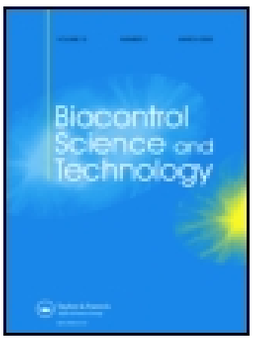

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First record of an entomopathogenic fungus of tomato leafminer, *Tuta absoluta* (Meyrick) in Tanzania

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ABSTRACT

Tuta absoluta (Meyrick) is a destructive pest causing adverse environmental and economic effects globally. Management of *T. absoluta* by synthetic chemicals is of diminutive achievement due to resistance trick of the pest. Harnessing of biocontrols is currently fascinating and efficient for management of the tremendous pests. This study isolated an entomopathogenic fungus from dead larvae of *T. absoluta* on tomato leaves from farmer's field Tanzania. Two *Aspergillus* fungal isolates were isolated and tested against larvae and adults of *T. absoluta* under conditions of 19.5°C and 50%RH, 30.4°C and 70% RH, respectively, in the laboratory. Sequence analysis of *Aspergillus* isolates that we referred as *A*-Tz1 and A-Tz2 were analysed with the BLAST program available at the National Center for Biological Information (NCBI). The two isolates (A-Tz1 and A-Tz2) showed 100% similarity with *Aspergillus oryzae* of GeneBank accession numbers EF121337.1 and MG519722.1. Such similarity implied that both *A*-Tz1 and A-Tz2 isolates were *A. oryzae*. To confirm pathogenicity, bioassays were conducted against the larvae, pupa and adults of *T. absoluta*. Results showed that at 1.0×10^8 conidia/mL, fungal isolates caused up to 70% larval mortality 3 days post inoculation and consequently inhibited pupation by 84.5% and adult emergence by 74.4%. The identified *A. oryzae* reduced the life span of adult *T. absoluta* up to 5 days post inoculation at 1.0×10^8 conidia/mL whereas in control *T. absoluta* survived up to 25 days. This study found that isolates of *Aspegillus* species have the potential to be used as active ingrediennt for the formulation of a new bioinjections for application by farmers to control the pest in fields.

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Biological control; entomopathogenic fungi; tomato; *Tuta absoluta*; Tanzania

1. Introduction

Tuta absoluta (Meyrick 1917) (Lepidoptera: Gelechiidae) is a serious pest damaging several Solanaceae's crops (Biondi, Guedes, Wan, & Desneux, 2018). Recently, *T. absoluta* invaded Africa disrupting tomato and several Solanaceous crops including potato, night

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shade and peppers (Retta, Berhe, & Management, 2015; Zekeya et al., 2016). The pest is spreading quickly across continents threatening cultivation of tomato and related crops in Africa (Brévault, Sylla, Diatte, Bernadas, & Diarra, 2014;).

Management of *T. absoluta* with synthetic chemicals are prevalent in Africa, however they are unaffordable and reported to pose hazardous health effects in other regions (Guedes & Picanço, 2012; Silva et al., 2011; Siqueira, Guedes, Picanço, & Entomology, 2000).

Feeding behaviour of larvae by mining between plant tissues has also been thought to attribute to the failure of most chemical pesticides with contact toxicity, leading to search for systemic pest control options (Desneux et al., 2010).

Approaches such as predators, parasitoids and microbial control including fungi and bacteria are sought to be efficient for management of *T. absoluta* (Contreras et al., 2014; Pires et al., 2009; Polaszek et al., 2012; Sabbour, 2014). Some fungi including *Beauveia bassiana* and *Metarhizium anisopliae* have been reported as effective biocontrol of pests (Inanli, Yoldaş, & Birgücü, 2012). *M. anisopliae* has been reported as effective control of several crop pests (Santi et al., 2011) and has been reported to parasitize eggs of *T. absoluta* (Pires et al., 2009). However, the genus, *Aspergillus* has been reported to control several pests including *T. absoluta* in the laboratory (Dahliz et al., 2013; Lakhdari et al., 2016) whereas *Aspergillus oryzae* was reported to control Locust (Zhang et al., 2015). Despite the entomopathogenic activity of some *Aspergillus* species, there is scarce information regarding detailed entomopathogenic activity on the tomato leafminer. This study characterised the entomopathogenic activity of local isolates of *A. oryzae* against *T. absoluta* in the laboratory. However, this study recommends future study on mass production, evaluation in field conditions and commercialisation of the new biocontrol for application by farmers in Tanzania.

