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ORIGINAL RESEARCH ARTICLE

Efficacy of indigenous entomopathogenic fungi against the black aphid, *Aphis fabae* Scopoli under controlled conditions in Tanzania

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Abstract

Worldwide application of synthetic insecticides as a main way of controlling aphids on various crops has resulted in diverse problems such as failures in pest control, negative public health and environmental impacts, and a build-up of resistance against insecticides by insect pests. Entomopathogenic fungi can be used as an alternative to insecticides since they offer the benefit of being environmentally friendly, without the risk of insect pests developing resistance. This study assessed 19 different indigenous fungi in the laboratory for their ability to control *Aphis fabae* Scopoli at various conidial concentrations (10^4, 10^5, 10^6 and 10^7 spores/ml) using the detached leaf method. *A. fabae* adults were reared on potted cowpea in cages. Both the 19 fungus types and their respective conidial concentrations used to treat *A. fabae* differed significantly ($P < 0.001$) in their ability to kill the pest. The *A. fabae* mortality rate increased in line with the increase in the conidial concentration of the fungus type, and *Aspergillus flavus* Link S18 and S19 performed better than the other fungus types evaluated. *Aspergillus flavus* S18 and S19 are recommended for further tests in the greenhouse to validate the laboratory results. The fact that the *Aspergillus* strains isolated and tested were all aflatoxin-producing strains calls for caution regarding their potential impacts on human and animal health. Further studies are recommended to conduct similar experiments using non-aflatoxin-producing strains *of A. flavus*, in order to determine whether they have similar effects on *A. fabae*.

Keywords Biocontrol · Mycopesticide · Cowpea pests · World Vegetable Center · Aspergillus

Introduction

Aphids are generally serious pests in agricultural and horticultural crops all over the world, causing direct damage by sucking plant sap, and indirect damage through the transmission of more than 300 plant pathogenic viruses, and contamination through honeydew deposits on the surface of plant foliage, thus favoring the growth of sooty mold which reduces photosynthetic surface (Hogenhout et al. 2008; Van Emden and Harrington 2007). Both young (nymphs) and adult aphids feed on plant sap, attacking almost everywhere on plants; leaves, stem buds, flowers and fruits. In so doing, they cause curling or stunted yellow leaves, distorted or deformed flowers and formation of galls on roots and stem on the infested plant (Van Emden and Harrington 2007). Many horticultural crops are affected by aphids in East Africa, including cabbages (Oduor et al. 1996), French beans (Gogo et al. 2014), cucurbits (Nordey et al. 2020), and cowpea (Karungi et al. 1999). *Aphis fabae* Scopoli (Hemiptera: Aphididae), is particularly challenging in northern Tanzania because of its year-round pressure and its impact on export crops such as French beans and Kalanchoe (Domier et al. 2007). Aphids are reported by Nyambo (2009) and Mureithi et al. (2015) as being among the major pests on agricultural crops in Tanzania and Kenya. In the current study, cowpea, which is among the most preferred hosts by aphids (Mfeka et al. 2019) was used to culture the insects.

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Worldwide application of synthetic insecticides as the main way of controlling aphids on various crops has resulted in diverse problems, such as failures in pest control, negative environmental impacts, and a build-up of insecticide resistance by aphids (Foster et al. 2007; Silva et al. 2012). Furthermore, the environmental and health impacts of synthetic pesticides are increasingly raising concerns, thus encouraging agricultural producers to search for and adopt effective alternative control methods such as biological control using natural enemies of pests (Inglis et al. 2001; Lacey et al. 2015).

