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Biocontrol Potential of Entomopathogenic Fungi against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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SÇ and WA designed the experiments. MAJ and WA practically performed all experiments. HAAK and MAJ prepared the manuscript and analyzed the data.

Key words
Bio-control agents, *Beauveria bassiana, Verticillium lecanii, Metarhizium anisopliae, Trichoderma longibrachiatum*, Entomopathogens

ABSTRACT

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an economic pest in several field crops, fruits, and vegetables. The objective of the present study was to evaluate the efficacy of entomopathogenic fungi using two concentrations (4×10^4 and 4×10^8 conidia/ml): *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma longibrachiatum* and *Verticillium lecanii*, against the adult female of *T. urticae* strains (red and green) using leaf-disc bioassay method. Their corrected mortalities were also calculated using Abbot formula. Results indicated that the concentration 4×10^8 conidia/ml of *Trichoderma longibrachiatum* caused the highest mortality of red (86.97%) and green (88.63%) strains of *T. urticae*. *Beauveria bassiana, V. lecanii* and *M. anisopliae* have also caused significant mortality ranging from 40.1 to 65.4% of both strains at the 4×10^8 conidia/ml suspension. Based on smaller LT_{50} value and non-overlapping 95% CI, *T. longibrachiatum* took the least significant time to kill 50% of the subjected mites population at both concentrations when compared with rest of the fungi. The adult female *T. urticae* exposed to the infection of respective entomopathogenic fungi upon death after the seven days of incubation and the fungal mycelial growth appeared around the mite's body. The fungal infection was also verified after re-isolation of dead *T. urticae* covered with mycelial growth.

INTRODUCTION

Tetranychus urticae Koch (Family; *Tetranychidae*) commonly known as two-spotted spider mite (TSSM), is an economically important pest of various plants covering over 1100 species having 140 families including fruits, vegetables, corn, cotton and other ornamental plants (Knapp and Kashenge, 2003; Alzoubi and Çobanoğlu, 2010; Bugeme *et al*., 2014). The two-spotted spider mites (TSSM) exist as red and green strains. They usually feed on leaves by removing leaf sap, damaging the mesophyll tissues and ultimately plant leaves develop chlorotic spots at exposure site. *T. urticae* destroys around 18-22 cells per minute and prolonged feeding may cause complete chlorosis and eventually defoliation occur (Chapman and Hoy, 1991). Infected plants show stunted growth and can lead to yield reduction which in due course effect the market value (Lahai *et al*., 1998; Dogan *et al*., 2017).

Acaricide has been used as the most frequent approach to control TSSM, but its extensive application may cause resistance in TSSM due to high reproductive growth and short generation time (Ambikadevi and Samarjit, 1997; Li *et al*., 2017; Medo *et al*., 2017). Unregulated pesticide applications have the harmful impact on the environment and human health. Therefore, ecofriendly and alternative approaches like biological agents and resistant varieties are of heavily needed to practice (Fathipour and Sedaratian, 2013). For instance, biological organisms have been successfully providing protection against TSSM (Saber *et al*., 2018). The most promising biological control agents against TSSM are entomopathogenic fungi (EPF) (Chandler *et al*., 2005). Fungi are the eukaryotic heterotrophs which have unique mode of action except from other pathogen like virus, bacteria and other entomopathogenic microbes

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(Ferron, 1978).

The infection mechanism of EPF involves germination of conidia which later on penetrate into TSSM cuticle and colonize in haemocoel before sporulation on mite cadaver (Inglis *et al*., 2001). The efficacy of EPF against *T. urticae* depends on fungal strain, conidial concentration, formulation, outside environment and pesticides compatibility (Bugeme *et al*., 2014; Gatarayiha *et al*., 2010a, b; Ullah and Lim, 2015; Afifi *et al*., 2015).

Many studies have been carried out on EPF against tetranychid mites such as *Tetranychus evansi* (Koch) and *T. urticae* (van der Geest, 1985; Chandler *et al*., 2000; van der Geest *et al*., 2000). Insect associated fungi *M. anisopliae* with the conidial concentration of 1×10^7 conidia/ml along with predatory mite *Phytoseiulus macropili* showed excellent mortality against *T. urticae* (Waked *et al*., 2021). It was also reported that *Metarhizium brunneum* (strains ARSEF 4556 and V275), *M. flavoviride* UPH-0288, *Lecanicillium lecanii* UPH-0241, and *Beauveria bassiana* UPH-1103 exhibited excellent results against the different life stages of the two spotted spider mites (Dogan *et al*., 2017). Different strains of *B. bassiana* (B76, B252) and *V. lecanii* (L2 and L5) were used against the against aphid beans *Megoura japonica* (Matsumura) which showed the biocontrol efficiency of EPF at different concentrations (1×10^6, 1×10^7, and 1×10^8 conidia/ml) (Trinh *et al*., 2020).