2. Materials and methods

2.1. Tomato plants

Twenty-one days old tomato seedlings (cultivar Tanya) were transplanted in plastic pots (diameter 14 cm and height 20 cm) containing sterile sand: compost soil (1:3 ratio) filled to a depth of about 12 cm. Then plants were allowed to grow under screen-house conditions for 14 more days at temperatures of about 23 ± 2°C and 80% relative humidity (RH).

2.2. Isolation of Aspergillus species

Fungi were isolated from dead *T. absoluta* larvae and adults from tomato leaves collected in farmers' field (3.14°S, 36.88°E) in Arusha). Isolation of fungi was achieved by sterile needle under stereomicroscope and cultured on potato dextrose agar in glass petri dishes. Out of 25 isolates identified, only two isolates (A-Tz1 and A-Tz2) with high entomopathogenic activity were selected for this study.

2.3. Morphological identification of isolates

Identification of fungal isolates was done using morphological features and colour as described by Diba, Kordbacheh, Mirhendi, Rezaie, and Mahmoudi (2007). Colour,

structure, appearance of mycelia and spore on PDA media were used to distinguish the fungus from another *Aspergillus* sp. (Zhang et al., 2015). A series of microscopic observation to verify morphology and colour pattern was used to distinguish the A-Tz1 and A-Tz2 isolates from *Aspergillus flavus* that is quite similar (Zhang et al., 2015).

2.4. Molecular identification

2.4.1. DNA extraction

In order to confirm whether these fungal isolates belong to *A. flavus* with aflatoxin compounds, molecular characterisation of all isolates was conducted. DNA from fungal isolates was extracted according to Mahuku (2004) with minor modifications. Two hundred milligrams of fresh fungal mycelia were transferred to a sterilised 1.5 mL Eppendorf tube containing 300 µL of TES, extraction buffer (0.2 M Tris–HCl (Bezemer, Jones, & Knight), 10 mM EDTA [pH 8], 0.5 M NaCl, 1% SDS) and 800 mg of acid washed sterilised sea sand. Micro-centrifuge tube 1.5 mL containing mycelium were fitted into homogeniser (Retsch) and macerated for 4 min. Samples were then vortexed for 30 s followed by the addition of 200 µL of TES extraction buffer containing proteinase K. The mixture was vortexed to thoroughly mix, and tubes were placed on water bath at 65°C for 30 min. Two hundred and fifty microlitres of 7.5 M ammonium acetate was added, mixed thoroughly and incubated at −5°C for 10 min. The mixture was centrifuged for 15 min at 12,000*g* to pellet DNA and the supernatant was transferred to a new tube and 500 µL of ice-cold isopropanol was added.

Tubes were then incubated at −5°C for overnight. The mixture was centrifuged for 10 min at 12,000*g* to pellet DNA. The supernatant was decanted, and DNA pellet was washed with 800 µL of cold 70% ethanol. Tubes were kept upside-down on a clean sterile paper towel for 10 min to air dry. DNA was eluted from the pellet with twice repeated extraction with 100 µL of 1× TE buffer (10 Mm Tris–HCl [pH 8], 1 Mm EDTA) each time centrifuged for 2 min to avoid collecting polysaccharides. DNA solution was transferred to micro-centrifuge tube, followed by the addition of 5 µL of RNase (20 mg/mL) and incubated at 37°C for 60 min. Finally, 1 drop (1 µL) DNA solution was dropped into Nano drop to determine concentration and purity of DNA.

2.4.2. Amplification, sequencing and phylogenetic analysis of the ITS rDNA region

Fragments containing the region encoding ITS-1, 5.8S *rDNA* and ITS-2 were amplified using universal primer, ITS-1(F 5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC- 3′) as described by Zhang et al. (2015). Polymerase chain reaction (PCR) was conducted with Taq^{TM} mix polymerase in a thermocycler amplification of total of 20 µL DNA mixture. The initial denaturation temperature was 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 45 s, annealing at 55° C for 30 s, extension at 72°C for 1 min and incubation at 72°C for 10 min. Electrophoresis of all PCR products were performed on the 1.5% agarose gel and was run against the 1 kb ladder (Promega). DNA was stained using Gel red stain for easy visualisation and TAE buffer was used. Products were visualised through gel documentation system. The 5.8s gene of fungal isolates were aligned with the sequences in the NCBI GenBank. A phylogenic analysis was conducted using neighbour joining (NJ) method with MEGA5

program. Bootstrap tests were conducted with 1000 replications in the NJ analysis and *Davidiella* sp. was used as outgroup.