Among the available biological control agents against insects, entomopathogenic fungi are the most significant pathogens where more than 16 species of fungi are known to naturally infect aphids (Pell et al. 2001; Chen et al. 2008). Entomopathogenic fungi are reported to be effective against all the developmental stages (eggs, larvae, intermediate stages and adults) of various insects (Shi and Feng 2004; Scholte et al. 2005). Since they are organisms that occur naturally, entomopathogenic fungi are regarded as the most promising alternative to the synthetic (chemical) pesticides and they are perceived as being friendlier to the environment than the synthetic pesticides (Butt et al. 2002; Sandhu et al. 2012). Moreover, the target insects are unlikely to develop resistance against them, since their mode of action appears to be less complex compared to their counterparts (Sandhu et al. 2012). Various works on entomopathogenic fungi (research, production, stabilization, formulation and application) have significantly contributed to the commercialization of more than 170 products of fungus-based biopesticides (de Faria and Wraight 2007). The most commonly used products are those based on *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Isaria fumosorosea* Wize, and *Beauveria brongniartii* (Sacc.) (de Faria and Wraight 2007). Studies have shown that the effectiveness of an entomopathogenic fungus depends on its ability to produce many spores and/or sticky spore surfaces or substances that enhance adhesion of spores to the host surface, its ability to germinate and penetrate the exoskeleton of the insect, its ability to survive digestion after being ingested by the insect (host) and ability to proliferate within the body of the host in order to collapse its immune system so that the insect subsequently dies (Vega et al. 2012; Sandhu et al. 2017). It has been reported that mortality of aphids due to treatment with entomopathogenic fungi increases with increase in spore concentration and the time of exposure (Asi et al. 2009; Sevim et al. 2013).

Although entomopathogenic fungi are increasingly used in Europe, the North America, and in Asia, their availability to growers in Africa is impeded by import restrictions, quality of products, and suitability with climatic conditions. Integrated pest management (IPM), the method which combines several techniques such as biological control, habitat modification, manipulation of cultural practices and use of crop resistant varieties are used to control pests by reducing the harmful effects of chemical pesticides (Dharam and Shankar 2012). IPM concept originated from the ideas about the ill effects of synthetic pesticides to non-targeted organisms including domestic animals and human, published by Carson (1962). Entomopathogenic fungi which are among the agents of biological control can well be included in IPM programs to control various arthropod pests (Allan et al. 2016; Maina et al. 2018).

The infection process of an entomopathogenic fungus starts with its entrance through the cuticle of the host insect (Sevim et al. 2015) occurring both physically and physiologically (enzymatically) (Clarkson and Chamley 1996). The sequence of events in this process occurs as follows: the fungus spores stick and settle on the cuticle of the insect, then the spores germinate and penetrate the cuticle to the haemocoel by forming appressoria, then hyphae develop in the hypodermis and they continue to multiply in the insect body and lymphatic cells and finally cause the death of the insect (Sani et al. 2020). According to Milner (1997), Roy et al. (2010), aphids are highly susceptible to entomopathogenic fungi especially the epizootic fungal diseases than any other arthropods.

This study was undertaken to assess the effectiveness/pathogenicity of 19 different entomopathogenic fungi (indigenous to Tanzania) against the black aphid, *A. fabae,* at different spore concentrations (10^4, 10^5, 10^6 and 10^7 conidia/ml) under laboratory conditions. The 19 entomopathogenic fungi were: S1, S2, S3, S4, S5, S6, S7, S8 (*Aspergillus flavus*), S9, S10, S11, S12, S13, S14, S15 (*Aspergillus tamarii*), S16, S17 (*Aspergillus flavus*), S18 (*Aspergillus flavus*) and S19 (*Aspergillus flavus*).

Materials and methods

The experiments were conducted from November 2018 to April 2019 in the entomology laboratory at the World Vegetable Center, Eastern and Southern Africa (WorldVeg-ESA) in Arusha, Tanzania (3.37406°S and 36.80560°E).

Plant material and aphid pure culture rearing

Cowpea, *Vigna unguiculata* (L.) Walp (Fabales: Fabaceae) which is among the hosts of *A. fabae* (Karungi et al. 1999) were cultured in one litter plastic pots (one plant per pot) in wooden cages, in a plastic film roofed screen house. The sides of the screen house were shielded with an insect-proof net of mesh size 0.6 mm whereas the sides and top of the cages (bottomed with wood) and the cage sleeves were shielded with a 0.3 mm mesh sized net to prevent untargeted insects from getting in as well as preventing the aphids (in the pure aphid culture – next paragraph) from getting out (Harmanto and Salokhe 2006). The cages (2 m long, 1 m wide and 0.8 m

high) in the screen house were placed on tables at a height of 0.8 m from the ground.