The significant virulence activity was reported by the evaluation of four EPF strains *B. bassiana, V. lecanii, M. anisopliae* and *Trichoderma harzianum* against the adult strain of *T. urticae* Koch with concentration of 1×10^8 (Elhakim *et al*., 2020). Direct conidial application of *M. anisopliae* (Isolate; 442.99), *V. lecanii* (Isolate; 450.99), *B. bassiana* (Isolate; Naturalis-L) *Hirsutella thompsonii* (Isolate; 463.99) at a concentration of 10^8 mL^{-1} showed significant results with mortalities of 54.4%, 51.8%, 52.1% and 37.6%, respectively (Chandler *et al*., 2005). It was also revealed that 3184.4 mL^{-1} conidia concentration of *B. bassiana* would be required to get 50% mortality in *T. urticae* (Irigaray *et al*., 2003). It was also found that *B. bassiana* and *M. anisopliae* with concentration of 1×10^7 conidia/ml resulted in the highest mortality against *T. urticae* (Bugeme *et al*., 2014).

The application of *B. bassiana* along with synergetic *Phytoseiulus persimilis* (Acari: Phytoseiidae) with low concentration effectively controlled *T. urticae* (Ullah and Lim, 2017). Twelve isolates belonging to three species were recorded to be pathogenic against *T. urticae* includes species *Isaria farinosa, Cladosporium cladosporioides* and *B. bassiana* but *B. bassiana* showed best antagonistic affect against *T. urticae* (Örtücü and Algur, 2017). Recorded results showed that *T. longibrachiatum* provide defense against one of the major eggplant pest *Leucinodes orbonalis* (Lepidoptera: Pyralidae) (Ghosh and Pal, 2016). The same result was also reported against *Aphis craccivora* Koch (Hemiptera: Aphididae), an economic pest of cowpea (Ibrahim *et al*., 2011).

The objective of this study was to evaluate the biocontrol efficiency of four different entomopathogenic fungal strains with two concentrations include *B. bassiana, V. lecanii, M. anisopliae* and *T. longibrachiatum* against the two adult female strains of *T. urticae* Koch. Recommendations regarding their usage in IPM strategy were also proposed in this study.

MATERIALS AND METHODS

Fungal strains

Insect associated fungal strains under study include *B. bassiana* (GenBank: LT604474), *M. anisopliae* (GenBank: LT604482), *T. longibrachiatum* (GenBank: LT159847) and *V. lecanii* (GenBank: LT626262) were obtained from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The EPF *B. bassiana* was isolated from dead bodies of cotton Mealy bug (*Phenacoccus solenopsiswhile*), collected from cotton field in Sundar, Lahore, Pakistan, *M. anisopliae* from Sahiwal, Punjab, Pakistan, followed by *T. longibrachiatum* from Layyah, Punjab, Pakistan, while *V. lecanii* was isolated from dead bodies of whitefly (*Bemisia tabaci*) collected from a cotton cultivated field in Layyah, Punjab, Pakistan (Anwar, 2016).

Maintenance of mites under control conditions

Population of *T. urticae* green and red type strains were taken from the host plantation of Silifke-Mersin and cultured in a greenhouse at, Plant Protection Department, Ankara University, Turkey. Both strains of *T. urticae* were reared on beans (*Phaseolus vulgaris* L.) plants at 25±1°C and 60±10% RH under a 16-h light duration. Both strains were not exposed to any acaricide prior its use in experiment for the last one year. *T. urticae* reared on the host plants for at least three generations before starting the fungal bioassays (Çobanoğlu and Kandiltaş, 2019; Shang *et al*., 2018). New *T. urticae* colonies were initiated after a week onto new host plant leaf by placing *T. urticae* infected leaf. Single aged female *T. urticae* were used for bioassays (Chandler *et al*., 2005).