2.4.3. Aflatoxin detection test

In order to discriminate Isolate A-Tz1 and A-Tz2 from aflatoxin producing *Aspergillus* species, a RIDASCREEN Aflatoxin total kit containing (100%-aflatoxin $B_{1,}$ 48%-B_2, 78%-G_1 18%-G_2) was used to characterise isolates according to manufactures instructions. Tests were conducted using dried spores of A-Tz1 and A-Tz2, aflatoxin producing *A. flavus* (*A. flavus* B_1+), new *A. flavus* isolate grown for 12 days on PDA (30°C) and PDA powder contaminated with aflatoxin B_1(PDA B_1+). Six standards (STD1, STD2, STD3, SD4, STD5 and STD6) with known concentration of aflatoxins: 0.00, 0.05, 0.15, 0.45, 1.35 and 4.05 µg/L, respectively, were used together to compare aflatoxin content with tested isolates. Samples were prepared as per manufacturer's instructions and extracted in 70% methanol and centrifuged at 3500*g* at room temperature (23°C) and the filtrate was diluted six times for the test. The optical densities (OD) were measured at 450 nm using SYNERGY HTX Multi-mode reader (BioTek Digital, USA). Samples and standards were analysed together in duplicate wells on plate and the experiment was conducted twice.

2.5. Preparation of Aspergillus concentrations

After 10 days of isolates A-Tz1 and A-Tz2 of conidial maturation on PDA in Petri dishes, spores were gently scrapped from the media by suspending into 10 mL sterile distilled water with 0.1% Triton X-100 per Petri dish to make a stock suspension. From each stock suspension of A-Tz1 and A-Tz2, concentration was assessed by counting spores using hemocytometer neubaeur (Manfield, German). After mixing suspension in one beaker the stock solution concentration was revealed to be 1.1×10^{10} conidial/mL in which working concentrations were prepared by the addition of water followed by counting spore using hemocytometer neubaeur at temperature range of 25.5°C under stereo microscope to obtain 1.0×10^8, $1.0 \times 10^7$and 1.0×10^6 conidia/mL for pathogenic test against stages of *T. absoluta*.

2.6. Pathogenicity of isolate A-Tz1 and A-Tz2 against T. absoluta larvae

Pathogenic effect of A-Tz1 and A-Tz2 against second instar larvae was conducted in the laboratory at 19.5°C and 50% RH, 30.4°C and 70% RH, (the lowest and optimal, respectively) temperature for cultivation of *A. oryzae* (Narahara et al., 1982). Tomato leaflets (5 × 8 cm area) were used as experimental units that were dipped into (1.0×10^6, $1.0 \times 10^7$and 1.0×10^8 conidia/mL) of isolates A-Tz1 and A-Tz2 and control (water containing 0.1% Triton X-100). Treated leaflets were dried at room temperature for elimination of excess water then were placed in Petri dishes (21 cm diameter) lined with wet paper towel and cottons covering the petiole to prevent leaf dehydration. Then, ten (10) *T. absoluta* larvae were inoculated separately into each of three treated leaflets and control. Each concentration was replicated three times with 10 larvae/replication. Treated leaflets were used only once at the beginning of the bioassay. After 24 h, when treated leaflets were depleted, treated larvae were fed on fresh untreated leaves. Dead insects were placed on another Petri dish lined with moist paper towel for re-isolation of fungus. Data were

recorded at interval of 24 h and trials were monitored up to the completion of *T. absoluta* life cycle and lifespan.

