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Original article

***In Vitro* Efficacy of Native Entomopathogenic Fungi Against Western Flower Thrips *Frankliniella Occidentalis* (Pergande) of Tomato in Kenya**

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Abstract

Tomato (*Solanum lycopersicum*) L. is an important crop in Kenya. Arthropod pests are major constraints to its production and farmers rely heavily on synthetic chemicals for control, which increases costs and leads to pollution of the environment. The objective of this study was to evaluate the effectiveness of native Kenyan fungi in managing thrips as an alternative to synthetic pesticides. *In vitro* studies were conducted to evaluate the effectiveness of native fungi against *Frankliniella occidentalis* collected from the tomato fields and maintained in the laboratory. The fungi were isolated from soil samples from tomato fields and identified based on their vegetative and reproductive structures. Treatments included fungal isolates and a control; replicated four times arranged in a completely randomized design. The effect of the fungal isolates on *F. occidentalis* was evaluated by treating thrips with concentrations of 1.0×10^7 conidia ml^{-1} . Data on mortality of *F. occidentalis* was recorded daily for 10 days after treatment. Percent mortality was subjected to Analysis of Variance (ANOVA) using SAS software version 9.4 to test the effect of different treatments. Means were separated using Student Newman-Keuls test at $P \leq 0.05$. *Gliocladium virens*, *Trichoderma virens*, *Fusarium solani*, *Fusarium oxysporum* and *Trichoderma afroharzianum* were more virulent causing mortalities above 50%. *Gliocladium virens* was the most potent, causing 62.2% mortality in adults and 43.8% in nymphs at 1.0×10^7 conidia ml^{-1} . The findings of this study showed that *Gliocladium virens* is a potential candidate for development as a fungal-based bio-pesticide against *F. occidentalis* on tomato. Further studies are warranted to determine the effectiveness of *G. virens* in controlling *F. occidentalis* under field conditions.

Keywords: *Gliocladium virens*, insecticidal activity, pesticides, thrips, tomato.

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INTRODUCTION

The Western flower thrip, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is an important quarantine arthropod pest of horticultural crops in the world (Infonet-Biovision, 2020). Thrips cause abscission of buds, fall of flowers and deformation of fruits. Thrips are also potential vectors of viral diseases such as Tomato Spotted Wilt Virus and the Tomato Chlorotic Spot Virus (Infonet-biovision, 2020). Despite their negative impacts on people and the environment, synthetic pesticides have been used for long to manage thrips (Ndakidemi *et al.*, 2016). Moreover, frequent application of synthetic pesticides has led to thrips developing resistance (Wagnitz, 2014). The numerous negative effects associated with chemical pesticides have led to increased interest in developing environmentally safer and sustainable strategies to manage arthropod pests. In particular, there has been a focus on the use of biopesticides as an alternative to synthetic pesticides in integrated pest management strategies (Srijita, 2015).

Biopesticides have several advantages including less toxic residues, more safer to non-target organisms, varied modes of action on pests, host specificity, compatibility with other pest management strategies and can be affordable to farmers if they are produced locally (Ouma *et al.*, 2014). The aim of this study was therefore to determine efficacy of native Kenyan fungal isolates against *F. occidentalis* *in vitro*.

MATERIALS AND METHODS

Insect colony

Initial colonies of *F. occidentalis* were collected from infested tomato crop in Bungoma County, in Western Kenya during field surveys conducted between 13th and 19th June, 2017. The specimens of *F. occidentalis* were processed in the Agricultural Research laboratory at Kenyatta University (KU), to confirm their identity using various identification keys (Moritz *et al.*, 2013). Thrips were reared in 3L modified clear plastic buckets. A 15cm diameter hole was cut on the lid and the sides of the cages covered with thrips-proof organdy cloth to allow ventilation. The insect colonies were maintained in the laboratory at 23 ± 2 °C, 60±10% RH.