Preparation of conidial suspension

For the preparation of conidial suspension, fungal cultures were grown on potato dextrose agar (PDA) media at 25±1°C, 75±5% RH, 12L: 12 photoperiods. Conidia of four entomopathogenic fungal strains were harvested from surface of 2 to 3 weeks old laboratory cultures by scraping with a glass rod. Spores were suspended in 5 mL autoclaved distilled water supplemented with Tween-80 (0.05%) as sticking material in sterile 15 mL conical centrifuge tubes. Percentage germination was examined after 24 h from 100-spore counts on each plate (Ekesi *et al*., 2002). The spore counting was done by using hemocytometer and adjusted the concentration $4x10^4$ and $4x10^8$ conidia/ml (Anwar *et al*., 2018) using sterile aqueous 0.05% v/v Tween-80 (Shang *et al*., 2018).

Virulence bioassay experiment

For virulence bioassay, two concentrations ($4x10^4$ and $4x10^8$ conidia/ml) of suspension were used against new emerged adult females *T. urticae* using leaf disc bioassay method (Shang *et al*., 2018). According to this method, bean leaves were placed upside down on top wetted polystyrene pad disc (2 cm) in small petri dishes (7cm diameter), so the leaves were remained hydrated on moist disc. Strips of filter paper were used to wrap the petiole to avoid the mites from escaping. The smaller petri dishes were further placed on plastic box (33.5×46×8.5 cm) and kept moist during the experiment. Five female adults of *T. urticae* (red strain) were picked up by camel-hair brush viewing under stereomicroscope and individually placed on leaf disc as described earlier. After that, 2.5 ml spore suspension of *B. bassiana* were evenly sprayed on bean leaf infested with *T. urticae* through hand sprayer (20ml). Control was sprayed with the same quantity of distilled water containing 0.05% Tween-80 (Shang *et al*., 2018; Dogan *et al*., 2017). The same process was carried out for rest of the entomopathogenic fungi against both red and green strains of *T. urticae*. The experiment was replicated 10 times. All the treated and control petri plates were placed in a controlled room at 25±1°C and 75±5% RH.

The number of dead and live mites were counted under stereomicroscope on 3rd, 5th, 7th, 9th and 11th day after application of EPF spores suspension. Mites were considered dead if they did not show movement when touched with a camel-hair brush. After 11th day, the dead mites were collected on filter paper and placed in incubator for sporulation at 25±1°C, 75±5% RH, and 16L:8D photoperiod conditions. The effectiveness of different fungi on adult stage of female *T. urticae* was evaluated according to mortality rate for each spore concentration of EPF. The colonized fungi on dead mites were reisolated by growing on artificially prepared PDA medium for 5-7 days at 25±1°C, 12L:12 photoperiods, in order to verify the fungal infection on mites.

Data analysis

Data regarding corrected mortality of adult female *T. urticae* Koch were observed after 3rd, 5th, 7th, 9th and 11th day intervals by using Abbott formula (Fleming and Retnakaran, 1985). To meet normality and homoscedasticity assumptions of the ANOVA, most of the data were transformed to square root transformation (Gomes and Gomes, 1984). When all the assumptions of ANOVA were satisfied, the data were analyzed by following ANOVA (Gelman, 2005). Means were separated by using the least significant difference (LSD) test at α:0.05. All mortality counts at different times were subjected to probit analysis using the software PoloPlus 2.0v in order to calculate median lethal time (LT_{50}) of each fungus against mite strains.

RESULTS

Pathogenicity of EPF against green strain of T. urticae

The virulence of four EPF including *B. bassiana, V. lecanii, M. anisopliae* and *T. longibrachiatum* with two concentrations $4×10^8$ and $4×10^4$ conidia/ml were evaluated against the two strains of adult female *T. urticae* (green and red strains). The corrected mortalities by Abbot formula are shown in Figures 1 and 2, the death of adult female *T. urticae* started after the 3rd day of conidial suspension applied. However, the highest corrected mortality in *T. urticae* (green strain) by applying *T. longibrachiatum* with conc. $4×10^8$ were revealed high about 88.6% after 11th day of inoculation. Similarly, *B. bassiana* showed second highest mortality of 65.4% and 47.2% with conc. $4×10^8$ and $4×10^4$ conidia/ml, respectively, followed by *V. lecanii* that exhibited 61.9% and 27.2% mortality, and *M. anisopliae* that showed 50.9% and 23.6% mortality at $4×10^8$ and $4×10^4$ conidia/ml, respectively.