2.7. Data collection and analysis

Larval survival duration was recoded at interval of 24 h until death. Percentage of larvae mortality was recorded at interval of 24 h in which number of dead larvae was deducted from initial total number of larvae times 100 over total number of larvae Active larvae (treated but persisted) were monitored for pupation rate by subtracting number of pupae from treated larvae times 100 over total number of larvae. Successful pupae (treated) were also monitored for percentage of adult emergence by subtracting number of adults emerged from total treated pupae times 100 over total pupae. Pupation, adult emergence and lifespan were also recorded by counting days taken for all pupae and adult to emerge over total insect set at the beginning of experiment. All data were analysed with the Proc GLM procedure of SAS, version 9.1 (SAS Institute, Cary, NC, USA) and tested for normality and homogeneity of variance. Mortality rate and adult life span were $\log_{10}$-transformed to obtain normally distributed data sets with equal variance. Larval duration, pupation duration and adult emergence duration were subjected to analysis of variance (ANOVA) whereas larval mortality, pupation rate and adult life span were subjected to Kruskal Wallis. Bonfferoni was used to separate mean difference of larval mortality, pupation rate and adult life span whereas Tukey's honest significant difference (HSD) was used to separate mean differences of larvae duration, pupal duration and adult emergence duration at 5% level of significance.

3. Results

3.1. Morphological and molecular characterisation of Aspergillus sp. A-Tz1 and A-Tz2

Morphological comparison of *Aspergillus* sp. A-Tz1 and A-Tz2 with *A. flavus* using culture on PDA showed that both isolates had dark color-olive/brown colour, covered with wooly tufts and smooth surface, whereas *A. flavus* showed brighter bright-yellow/green colour, covered with thick short pile like cotton on side and rough surface (Figure 1).

The sequence similarity search using ITS sequences of *Aspergillus* sp. A-Tz1 and A-Tz2 in BLASTN program revealed that both *Aspergillus* sp. A-Tz1 and A-Tz2 belong to *A. oryzae* with 100% sequence similarity to other *A. oryzae* isolates from China and India. A-Tz1 and A-Tz2 showed 100% similarity with *A. oryzae* GeneBank accession numbers EF121337.1 and MG519722.1, respectively, and *A. oryzae*/India/2016 and *A. oryzae*/China/2017 (Figure 2). Such similarity implied that both *A*-Tz1 and A-Tz2 isolates belong to *A. oryzae* due to close relation with other isolates depicted in phylogenetic tree (Figure 2).

Based on aflatoxin test, result showed a negative correlation between optical density values and aflatoxin concentration (Figure 3). The presence of aflatoxins is indicated by high level of the optical density (OD) values in aflatoxin containing standards. Isolates A-Tz 1 and A-Tz 2 exhibited OD of 3.01 and 3.19 nm, respectively, with 0.00 µg of

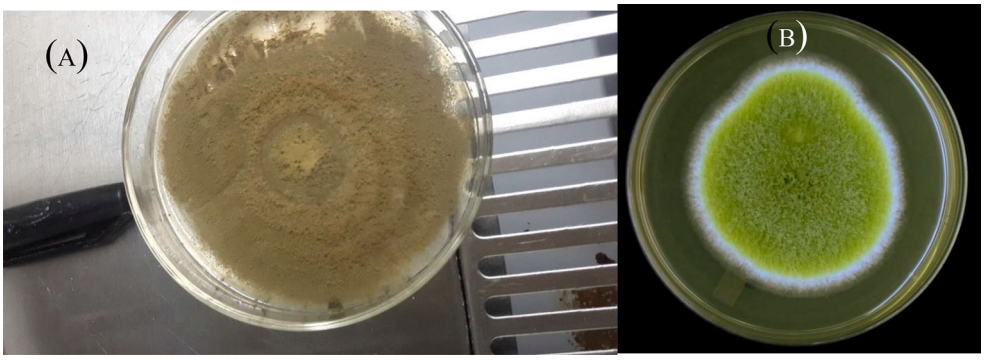

Figure 1. Morphological difference between *A. oryzae* (A-Tz1 and A-Tz2) (A) and *A. flavus* (B) on PDA incubated at 28°C for 12 days.

aflatoxin metabolites that was similar to control (STD 1) with aflatoxin level of 0.00 µg. In contrast, standard 2 up to 6 had aflatoxin concentration of 0.05 , 0.15, 0.45, 1.35 and 4.05 µg, respectively, with inverse OD ranging from 3.4 to 0.5 nm (Figure 3).