The adult *F. occidentalis* were aspirated from the collection vials and transferred into 3L clear plastic buckets and fed on French bean pods *Phaseolus vulgaris* L. var Samantha for three days to allow for egg laying. After three days, *P. vulgaris* pods were transferred to new clear plastic buckets lined with paper towel at the bottom for development of the nymphs, pupa and adult emergence. The newly emerged adults were consecutively transferred to new rearing plastic buckets provided with *P. vulgaris* pods to allow the development of the next generation for use in the *in vitro* experiment. Adults and first-instar nymphal stage were used in the bioassay.

Isolation of entomopathogenic fungi

The fungal isolates were obtained from the soil samples and two insect cadavers collected from tomato fields in Bungoma County, Kenya. The fungi were isolated by pour plate technique as described by Belete *et al.* (2015). Suspensions of soil samples collected from each site were prepared by mixing 1g soil into 9 ml of sterile distilled water thoroughly by agitating using a magnetic shaker (HeidolphUnimax 1010) at 200 revolutions per minute. Thereafter, serial dilutions of 10^{-1} , 10^{-2} and 10^{-3} of the soil suspensions were prepared (Belete *et al.*, 2015).

One milliliter aliquot of the serially diluted suspension from each dilution was pipetted into sterile Petri dish containing Potato Dextrose Agar (PDA) amended with antibiotic (tetracycline) to inhibit bacterial growth. Three replications were made and the Petri dishes were sealed with parafilm and placed in an incubator (BJPX-H230JI) at 23 ± 2 °C for fungal growth (Abu and El-Hindi, 2017). Seven days after incubation, the fully sporulated fungal cultures were sub-cultured on new PDA medium to make pure cultures.

Fungi identification

A segment of mycelium from the sporulating colonies in each pure culture was transferred onto glass slides and stained with a drop of lactophenol cotton blue solution. Thereafter, the slide was examined under a compound microscope (XSZ-107T) at a magnification x40 for characteristics of their vegetative and reproductive structures such as hyphae colour, size, shape of conidia and conidiophores. The fungal isolates were identified using the key described by Watanabe (2010).

Preparation of conidial suspensions

Conidia of potential entomopathogenic fungi were harvested by scraping off the sporulating colonies on PDA medium with a sterilized glass rod and suspended in distilled water containing 0.05% Tween-20 (v/v aqueous solution), a surfactant and filtered through a muslin cloth as described by Borisade and Magan (2015). The conidial suspension was agitated using a magnetic shaker (Heidolph Unimax 1010) set at 150 revolutions per minute to get a homogeneous suspension. Spores were counted using an improved Neubauer haemocytometer and suspensions diluted with distilled water to make the final concentration of approximately 1.0×10^7 conidia ml⁻¹ for use in the pathogenicity test (Masoud and Bahar, 2012).

Pathogenicity of fungal isolates against *Frankliniella occidentalis in vitro*

Freshly harvested *P. vulgaris* pods were washed with tap water and surface-sterilized by immersing them in 1.5% sodium hypochlorite for 3 min then rinsed three times in distilled water. Four, 2mm diameter discs of sterile *P. vulgaris* pods were cut using a scalpel and separately dipped in 100ml glass beakers containing 10ml of each of the fungal suspension at 1.0×10^7 conidia ml⁻¹. These were

agitated gently for 5 min to allow even distribution of the spore suspensions on the pods. Pods were removed using a pair of forceps and allowed to dry for 3 min on a sterile filter paper, after which they were transferred into sterile Petri dishes (9cm diameter) as diet for thrips. Twenty adult and first- instar *F. occidentalis* were then introduced gently in the Petri dishes using a camel hair brush. In the control treatments, pods were dipped in sterile distilled water containing Tween-20 at 0.05% (v/v).