Inhibitory effect of four EPF strains against T. urticae

Factorial based ANOVA indicated a promising result of four different fungal strains ($F = 8.35$, $p < 0.01$; Table I), with two different conidial concentrations ($F= 13.54$, $p < 0.01$; Table I) on 11th day after treatment. However, interaction effect between fungi and concentrations to cause mortality was non-significant ($P>0.05$). *T. longibrachiatum* produced the highest mortality of *T. urticae* followed by *M. anisopliae*, *V. lacanii* and *B. bassiana* (Table II). In all the cases, conidial suspension $4×10^8$ produced the higher mortality than those observed at $4×10^4$ (Table III). While the factorial based ANOVA indicated a promising result of four different fungal strains ($p < 0.01$; Table I), with two different conidial concentrations ($p < 0.01$; Table I) on 11th day after treatment. However, interaction effect between fungi and concentrations to cause mortality was non-significant ($P > 0.05$). *T. longibrachiatum* produced

Table I. Analysis of Variance (ANOVA) for the mortality *T. urticae* (green and red strain) by four EPF strains, each at two conidial concentrations.

Source of variation	Green strain					Red strain				
	df	SS	MS	F	P	df	SS	MS	F	P
Fungi	3	139.72	46.57	8.35	<0.01	3	109.27	36.41	9.33	<0.01
Concentration	1	75.52	75.52	13.54	<0.01	1	22.31	22.31	5.72	<0.05
Fungi*Conc.	3	33.77	11.25	2.02	>0.05	3	15.45	5.15	1.32	>0.05
Error	72	401.64	5.58			72	280.94	3.90		
Total	79	650.65				79	427.92			

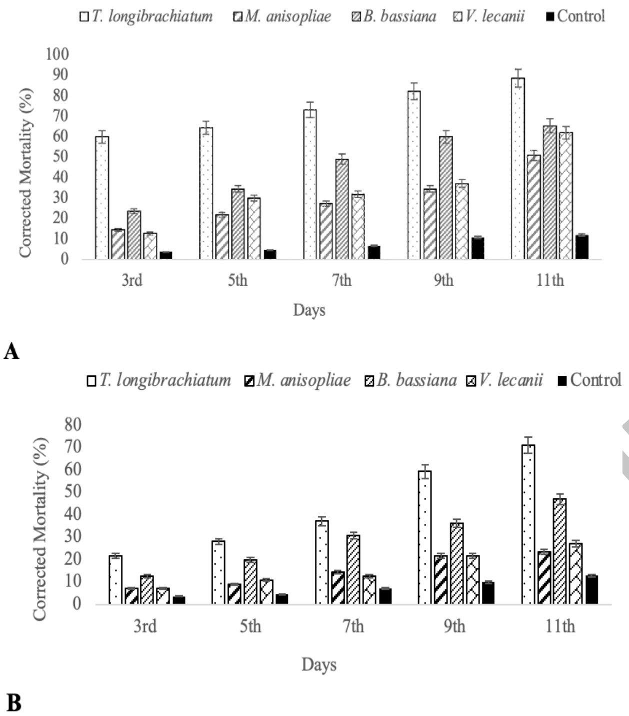

Fig. 1. Corrected mortality percentage of four EPF at concentration 4×10[8] conidia/ml (A); conc. 4×10[4] conidia/ml (B) against adult female *T. urticae* (green strain).

the highest mortality of *T. urticae* followed by *B. bassiana, V. lacanii* and *M. anisopliae* (Table II). In all the cases, conidial suspension 4×10[8] produced the higher mortality than those observed at 4×10[4] (Table III).

Pathogenicity of EPF against red strain of T. urticae

Likewise, corrected mortality of *T. urticae* (red strain) was observed in the treatment with *T. longibrachiatum* (4×10[8] conidia/ml), ranging 86.9% after 11[th] day followed by *T. longibrachiatum* (4×10[4] conidia/ml) with 64.09%. There are some contrasting results as compared to the green strain, *M. anisopliae* with at 4×10[8] and 4×10[4] conidia/ml showed 55.3% and 28.7% mortality after the 11[th] day of pathogenicity treatment. *B. bassiana* revealed 40.3% and 35.7% of virulence pathogenicity while *V. lecanii* with both concentrations showed lowest virulence against red strained female *T. urticae* that are 40.1% and 25.6%. Detailed corrected mortalities of four strain of EPF after 3[rd], 5[th], 7[th], 9[th] and 11[th] days against red strain *T. urticae* in (Fig. 2).