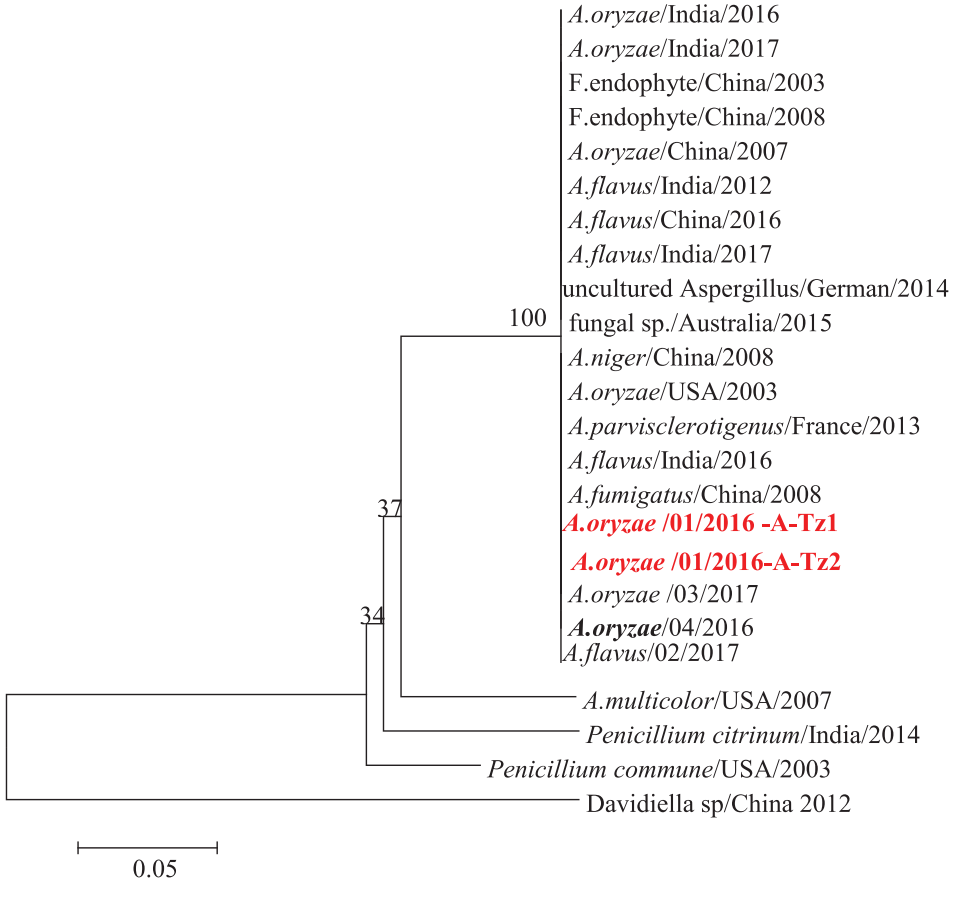

Figure 2. Neighbour joining tree based on ITS sequence data of isolate A-Tz1 and A-Tz2 and accepted species of section *flavi*.

