيام من المعاملة عند $1x10^7$و $1x10^8$. أكدت النتائج أنه يمكن إستخدام فطر *A. aflatoxiformans* بنجاح لمكافحة البيض و الأطوار الغير ناضجة و الإناث الكاملة ل *T. urticae*، لذلك يمكن إدراج هذا الفطر في أي من برامج المكافحة المتكاملة لأكاروس العنكبوت الأحمر ذي البقعتين.