Treatments were replicated four times and arranged in a complete randomized design (CRD). All Petri dishes were incubated at room temperature (23 ± 2 °C). Mortality of *F. occidentalis* was recorded daily for 10 days (Youssef, 2015). Dead *F. occidentalis* were mounted on a dissecting microscope (NTB-3A) and observed at x10 magnification for presence of fungal mycelia which was used as a symptom of mycosis. Mortality was computed as percentage based on Marek (2010) formula:

$$\text{Mortality Percent} = \left(\frac{\text{Number of dead insects}}{\text{Total number of treated insects}} \right) \times 100$$

Statistical analysis

In vitro data on percentage mortality of adult and nymphs of *F. occidentalis* were subjected to one way analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9.4 (SAS Institute, 2013). Mean separation was accomplished according to Student Newman-Keuls (SNK) test at $P \leq 0.05$ (Sokal and Rohlf, 1995).

RESULTS

Entomopathogenic fungi isolated

A total of 40 fungal species were isolated from the soil samples with two species recovered from the insect cadavers. These belonged to different genera, *Trichoderma*, *Verticillium*, *Fusarium*, *Penicillium* and *Plactosporium*. Most of the fungal isolates were from the genus *Trichoderma* with *Trichoderma harzianum* being the predominant species comprising nine isolates from Sirisia, three from Bumula and two from Mt. Elgon. Two fungal species, *Plactosporium tabacinum* and *Penicillium resticulosum* were recovered from *F. occidentalis* and *Tuta absoluta* cadaver, respectively (Table1).

Table 1. Fungal species tested against *Frankliniella occidentalis* and their origin

| Fungal isolate | Isolation locality | | | |
|--|--------------------|-----|-----|----|
| | Source | SRS | BML | ME |
| <i>Trichoderma harzianum</i> Rifai | Soil | 8 | 3 | 2 |
| <i>Trihoderma afroharzianum</i> Chaverri | Soil | 1 | - | - |
| <i>Trichoderma koningi</i> Oude | Soil | 3 | 1 | 1 |
| <i>Trichoderma aureoviride</i> Pers | Soil | 1 | - | 1 |
| <i>Trichoderma koningi</i> Oude. | Soil | 1 | - | - |
| <i>Trichoderma virens</i> 1Mill | Soil | 1 | - | - |
| <i>Trihoderma virens</i> 2 Mill | Soil | - | 1 | - |
| <i>Fusarium oxysporum</i> Snyder | Soil | - | 1 | - |
| <i>Verticillium balanoides</i> Dowsett | Soil | 1 | - | - |
| <i>Verticillium dahliae</i> Kleb | Soil | - | - | 1 |
| <i>Fusarium ventricosum</i> Link | Soil | 1 | - | - |
| <i>Fusarium solani</i> Mart | Soil | 1 | - | - |
| <i>Penicillium jantinelum</i> Biourge | Soil | - | - | 1 |
| <i>Plactosporium tabacinum</i> Beyma | Thrip | 1 | - | - |
| <i>Penicillium resticulosum</i> Birkinshaw | <i>T. absoluta</i> | - | - | 1 |

SRS-Sirisia; BML-Bumula; ME-Mt. Elgon

Pathogenicity of fungal isolates against *Frankliniella occidentalis* in vitro

All tested fungal isolates including *T. harzianum*, *G. virens*, *T. afroharzianum*, *F. solani*, *F. oxysporum*, *F. ventrihosum*, *T. koningi*, *T. pseudokoningi*, *T. aureoviride*, *V. dahliae*, *V. balanoides*, *P. resticulosum*, *P. jantenillum* and *P. tabacinum* were pathogenic to the nymphs and adults of *F. occidentalis* (Figure 1). The fungi recorded mortality of between 23 and 62%. Mortality in the control treatment was lower and did not exceed 4% for nymphs and 15% for adults. Among the twenty fungal isolates, *G. virens*, *T. virens*, *F. solani*, *F. oxysporum* and *T. afroharzianum* were more virulent causing mortality of $\geq 50\%$ (Figure 2; Table 2).