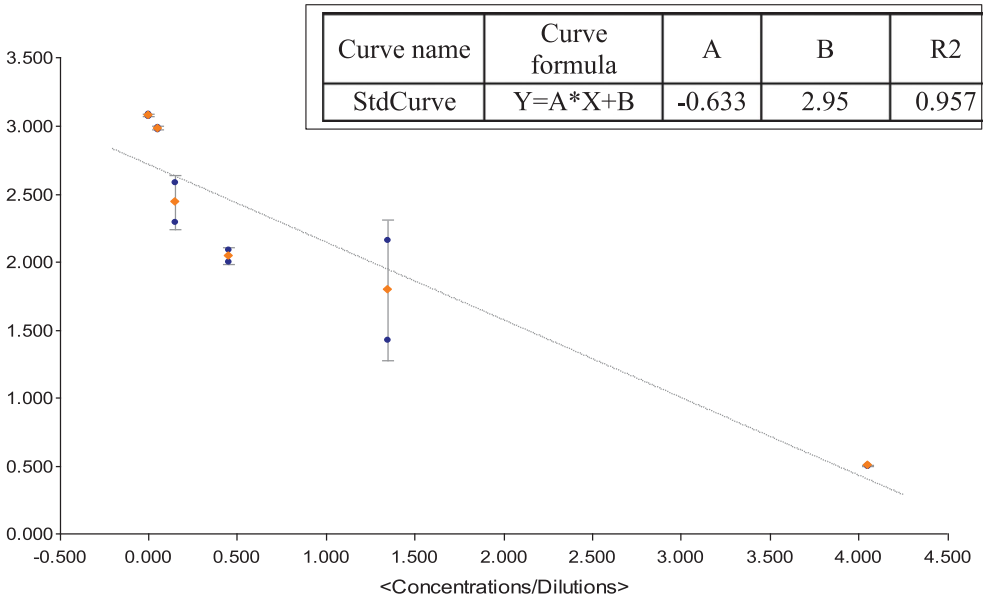

Figure 3. A standard curve presenting a relationship between optical density (450) on *Y*-axis, and aflatoxin concentration level on *X*-axis.

3.2. Pathogenicity of A-Tz1 and A-Tz2 on Tuta absoluta *at different conditions*

There was significant pathogenic effect of *Aspergillus* isolates A-Tz1 and A-Tz2 on of *T. absoluta* larvae elevated at 30.4°C and 70%RH and 19.5°C and 50%RH compared to control. The effect of temperature and RH on larval duration, pupation duration and adult emergence duration varied significantly between treatments (Tables 1 and 2). The pathogenic activity was concentration dependent in which higher concentration exhibited higher activity at both conditions.

At 30.4°C and 70%RH larval, pupal and adult duration was shorter compared to 19.5°C and 50%RH in both isolate and control (Tables 1 and 2). The effect of treatment increased with increase in concentration where at 1.0×10^8 conidia/mL larvae had short duration averaging 3.5 ± 0.3 days and 6.8 ± 0.5 days in A-Tz1 and A-Tz2, treated larvae, respectively, whereas in control larvae survived longer averaging 12.0 ± 0.8 days (Table 1). The effect of treatment on pupation was significant in which control exhibited shorter pupation period averaging 9.6 ± 0.3 days compared to sick larvae (A-Tz1) that spent 15.0 ± 0.3days

Table 1. Effect of isolate A-Tz1 and A-Tz2 on *Tuta absoluta* at 30.4°C and 70%RH.

Treatment	Concentration (conidial/mL)	Larval duration (days)	Pupation duration (days)	Adult emergence duration (days)
Control	0	11.6 ± 0.6a	9.6 ± 0.3c	16.6 ± 0.5a
A-Tz 2	1.0×10^6	11.0 ± 0.4a	13.07 ± 0.4b	11.6 ± 0.3b
A-Tz 1	1.0×10^6	9.4 ± 0.4ab	13.6 ± 0.3b	11.1 ± 0.3b
A-Tz 2	1.0×10^7	8.6 ± 0.5bc	13.0 ± 0.3b	10.0 ± 0.6b
A-Tz 1	1.0×10^7	7.3 ± 0.5ab	14.6 ± 0.4ab	7.2 ± 0.9c
A-Tz 2	1.0×10^8	5.8 ± 0.6ab	15.1 ± 0.4a	2.3 ± 0.4d
A-Tz 1	1.0×10^8	3.5 ± 0.5c	15.0 ± 0.3a	2.2 ± 0.5d
P value		*<.0001*	*<.0001*	*<.0001*

Table 2. Effect of isolate A-Tz 1 and A-Tz 2 on *Tuta absoluta* at 19.5°C and 50%RH.

Treatments	Concentration (conidial/mL)	Larval survival duration (days)	Pupation duration (days)	Adult emergence duration (days_
Control	0	12.0 ± 0.8a	10.6 ± 0.2c	19.9 ± 0.3a
A-Tz 2	1.0×10^6	10.9 ± 0.6a	10.8 ± 0.2c	8.7 ± 0.9b
A-Tz 1	1.0×10^6	10.6 ± 0.4a	11.7 ± 0.6c	10.2 ± 1.0b
A-Tz 2	1.0×10^7	7.6 ± 0.4b	11.7 ± 0.6c	4.5 ± 1.0cd
A-Tz 1	1.0×10^7	6.1 ± 0.4b	11.7 ± 0.6c	7.0 ± 0.7bc
A-Tz 2	1.0×10^8	6.8 ± 0.5b	14.2 ± 0.3ab	2.9 ± 0.4d
A-Tz 1	1.0×10^8	4.8 ± 0.8c	15.5 ± 0.5a	2.8 ± 0.2d
P value		<.0001	<.0001	<.0001

for pupation (Table 1).The effect of *A. oryzae* isolates on emergence of adult *T. absoluta* was also high at concentration of 1.0×10^8 conidial/mL of both A-Tz1 and A-Tz2 that exhibited 2.2 ± 0.5 days and 2.3 ± 0.4 days, respectively, to undergoes mortality compared to control that exhibited longer emergence time averaging 16.6 ± 0.5 days(Table 1).