Similar structures and cowpea potted plants were separately used to rear a pure culture of *A. fabae*. Black aphid nymphs were collected from cowpea plants in the field within and around World Vegetable Center compounds. They were introduced and reared on potted insect-free cowpea plants in cages in screen houses. The black aphid colonies were purified by shifting the newly born nymphs from the initial cage to new ones on plants that were fresh and free from aphids. From such lots, the experimental colonies were multiplied. When the plants were severely damaged by the aphid, new uninfested plants were placed in the cages and let the aphids shift themselves from the overcrowded plants to the new ones. Finally the dying plants due to aphid damage were removed from the cage and destroyed.

A planting medium of a mixture of loam forest soil and sand at 2:1 (*v*/v - loam forest soil: sand) was used for planting the cowpea in the pots to imitate the sandy loam soil which is preferred by cowpea (Makoko 2008; Mfeka et al. 2019). Plants were watered once to twice a week depending on the moisture of the soil detected by feel. Temperature and relative humidity (RH) in the cages were 25 ± 3 °C and 60 ± 5% respectively and a photoperiod of 12:12 h (L: D). Plants in one cage were used to raise *A. fabae* colony while those in other three cages were used as a source of young shoots on which the aphid (*A. fabae*) were fed during application of entomopathogenic fungi experiments in laboratory.

Table 1 List of the fungal isolates collected from farmers' fields in Arumeru, Arusha and Moshi Rural, Kilimanjaro using a Microbiological air sampler

Fungus strain name/code	Source of collection	
	Region	Locality
S1	Arusha	Madira Farm
S2	Arusha	Madira Farm
S3	Arusha	Madira Farm
S4	Arusha	Madira Farm
S5	Arusha	Madira Farm
S6	Arusha	Madira Farm
S7	Arusha	Madira Farm
S8 (*A. flavus*)	Arusha	Madira Farm
S9	Arusha	Madira Farm
S10	Kilimanjaro	Kibosho
S11	Arusha	Duluti
S2	Arusha	Duluti
S13	Arusha	Duluti
S14	Arusha	Duluti
S15 (*A. tamarii*)	Kilimanjaro	Kibosho
S16	Kilimanjaro	Kibosho
S17 (*A. flavus*)	Arusha	Madira Farm
S18 (*A. flavus)*	Arusha	Madira Farm
S19 (*A. flavus*)	Arusha	Madira Farm

Fungus collection, isolation and purification

Several indigenous microbial propagules were randomly (informal visits) collected from farmers' fields within Arumeru District in Arusha Region and within Moshi Rural District in Kilimanjaro Region for 4 days using a Microbiological air sampler (MicroBio-MB1 - Parrett Technical Developments, Bromley, England) pre-loaded with a sterile Petri dish containing Potato Dextrose Agar (PDA) fungal culturing medium. The collected spores/propagules were then incubated at 28 ± 1 °C, 75 ± 2% RH, until fungal growths were observed and matured.

The resulting 19 different fungal growths collected from Arusha and Kilimanjaro Regions (Table 1) were purified by carefully transferring spores using a sterile inoculation needle from the first Petri dish to new ones containing a similar culturing medium (PDA). The new Petri dishes containing PDA were initially sterilized by autoclaving at 121 °C for 15 to 20 min. They were then cultured in an incubator at 28 ± 1 °C and 75% ± 2% RH until fully grown colonies appeared, and the process was repeated until pure colonies were obtained.

After maturation and full sporulation, the conidia were harvested by gently scraping the surface of the cultures with a sterile inoculating loop to dislodge the conidia from the surface of the agar plates. The harvested conidia were stored at 4 °C and 0% RH. To purify the fungal types and determine the viability of the conidia, ten sterile Petri dishes with PDA medium were inoculated with 1 ml of conidial suspension of each type, then incubated for 5 days at 28 ± 1 °C and 75 ± 2% RH.

Assessing the effectiveness of the collected indigenous fungi against aphid

A factorial experiment under completely randomized design with two factors, i.e. type of fungus and concentration of conidial suspension, was used to compare the efficacy of the collected indigenous entomopathogenic fungi against adult *A. fabae* using the detached leaf method (Yokomi and Gottwald 1988). The experiments were divided into two with regard to the number of fungus types involved as indicated below.