Table II. Mortality of *T. urticae* (green and red strain) against four different fungi.

Fungus	Green strain	Red strain
	Mean mortality (%)	Mean mortality (%)
Trichoderma longibrachiatum	8.49A	8.86A
Metarhizium anisopliae	6.08B	6.99B
Verticillium lecanii	5.57B	6.10B
Beauveria bassiana	5.03B	5.90B
LSD value (at 0.05)	1.49	1.49

Means sharing different letters are statistically different (p<0.05) following one-way ANOVA and LSD test.

Table III. Mortality of *T. urticae* (green and red strain) against fungi at two concentrations.

Concentration (conidia/ml)	Mean mortality (%)	Mean mortality (%)
4×10[8]	7.26A	7.49A
4×10[4]	5.32B	6.44B
LSD value (at 0.05)	1.00	0.88

Means sharing different letters are statistically different (p<0.05) following one-way ANOVA and LSD test.

LT_{50}

Based on smaller LT_{50} value and non-overlapping 95% CI, *T. longibrachiatum* took significantly the least time to kill 50% of the subjected mites population at both concentrations when compared with rest of the fungi (Table IV). In the case of *T. urticae* (green strain), T. longibrachiatum took 4.23 and 6.70 days to kill 50% of the exposed population at 4×10[8] and 4×10[4] concentrations, respectively. In the case of *T. urticae* (red strain), *T. longibrachiatum* took 2.34 and 7.45 days to kill 50% of the exposed population at 4×10^8 and 4×10^4 concentrations, respectively.

Table IV. LT_{50} of two concentrations of EMF against green and red strains of *T. urticae*.

Concen-tration	Fungus	n*	Green strain			Red strain		
			LT_{50} (days)** (95% CI)	Slope ± SE	χ^2 (df=3)	LT_{50} (days)** (95% CI)	Slope ± SE	χ^2 (df=3)
4×10^8	*T. longibrachiatum*	50	4.23 (3.75-4.66)	2.74 ± 0.31	2.20	2.34 (1.20-3.19)	1.50 ± 0.31	2.43
	M. anisopliae	50	9.96 (8.29-13.62)	1.60 ± 0.30	1.66	13.08 (9.41-39.78)	1.83 ± 0.32	3.57
	B. bassiana	50	13.69 (9.69-40.32)	1.00 ± 0.29	1.17	7.14 (6.28-8.25)	2.04 ± 0.30	0.91
	V. lecanii	50	12.25 (9.66-19.94)	1.45 ± 0.30	0.74	10.17 (7.56-27.44)	2.28 ± 0.33	6.76
4×10^4	*T. longibrachiatum*	50	6.70 (5.77-7.84)	1.79 ±0.29	2.26	7.45 (5.56-09.70)	2.44 ± 0.27	7.21
	M. anisopliae	50	40.39 (19.02-60.25)	0.96 ± 0.33	0.60	31.40 (18.80-50.27)	1.55 ± 0.38	0.97
	B. bassiana	50	18.74 (13.15-39.09)	1.61 ± 0.34	0.37	13.05 (10.58-19.12)	1.86 ± 0.33	0.83
	V. lecanii	50	25.45 (14.85-55.90)	1.05 ± 0.32	0.27	27.46 (17.49-87.36)	1.63 ± 0.38	1.47

*number of mites used in bioassays; **lethal time to kill 50% mites exposed.