At 19.5°C and 50%RH, isolate A-Tz1 exhibited longer larvae duration averaging 4.8 ± 0.8 days compared to A-Tz2 (6.8 0.5 days) and control treated larvae that caused mortality in average of 12.0 ± 0.8 days post inoculation (Table 2). The effect of treatments on pupation of *T. absoluta* differed between treatment in which A-Tz2 and A-Tz1 treated insects (sick pupae) exhibited the longer pupation time averaging 15.8 ± 0.7days at 1×10^8 conidia/mL compared to 1×10^6 conidia/mL treated insect and control that completed pupation in 10.6 ± 0.2 days post inoculation (Table 2). The duration for adult emergence also varied between treatments in which isolate A-Tz1 and A-Tz2 treated insects emerged after an average of 2.8 ± 0.2 and 2.9 ± 0.4 days, respectively, in contrast to control where longer adult emergence duration of up to 19.9 ± 0.3 days was observed due to high number of live insects that survived for longer period (Table 2).

Mortality rate of larvae, pupation rate and adult emergence rate varied significantly between treatments at 30.4°C, 70%RH and 19.5°C, 50%RH (Tables 3 and 4). Isolate A-Tz1 exhibited the highest mortality compared to isolate A-Tz2 and control. At higher concentrations of 1.0×10^8 conidial/mL, both isolate A-Tz1 and A-Tz2 depicted higher mortality rate of 76.66% and 70%, respectively, compared to control that only 13.33% larval mortality was observed (Table 3). The rate of pupation was 23.3% in A-Tz1 at high concentration of 1.0×10^8 conidia/mL compared to low concentration of A-Tz2 and compared control that was 86.6% of pupation. The percentage of adult emerged from treated (sick pupae) was lower ranging from 18.6% to 33.3% in higher and moderate concentration, respectively, compared to control that was successful by

Table 3. Effect of isolate A-Tz 1 and A-Tz mortality of *Tuta absoluta* at 30.4°C and 70%RH.

Treatments	Concentration (conidia/mL)	% of Larval mortality	% of Pupation	%Adult emerged	Adult lifespan (days)
Control	0	13.3	86.7	76.7	16.6 ± 0.5a
A-Tz 2	1.0×10^6	43.3	66.3	50.0	10.2 ± 1.0b
A-Tz 1	1.0×10^6	40.0	46.7	43.6	8.7 ± 0.9b
A-Tz 2	1.0×10^7	60.0	33.3	33.3	7.0 ± 0.7bc
A-Tz 1	1.0×10^7	63.3	33.3	23.3	4.5 ± 1.0cd
A-Tz2	1.0×10^8	70.0	26.6	23.3	1.3 ± 0.4d
A-Tz1	1.0×10^8	76.7	23.7	18.6	0.8 ± 0.2d
P value		*<.0001*	*<.0001*	*<.0001*	*<.0001*

Table 4. Effect of A-Tz1 and A-Tz2 on mortality of *Tuta absoluta* at 19.5°C and 50%RH.

Treatments	Concentration (conidia/mL)	% Larval mortality	% of Pupation	%Adult emerged	Adult life span
Control	0	14.00	80.00	76.66	19.9 ± 0.3a
A-Tz 2	1.0×10^6	50.00	50.00	50.00	11.6 ± 0.3b
A-Tz 1	1.0×10^6	50.00	46.66	46.66	11.1 ± 0.3b
A-Tz 2	1.0×10^7	53.33	33.33	33.33	10.0 ± 0.6b
A-Tz 1	1.0×10^7	66.66	33.33	33.33	7.2 ± 0.9c
A-Tz 2	1.0×10^8	66.66	33.33	33.33	5.0 ± 0.7c
A-Tz 1	1.0×10^8	70.00	26.66	26.66	2.7 ± 0.4d
P value		*<.0001*	*<.0001*	*<.0001*	*<.0001*

76.7% (Tables 3 and 4). However, effect of both isolate A-Tz1 and A-Tz2 on life span of *T. absoluta* was significantly across both conditions 19.5°C and 50%RH and 30.4°C and 70%RH but with moderately higher activity in the later conditions (Tables 3 and 4).