Assessing the effectiveness of all the 19 fungus types collected against aphids

An initial experiment was conducted to compare the efficacy of the 19 isolated fungi (S1, S2, S3, S4, S5, S6, S7, S8

(*Aspergillus flavus*), S9, S10, S11, S12, S13, S14, S15 (*Aspergillus tamarii*), S16, S17 (*Aspergillus flavus*), S18 (*Aspergillus flavus*) and S19 (*Aspergillus flavus*) at different conidial concentrations (i.e., 10^5, 10^6 and 10^7 spores/ml) to a control (sterile distilled water), and each treatment (fungus type and conidial concentration) involved ten 3-day-old *A. fabae* adults and was replicated 10 times. Conidial concentrations were measured using a haemocytometer (Neubaeur chamber, Germany).

Assessing the effectiveness of the best five (out of the 19 collected) fungi against aphids

Based on the results of the first experiment which involved all the 19 fungi collected, five fungus types which were later identified as belonging to *Aspergillus* genus (*A. tamarii* Kita (S5), *A. flavus* Link (S8), *A. flavus* (S17), *A. flavus* (S18) and *A. flavus* (S19)), were then selected for further screening under laboratory conditions (at 28 ± 1 °C, 75 ± 2% RH). The efficacy of the five selected fungi was compared at four conidial concentrations (i.e., 10^4, 10^5, 10^6 and 10^7 spores/ml) to a control (sterile distilled water), and each treatment (fungus type and conidia concentration) was replicated 10 times. The whole experimental set-up was then repeated three times on different dates (i. e., experiments 1–3).

Young cowpea tip-shoots cultured in a greenhouse were rinsed in tap water for 15 min, washed three times with sterile distilled water, and air-dried under a sterile laminar flow hood. Working under the sterile hood, a blotting paper moistened with sterile distilled water was placed as a liner in the bottom of a sterile Petri dish to maintain a high relative humidity (~90%) throughout the duration of the experiment, to prevent the cowpea shoots from drying out before the end of the experiment. Under the sterile hood, the young cowpea tip-shoots were dipped into a suspension of fungal spores in accordance with the concentration level (treatment – see how they were prepared in the next paragraph) required. Each of these shoots were then placed in the Petri dishes previously lined with moist sterile bloating paper and covered with its lid.

Stock suspensions of fungal spores used as explained above were prepared by adding 20 ml of sterile distilled water to each Petri dish with a pure fungal colony, preceded by gentle scraping on the surface of the cultures, as explained above. The spore suspension was pipetted from the plate and

Table 2 Mean aphid mortality (*Aphis fabae*) (± SE) caused by different entomopathogenic fungus treatments at three different conidial concentrations under laboratory conditions ($N = 10$)

Fungus strain	Aphid mortality (%) at three conidial concentrations (spores/ml.) of different fungus strains					
	10^5		10^6		10^7	
	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
S1	57 (0.16)bcde A	30–90	52 (0.19)cde A	20–70	52 (0.13)def A	30–70
S2	1 (0.03)j B	0–10	23 (0.14)fg A	0–40	26 (0.20)ghi A	0–50
S3	31 (0.07)ghi A	20–40	33 (0.12)ef A	20–60	34 (0.07)fgh A	20–40
S4	34 (0.11)fghi A	20–50	34 (0.08)ef A	20–50	39 (0.11)fgh A	20–50
S5	67 (0.13)abc A	50–80	65 (0.16)bcd A	50–90	78 (0.10)abc A	60–90
S6	35 (0.08)fgh A	20–50	33 (0.16)ef A	10–60	30 (0.11)ghi A	10–40
S7	35 (0.27)fgh A	0–100	39 (0.14)ef A	10–60	32 (0.14)fgh A	10–60
S8 (*A. flavus*)	68 (0.15)abc B	50–100	74 (0.09)abc AB	60–90	82 (0.09)abc A	70–90
S9	56 (0.08)bcd C	40–70	94 (0.07)a A	80–100	85 (0.12)ab B	60–100
S10	81 (0.18)ab A	50–100	26 (0.11)fg B	10–40	74 (0.22)abc A	30–100
S11	42 (0.19)defg B	10–70	77 (0.22)ab A	40–100	22 (0.09)hi C	10–40
S2	34 (0.15)fgh A	10–50	68 (0.14)ef A	20–60	66 (0.13)fgh A	30–70
S13	52 (0.21)cdef A	20–90	41 (0.11)bc A	5090	41 (0.14)cde A	40–80
S14	38 (0.08)efgh B	20–50	45 (0.19)def B	10–80	69 (0.14)bcd A	50–100
S15 (*A. tamarii*)	86 (0.13)a A	60–100	71 (0.17)abc A	50–100	87 (0.09)ab A	70–100
S16	16 (0.15)hij B	0–40	32 (0.20)ef AB	10–70	48 (0.18)efg A	20–70
S17 (*A. flavus*)	70 (0.15)abc A	40–90	78 (0.08)ab A	70–90	80 (0.12)abc A	60–100
S18 (*A. flavus)*	73 (0.16)ab A	50–100	74 (0.14)abc A	50–90	78 (0.12)abc A	60–90
S19 (*A. flavus*)	64 (0.13)abc A	50–90	78 (0.20)abc C	3090	92 (0.11)ab B	70–100
Control	8 (0.09)ij A	0–2	0 (0)g B	0–2	1 (0.03)i B	0–2