Gliocladium virens caused the highest mortality of 62.2% in adults and 43.8% in nymphs at 1.0×10^7 conidia ml⁻¹, which was significantly different compared to other isolates (F=40.7; df=19, 23; P<0.0001). There were significant differences among *V. dahliae* and *P. tabacinum*, *T. koningi* 1 and *F. ventricosum* in their effect on the adults (F= 40.7; df=19, 23; P<0.0001). The effect between *T. koningi* 1 and *P. resticulosum* on the nymphs of *F. occidentalis* was significant (F=24.4; df=19, 23; P<0.0001). *Trichoderma* spp. and *T. koningi*2 were significantly different on mortality of adult *F. occidentalis* (F= 40.7; df=19, 23; P<0.0001) (Table 2).

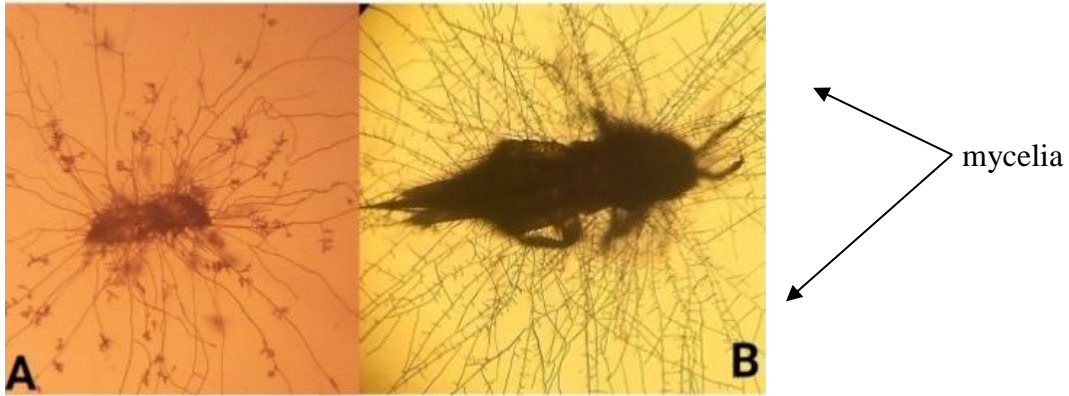


Figure 1. Infection by antagonistic fungus on thrip's cuticle;

A) *Trichoderma afroharzianum*, B) *Gliocladium virens*. (x40 Mg).

Source: (Barasa, 2017).

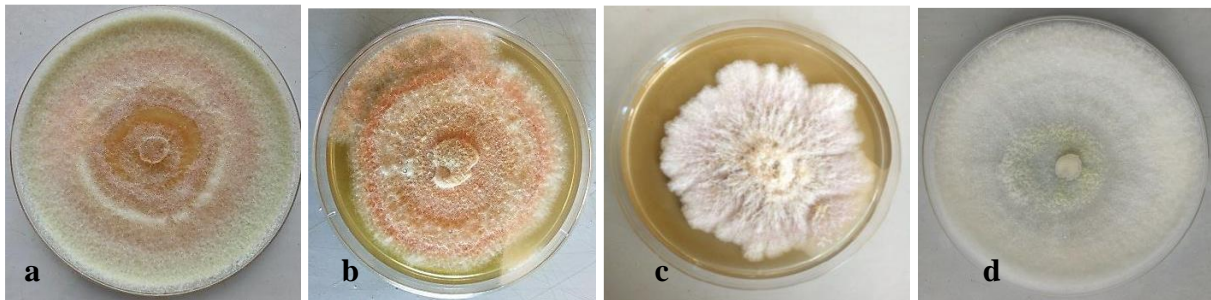


Figure 2. Isolated fungal antagonists *in vitro* bioassay;

a) *Gliocladium virens*, b) *Fusarium solani*, c) *Fusarium oxysporum*, d) *Trichoderma afroharzianum*

Source: (Barasa, 2017)