4. Discussion

Based on morphological and molecular identification, we confirmed that isolates *Aspergillus* sp. A-Tz1 and A-Tz2 belong to *A. oryzae*. Morphological features including olive colour, fluffy mycelia and revealed that isolates were *A. oryzae* (Zhang et al., 2015). Although morphology and physiology are traditionally used for the identification of fungi, such characteristics were not quite sufficient (Geiser et al., 2007), thus it was complemented with the molecular identification by the use of ITS1-5.8S rDNA-ITS2 as appropriate locus for the identification of *Aspergillus* species (Zhang et al., 2015). However, a combination of morphological and molecular characterisation has been documented for identification of *A. oryzae* infecting locust in China (Zhang et al., 2015). Thus, we were also able to use both morphological and molecular identification approaches to confirm the identity of *Aspergillus* sp. A-Tz1 and A-Tz2 as *A. oryzae* in the current study.

Additionally, aflatoxin test revealed that both isolates *Aspergillus* sp. A-Tz1 and A-Tz2 are non-aflatoxin producer thus could not be associated with aflatoxin producing *Aspergillus* species especially *A. flavus* that is similar to in most morphological features (Zhang et al., 2015).

An entomopathogenic activity of the newly identified isolates was conducted as it has been reported from several fungi infecting *T. absoluta* (El-Hindi, 2016). The Efficacy of *Aspergillus* sp. against *T. absoluta* has also been reported in some African countries (Lakhdari et al., 2016). Specifically, Zhang et al. (2015) revealed the entomopathogenic activity of *A. oryzae* against locusts in China. This study revealed the efficacy of *A. oryzae* against *T. absoluta* which concur the study by Zhang et al. (2015) that established an entomopathogenic activity of the same fungus on different pest. In this study, the pathogenic activity of *A. oryzae* against *T. absoluta* increased with rise in concentration, in which the higher concentrations caused higher mortality within a short time. In addition, *A. oryzae* was also effective than control at lower concentrations although its activity on larvae, pupae and adult mortality was sluggish compared to higher concentration. *A. oryzae* infected *T. absoluta* in all stages in which treated insects were weak leading to delayed pupation than healthy insects in control. It was also observed that few insects pupated and emerged into adult in which the period for adult emergence and survival duration was very short in *A. oryzae* treated insect regardless of concentration high compared

to control. This could be due to low number of insects in *A. oryzae* treated plates due to high mortality exerted by *A. oryzae* in short time leading to low average duration at the end of the experiment compared to control.

Despite concentrations of *A. oryzae* in treated dead insects, the fungal mycelia and spore were finally, observed propagating on insects' cuticles (Pedrini, Ortiz-Urquiza, Zhang, & Keyhani, 2013; Petlamul & Prasertsan, 2012) and eventually the entire insect body was found degraded due to fungal activity (Charnley & Leger, 1991). The destruction on insect body could be due to enzymatic activity secreted by fungus in insects' cuticles and exoskeletons as a result of hydrophobic interaction between fungus and insect attachment sites (Charnley & Leger, 1991; Zhang et al., 2012).

Temperature and RH have been reported to affect entomopathogenic activity of fungi (Li et al., 2014). This study revealed that at moderate conditions of 28–32°C and 60–80% RH temperature and RH, respectively, the activity of *A. oryzae* was higher than that below 20°C and 50% RH. This could be associated with high fungal metabolic rates in warm temperature, compared to cool temperature. These findings are in consistent with previous report that revealed the entomopathogenic activity of fungal species including *Metarhizium* and *Beauveria spp.* at 25–30°C (Shi & Feng, 2004). Thus, the higher infectivity rate in warmer temperature suggests the shorted duration for entomopathogenic fungi activity for control of *T. absoluta* with short life cycle.

5. Conclusion

This study discovered that *A. oryzae* is efficacy against life stages of *T. absoluta*, a devastating pest of Solanaceous crops and weeds. Hence, these findings suggest for field evaluation and formulation of biopesticide from *A. oryzae* isolates that could save as biocontrol of *T. absoluta* to minimise use synthetic chemicals for protection of crops and biodiversity.

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