Different lower-case letters indicate differences between fungi for the same conidial concentration. Different capital letters indicate differences between conidial concentrations for a given fungus

Table 3 Mean aphid mortality (*Aphis fabae*) (± SE) caused by five entomopathogenic fungi at four different conidial concentrations under laboratory conditions ($N = 10$)

Exp.	Conidial concentration (spores/ml.)	Aphid mortality (%) as caused by each fungus strain including the control experiment											
		Control		*A. flavus* (S8)		*A. flavus* (S17)		*A. flavus* (S18)		*A. flavus* (S19)		*A. tamarii* (S15)	
		Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
1	10^4	2(0.42)cB	0–2	27(1.70)bB	0–50	40(1.05)abC	20–50	33(1.41)bC	20–70	32(1.03)abD	20–50	44(0.70)aB	0–10
	10^5	1(0.32)dB		36(0.97)cB	20–50	45(1.58)bcC	20–80	71(1.79)aB	50–90	53(1.06)abC	40–70	49(1.29)bAB	0–40
	10^6	3(0.48)dB		53(0.82)cA	40–70	62(1.03)bcA	50–80	77(1.25)aB	50–90	68(1.55)abB	40–90	61(0.88)bcA	10–70
	10^7	10(0.47)cA		61(1.10)bA	50–80	60(1.94)bAB	30–90	90(0.82)aA	70–100	82(0.79)aA	70–90	53(1.25)bAB	20–70
2	10^4	1(0.32)dA	0–1	36(0.84)bC	20–50	33(1.16)bD	20–50	46(0.97)aD	30–60	46(0.70)aC	40–60	22(0.79)cB	30–50
	10^5	2(0.42)dA		41(1.37)cC	30–70	54(0.97)abC	40–70	59(1.10)aC	40–80	46(1.17)bcC	30–70	16(1.51)dB	30–70
	10^6	0(0)aA		54(1.65)cdB	30–80	70(1.41)abB	40–90	75(1.08)aB	60–90	60(1.33)bcB	40–80	32(2.04)dAB	50–70
	10^7	1(0.32)cA		79(0.57)bA	70–90	82(1.32)abA	60–100	87(1.06)abA	70–100	91(0.88)aA	70–100	48(1.81)cA	30–70
3	10^4	0(0)bA	0–0	25(0.71)aC	10–30	21(1.60)aB	0–50	26(1.07)aB	10–50	22(1.03)aD	10–40	19(1.37)aB	10–30
	10^5	0(0)bA		68(1.48)aB	50–100	70(1.49)aA	40–90	73(1.57)aA	50–100	64(1.35)aC	50–90	31(1.37)bB	0–40
	10^6	0(0)cA		74(0.97)abA	60–90	78(0.79)aA	70–90	74(1.43)aA	50–90	78(1.99)aB	30–90	56(1.51)bA	10–70
	10^7	0(0)dA		82(0.92)abA	70–90	80(1.25)bA	60–100	78(1.23)bA	60–90	92(1.14)aA	70–100	50(1.41)cA	20–70

Different lower-case letters indicate differences between fungi for the same conidial concentration. Different capital letters indicate differences between conidial concentrations for a given fungus within the same experiment

filtered through three layers of cheesecloth. The spore concentration in a mother stock suspension was assessed using a haemocytometer (Neubaeur chamber, Germany) and stock suspension concentrations of 10^5, 10^6 and 10^7 spores/ml. were prepared.