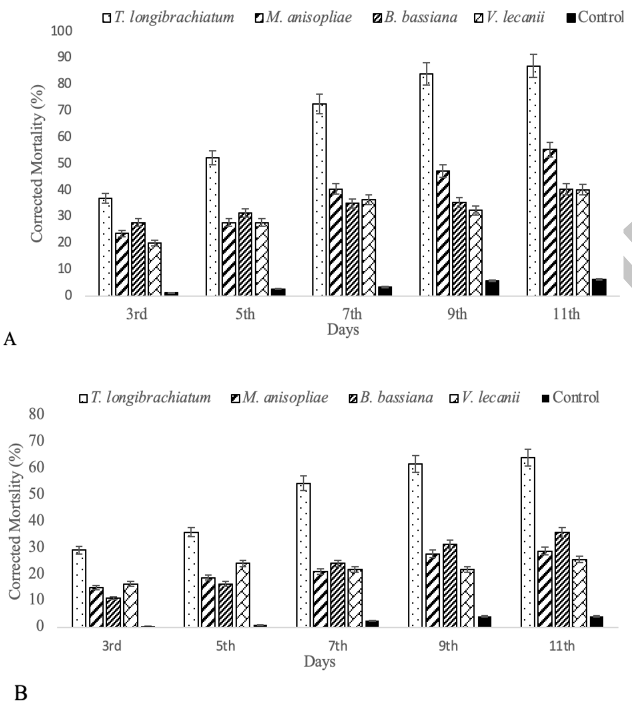

Fig. 2. Percentage mortality of four entomopathogenic fungi at a concentration of 4×10^8 conidia/ml (A) concentration 4×10^4 conidia/ml (B) against adult female *T. urticae* (red strain).

EPFinfection dead mites

The infection process of EPF on dead *T. urticae* was started with the germination of conidia, which penetrated *T. urticae* cuticle and colonized in haemocoel before sporulation (Ullah and Lim, 2015; Afifi *et al.*, 2015). Sporulation occurred after 7 days by incubating at 25 °C and white mycelial growth covered the whole body. Figure 3 shows the pictorial description of healthy and infected mites.

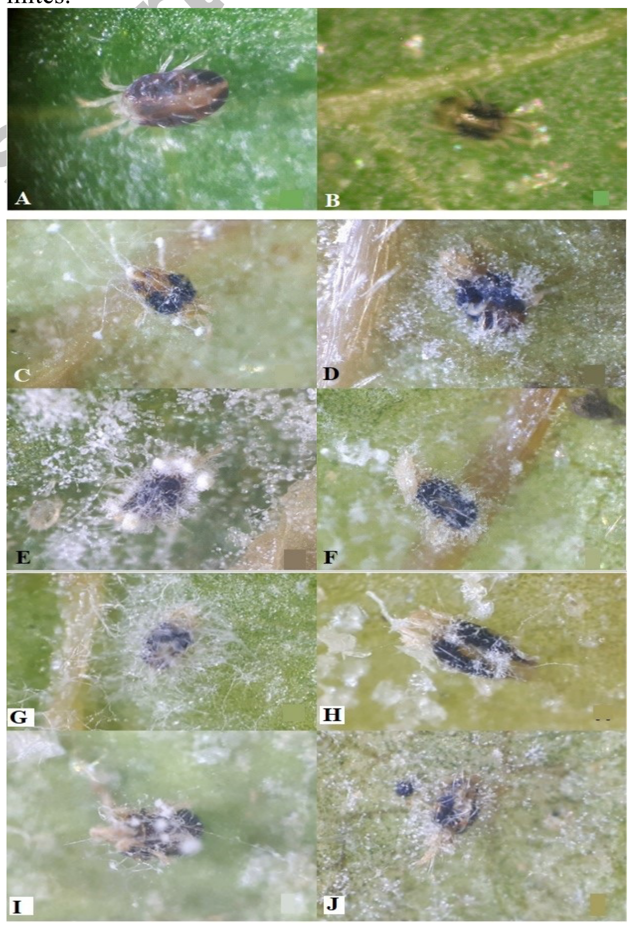

Fig. 3. Healthy red (A) and green (B) strains of *T. urticae;* C represent infection by *T. longibrachiatum*; D shows *M. anisopliae* infection while E and F indicate *B. bassiana* and

V. lecanii infection respectively against the green strain of *T. urticae;* G represents infection by *T. longibrachiatum*, H shows infection with *M. anisopliae*, while I and J indicate *B. bassiana* and *V. lecanii* infection respectively against the red strain of *T. urticae.*

DISCUSSION

T. urticae is a catastrophic pest around the world because of its resistance capacity against acaricides (Chandler *et al*., 2000). In this study four different EPF: *B. bassiana, V. lecanii, M. anisopliae* and *T. longibrachiatum*, have been evaluated against adult female *T. urticae*. From these fungi, *T. longibrachiatum* has been used for the first time against female *T. urticae*. Moreover, the concentration of conidial suspension has also an impact on mortality of *T. urticae*.