Table 2. Virulence of fungal isolates against *Frankliniella occidentalis* 10 days post treatment

| Isolation locality | Fungal isolate | Mean mortality (% ± S.E) | |
|--------------------|------------------------------------|--------------------------|--------------|
| | | Adults | Nymphs |
| Sirisia | <i>Trichoderma afroharzianum</i> | 37.8±3.9de | 33.3±3.1bcd |
| Sirisia | <i>Trichoderma harzianum</i> | 37.8±3.9de | 33.3±3.1bcd |
| Sirisia | <i>Trichoderma pseudokoningi</i> 1 | 33.7±4.2ef | 27.5±3.7de |
| Sirisia | <i>Trichoderma pseudokoningi</i> 2 | 31.7±3.4f | 28.7±3.2cde |
| Sirisia | <i>Trichoderma aureoviride</i> | 43.2 ±4.7d | 33.2±2.3bcd |
| Sirisia | <i>Verticillium balanoides</i> | 42.7±4.6d | 34.7±3.6bcd |
| Sirisia | <i>Gliocladium virens</i> | 62.2±5.4a | 43.8±4.2a |
| Sirisia | <i>Penicilium resticulosum</i> | 28.7±3.0fg | 24.0±3.1e |
| Sirisia | <i>Fusarium ventricosum</i> | 30.8±5.0f | 28.3±3.3cde |
| Sirisia | <i>Fusarium solani</i> | 56.0±6.1bc | 38.8±5.6ab |
| Mt. Elgon | <i>Trichoderma koningi</i> 1 | 40.0± 4.2d | 39.2±4.8ab |
| Mt. Elgon | <i>Trichoderma pseudokoningi</i> 3 | 37.5±5.0de | 30.8±3.6bcde |
| Mt. Elgon | <i>Trichoderma</i> spp. | 25.3 ±3.1g | 27.2±3.9de |
| Mt. Elgon | <i>Verticillium dahliae</i> | 40.0±4.6d | 37.3±3.9abc |
| Mt. Elgon | <i>Penicilium jantinelum</i> | 38.7±4.5de | 30.3±3.5bcde |
| Mt. Elgon | <i>Plactosporium tabacinum</i> | 31.5± 3.2f | 23.2±3.4e |
| Bumula | <i>Trichoderma koningi</i> 2 | 24.5±3.7g | 27.7±3.4de |
| Bumula | <i>Trichoderma virens</i> | 57.3±5.4ab | 39.3±3.9ab |
| Bumula | <i>Fusarium oxysporum</i> | 54.5±6.0bc | 38.7±4.8ab |
| | Control | 15.83±2.9h | 4.0±1.4f |
| | | F=40.7 | F=24.4 |
| | | df=19, 23 | df=19, 23 |
| | | P<0.0001 | P<0.0001 |

Means followed by the same letter (s) in each column are not significantly different according to Student Newman-Keuls (SNK) test at P≤0.05.

DISCUSSION

The results revealed that majority of the fungal species isolated from the soil samples belong to the genera *Trichoderma*. These included *T. harzianum*, *T. koningi*, *T. pseudokoningi*, *T. aureoviride* and *T. harzianum*. Other studies have confirmed abundance of this fungus and its biocontrol activity (Muthomi *et al.*, 2017). All the fungal isolates tested were pathogenic to the adults and nymphs of *F. occidentalis*. Adults were more susceptible to fungal infection than nymphs. This could partly be attributed to the loss of fungal conidia in the nymphs during ecdysis (Niassy *et al.*, 2012). Mortality levels varied significantly between the isolates which agree with previous reports when fungi were evaluated for their effect against diverse groups of insects (Wu *et al.*, 2014). Of the five most potent isolates, *G. virens* has previously been reported to be most virulent against *Triatoma dimidiata* Latreille (Hemiptera: Reduviidae) which caused 100% and 35.7% mortality in the adults and nymphs, respectively (Guadalupe *et al.*, 2014). This high virulence of this fungus was confirmed in this study.

CONCLUSION

This study suggests that *G. virens* has the potential to be developed as a fungal-based bio-pesticide for controlling western flower thrips on tomato crop. Further studies are warranted to determine the effectiveness of *G. virens* against *F. occidentalis* under field conditions. In addition, the findings of this study forms a basis for further exploitation of microbial antagonists from the local environment.

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