Ten 3-day-old *A. fabae* adults previously reared on clean and healthy cowpea plants in pots placed in cages in screen house were placed on the leaves dipped in a fungal spore suspension in each formally prepared Petri dishes. As a control, same number of aphids of same age were placed on sterile cowpea leaves that had not been treated with any of the fungus types, but had just been dipped in sterile distilled water.

Data collection

Aphid mortality was recorded daily for up to seven days and the cause of death was confirmed by searching for fungal growths on the dead aphid body under a microscope. If a dead aphid did not show fungal outgrowths of similar characteristics to those of the one applied as the treatment, its death was considered as caused by another factor, or factors, and was therefore discarded.

Genetic identification of fungi and quantification of their aflatoxin production

Samples of the five most effective fungi against *A. fabae,* as observed under laboratory conditions, were subjected to molecular analysis using sequence data of the ITS region (ITS1 and ITS2 markers) of the nuclear ribosomal DNA (Henry et al. 2000), since it is frequently variable between different isolates of the same species (Gomes et al. 2002). A genetic analysis was conducted by an external laboratory with ISO/CEI 17025 certification (ADNId, Montferrier-sur-Lez, France). The procedure was followed by sequencing (Next Generation Sequencing – NGS) on a MiSeq (Illumina). Samples of the five selected strains were also sent to an external laboratory at Nelson Mandela African Institute of Science and Technology (NM-AIST) in Arusha, Tanzania, for analysis to quantify their production of aflatoxins (G1, G2, B1 and B2) using HPLC procedure.

Data analysis

Kruskal Wallis tests were used to assess the effect of the fungus types and their conidial concentrations on aphids as the data did not follow normal distribution. When significant differences were established, post-hoc analyses were carried out to compare fungus type efficiency for the same conidial concentration and to compare the efficiency of each fungus type at different conidial concentrations using Fisher's least significant difference and the Holm method to adjust the *P* value. All statistical analyses were performed using R software with the agricolae package (de Mendiburu and de Mendiburu 2013). A statistical analysis of the genomic data was conducted using computer software and blasting into the on-line genomic databases.

Results and discussion

Nineteen different isolated fungus types collected from the surveyed areas of the Arusha and Kilimanjaro Regions are presented in Table 2. Almost all of the 19 fungal isolates showed a resemblance to the genus *Aspergillus* when the morphology of the colonies (colony structure) was observed in the cultures. This observation suggested that the fungal propagules collected in this study were possibly dominated by *Aspergillus* species which raised the need for molecular identification of the fungi that caused higher mortality on *A. fabae* to confirm or refute the initial results observed in the colony cultures.

The fungal types and their respective conidial concentrations used to treat *A. fabae* differed significantly ($P < 0.001$) in their ability to kill *A. fabae,* both in the initial experiments with all the 19 fungus types collected (Table 2) and with the five (out of the 19 types collected) most efficient fungi re-tested separately thereafter (Table 3). Among the 19 fungus types collected and tested, strains S8, S15, S17, S18, and S19 were selected for further experiments (Table 3) since they

Table 4 Type and quantity ($\mu g.kg^{-1}$) of aflatoxins produced by the five selected fungal strains

Fungal strain	Type and quantity of aflatoxin produced ($\mu g.kg^{-1}$)				
	Aflatoxin G2	Aflatoxin G1	Aflatoxin B2	Aflatoxin B1	Total aflatoxin
Aspergillus flavus (S8)	3.24	3.6	0.84	31.2	38.88
Aspergillus flavus (S17)	3.24	105.48	0.0	26.56	135.24
Aspergillus flavus (S18)	5.4	21.36	0.6	19.44	46.8
Aspergillus flavus (S19)	0.0	3.48	1.08	27.48	32.04
Aspergillus tamarii (S15)	7.44	0.0	0.6	0.36	8.4

were the most efficient to *A. fabae* with mortality percentages of more than 64, 71, and 78% at conidial concentrations of 10^5, 10^6, and 10^7 spores/ml, respectively (Table 2). S8, S17, S18 and S19 were all collected from Madira Farm in Arusha Region while S15 was collected from Kibosho in Kilimanjaro Region (Table 1). Molecular analysis revealed that out of the five selected fungi, four which originated from Madira Farm were found to be strains of the species *A. flavus* and one which originated from Kibosho belonged to the species *A. tamarii*.