Similar study by Elhakim *et al*. (2020) has evaluated the four EPF (conc.1 x 10^8 ml^{-1}) *B. bassiana, V. lecanii, M. anisopliae* and *T. harzianum* against the *T. urticae* in common maize plant and found the mortalities varied by 15-70%, 11-72%, 18-85% and 8-63%, respectively. In our study, *T. longibrachiatum* showed excellent results against both strains of *T. urticae* with recorded mortality percentages in green strain 88.6% with conc. 4 x 10^8 condia/ml, and 71.3% (conc. 4 x 10^4 conidia/ml), followed by red strain 86.9% (conc.4 x 10^8 conidia/ml), and 64% (conc. 4 x 10^4 conidia/ml). Despite of no study on *T. longibrachiatum* evaluation against *T. urticae,* Ghosh and Pal (2016) studied entomopathogenic potential of *T. longibrachiatum* against *Leucinodes orbonalis* (Lepidoptera: Pyralidae)- an economic pest of brinjal (*Solanum melongena* L.). Anwar *et al*. (2016) also used *T. longibrachiatum* against *Bemisia tabaci* showing significant result similar to this study.

However, *B. bassiana* also showed very promising results and the mortality percentages of *T. urticae* were 40.3% (red strain), 65.4% (green strain) having conc. 4 x 10^8 ml^{-1} followed by 35.7% (red strain), 47.2 % (green strain) (conc. 4 x 10^4 ml^{-1}), while *M. anisopliae* revealed 49.1% (red strain), 50.9% (green strain) (conc. 4 x 10^8 ml^{-1}) and 28.7% (red strain), 23.6% (green strain) (4 x 10^4 ml^{-1}). The similar study was carried out by Negash *et al*. (2017), found range of mortality from 46% to 86% in adult *T. urticae* by using *B. bassiana* and *M. anisopliae*. Similar findings were also reported with *B. bassiana* against *T. urticae* (Irigaray *et al*., 2002; Wekesa *et al*., 2006). Studied *B. bassiana* and *M. anisopliae* as biocontrol agents against different development stages of *T. urticae* and found reduction in viability of eggs with significant mortality of adult female spider mite (Bugeme *et al*., 2014). Chandler *et al*. (2005) showed that direct application of *B. bassiana, M. anisopliae* and *V. lecanii* caused enough mortality than the control treatment with distilled water. Alves *et al*. (1998), also reported the relevant findings which indicated that *B. bassiana* caused mortality against about against *T. urticae* about 35 to 95%. Whereas *V. lecanii* (conc. 4 x 10^8 conidia/ml) used in our study exhibited mortality against red and green strains *T. urticae* about 40.1% and 61.9% respectively. The similar kind of study on common beans by Bugeme *et al*. (2015) has found the reduction in population densities of *T. urticae* by using the 10^8 conidia/ml concentration of *M. anisopliae*. The efficacy difference in current findings among the four EPF may be the production of plant allelochemicals which retarded the fungal growth (Chandler *et al*., 2000), or vary the efficiency of fungal strains on host plant (Poprawski *et al*., 2000).

Lethal concentration of conidial suspension also impacts the entomopathogenic efficacy against the mites and other pests (Negash *et al*., 2017). Tefera and Pringle (2004) testified that strains of *B. bassiana* (BB-01) and *M. anisopliae* (PPRC-4) with high conidial concentration 1 x 10^8 conidia/ml found more mortality as compared to the lower concentration against the *Chilo partellus* (Lepidoptera: Pyralidae). These reports stated our current results that conidial concentration (4 x 10^8 conidia/ml) recorded more mortality in *T. urticae* as compared to (4 x 10^4 conidia/ml) in all findings. The reason of high mortality with high concentration is because the strain takes less time to kill the *T. urticae* (Negash *et al*., 2017). Similar results were also found with carmine spider mites (*Tetranychus cinnabarinus*), which showed more mortality with high concentration (Shi *et al*., 2008).

Therefore, current study found that four strains of EPF *T. longibrachiatum, B. bassiana, M. anisopliae* and *V. lecanii* acted as a potential biocontrol against two spotted spider mite (TSSM), *T. urticae*. In addition, (4 x 10^8 conidia/ml) should be considered as effective concentration for the control of *T. urticae*.

CONCLUSION

It is concluded that EPF *T. longibrachiatum, B. bassiana, M. anisopliae* and *V. lecanii* can be considered as alternate source of conventional acaricides for control of *T. urticae*. Based on these baseline data generated under laboratory conditions, it is recommended to plan simulated field trials under varying environmental conditions in order to include these fungi in pest management program.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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