To date, approximately 750 fungus species from about 90 genera have been documented as entomopathogenic, although only a few of these species are being developed as mycopesticides against insect pests (de Faria and Wraight 2007). Previously, Seye et al. (2014) reported the first strains of *A. flavus* and *Aspergillus clavatus* Long being pathogenic to aphids in laboratory bioassays, with a higher mortality of the pea aphid, *Acyrthosiphon pisum* (Harris) caused by *A. flavus* than that caused by *A. clavatus* and *Metarhizium Anisopliae* (Metschn.).

In the current study, the conidial concentration of 10^7 spores/ml gave higher *A. fabae* mortality than the rest of the concentrations (10^6, 10^5 and 10^4 spores/ml.) in all experiments.

Similar results were reported in Turkey by Sevim et al. (2013), where five conidial concentration levels (10^8, 10^7, 10^6, 10^5 and 10^4 spores/ml) of the entomopathogenic fungus *B. bassiana* against *Corythucha ciliatawere* (Say) (Hemiptera: Tingidae) showed that the higher the conidial concentration, the higher was the mortality of the insect. *Aspergillus flavus* S18 and S19 were found to be the most effective in controlling *A. fabae,* with mortality rates over 22, 46, 60, and 78% at 10^4, 10^5, 10^6, and 10^7 spores/ml, respectively, in all experiments in the current study.

Aspergillus flavus and *A. tamarii* are members of the *Aspergillus* section *Flavi*, which are generally regarded as toxin (aflatoxins and other mycotoxins) producers and are found mainly in humid climates (Marin et al. 2013; Frisvad et al. 2019; Norlia et al. 2019).

It was further confirmed in the current study that all the strains of *A. flavus* and the species *A. tamarii* identified produce aflatoxins in various amounts, i.e. from 8.4 to 135.2 $\mu g.kg^{-1}$ (Table 4). Aflatoxins are toxic secondary metabolites produced by certain fungi belonging to the genus *Aspergillus*. The two most important aflatoxin producers are *A. flavus* and *A. parasiticus* (Gourama and Bullerman 1995), which are commonly found in soil and can contaminate agricultural crops, both in the field and after harvest as well as processed items originating from agricultural products (Visconti and Perrone 2008). Aflatoxins cause various ill effects in humans and animals, such as aflatoxicosis outbreaks and death (Lewis et al. 2005), chronic health risks such as cancer, immune suppression and child stunting (Guchi 2015; Gong et al. 2016). For these reasons, the findings in this study cannot be recommended for use as a technology to control *A. fabae* in agricultural production settings, but rather they may be useful for scientific settings. It is worth noting that there are *A. flavus* strains that do not produce aflatoxins (Ehrlich 2014). Interestingly, such strains have been found to outcompete the toxin-producing strains when they co-occur, hence they have been developed and used as a biological control agent against aflatoxin (the toxin-producing strains) and have proved to be highly effective (Cotty and Bhatnagar 1994). In Tanzania, a product called Aflasafe TZ01 developed by the International Institute of Tropical Agriculture (IITA) has been tested and registered for use as a bio-control agent against aflatoxin (Moral et al. 2020). This product can be tested to find out whether it is effective in causing mortality to aphids.

Conclusions

Out of 19 fungal isolates from fields in Tanzania, two strains of *A. flavus* were found to be the most effective in controlling *A. fabae* under laboratory conditions. Our study confirmed previous reports on the efficiency of Aspergillus in controlling different types of aphid. However, the results obtained in the current study are challenged by the fact that the five *Aspergillus* spp. isolated and tested were all aflatoxin-producing strains, so they cannot be recommended as a technology to be transferred to producers due to the toxicity of aflatoxin to human, animal and environment. It is therefore recommended that similar experiments be conducted using the non-aflatoxin-producing strains, in order to determine whether they have similar effects on *A. fabae*. However, it is believed that the results of the current study provide an indirect scientific contribution towards the worldwide efforts in searching for alternatives to synthetic pesticides to manage insect pests.

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Data availability The data and materials used in this study are available for references.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Code availability Not applicable.

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Consent to participate All authors were contented to participate in this study.

Consent to publication All authors are contented with publication of this study.

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